G4thering

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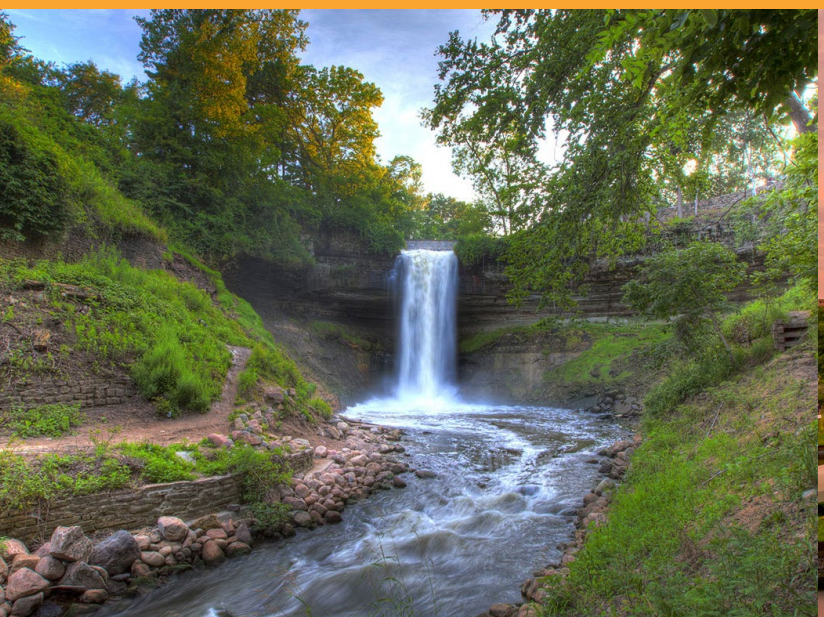
Abstract Book



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Invited Speakers and Oral Abstracts

BIOLOGY & DISEASE 1: Tuesday, June 3, 2025 0830-1010

0830-0850 **Inv. 1 – Marco Di Antonio, *Imperial College of London***

Individual G-Quadruplex Targeting with Chemically Functionalised CRISPR-Cas9 Uncovers Transcriptional and Ligand-Specific Responses

Marco Di Antonio[1]

[1]*Imperial College of London*

The development of selective ligands to target DNA G-quadruplexes (G4s) has been pivotal in supporting their role in transcriptional regulation.1 However, most of the ligands described to date lack intra-G4 selectivity, severely limiting their potential for uncovering the biological function of individual G4s across the genome. In this talk, I will present ATENA (Approach to Target Exact Nucleic Acid alternative structures) a new method we developed to provide G4-ligand with intra-G4 selectivity. ATENA relies on the chemical modification of established G4-ligands that can be conjugated to a catalytically inactive Cas9 protein (dCas9) using Halo-Tag, enabling the targeting of individual G4s in living cells. We have systematically screened the length of the PEG-linkers connecting the G4-ligands to the Halo-Tag and sgRNA sequences to attain optimal G4-engagement both in vitro and in cells. Using optimised conditions, we leveraged ATENA to demonstrate how selective targeting of the established G4 in the proto-oncogene c-MYC suppresses its transcription exclusively from the P1 promoter. We also show that positioning ligands in the proximity of TATA-boxes suppresses c-MYC transcription in a G4-independent manner, highlighting the importance of appropriate design to measure real G4-mediated transcriptional changes. We also demonstrate that selective targeting of the PVT-1 G4 by conjugation of dCas9 to different G4-ligands, PDS or PhenDC3, leads to either stimulation or repression of PVT-1 transcription, indicating that the biological responses elicited by G4-stabilization can be highly dependent on the type of ligand used. We further leveraged our platform to study the biological responses caused by targeting de novo G4s. Our study provides key insights into G4-based therapeutic design, offering an innovative platform to investigate G4 biology from a fresh perspective.

0850-0910 **Inv.22 – Kevin Raney, *University of Arkansas***

Mechanism for Unfolding of G-Quadruplex DNA by Pif1 Helicase

Kevin Raney[1]

[1]*University of Arkansas*

Pif1 is a superfamily 1B helicase implicated in unfolding of G-quadruplex DNA (G4s). G-quadruplex DNA can be formed when guanine-rich nucleic acids induce four-stranded tetrads that are held together by Hoogsteen base pairing and stabilized through stacking interactions. G4s are sufficiently stable to cause genome instability through impeding DNA replication fork progression. Pif1 helicase binds preferentially to G4 DNA and plays multiple roles in maintaining genome stability, but the structural mechanism by which Pif1 unfolds G4s is poorly understood. Here we report the co-crystal structure of Saccharomyces cerevisiae Pif1 (ScPif1) bound to a G4 DNA with a 5′ single-stranded DNA (ssDNA) segment. ScPif1 recognizes G4 mainly through a region referred as the “wedge” in the 1A domain that contacts the 5′ most G-tetrad directly. A conserved Arg residue in the wedge is required for Okazaki fragment processing but not for mitochondrial function or for suppression of gross chromosomal rearrangements. Multiple substitutions at this position have similar effects on resolution of DNA duplexes and G4s, suggesting that ScPif1 may use the same wedge to unwind G4 and dsDNA. Our results reveal the mechanism governing dsDNA unwinding and G4 unfolding by ScPif1 helicase that can potentially be generalized to other eukaryotic Pif1 helicases.

0910-0930 **Inv.3 – Brett Kaufman, *University of Pittsburgh***

G-Quadruplexes in Mitochondria

Brett Kaufman[1]

[1]*University of Pittsburgh*

The stability and normal expression of mtDNA are essential for cellular respiration and tissue viability. Secondary structures are present in mtDNA, but we have an incomplete understanding of how they impact mitochondrial replication, gene expression, and genome integrity. We and others have associated G-quadruplex structures (G4s), which form among guanine-rich sequences, with mtDNA deletion breakpoints. Our exciting preliminary data shows that mitochondrial-localized G4 binding agents (mtG4BAs) negatively influence mitochondrial replication, transcription, and respiration through G4 stabilization and have a biased interaction with mtDNA sequence variants that affect G4 stability. Despite this, mitochondrial G4 are evolutionarily conserved, suggesting that G4s have a beneficial role in mitochondrial function at physiological levels. These data lead us to the overarching hypothesis that physiological G4 formation within mtDNA regulates mitochondrial transcription and replication. The mtDNA is rich in predicted G4-forming sequences, but it is unclear which mitochondrial sequences usually form G4 in cells, how these structures affect mitochondrial biology,

and which specific enzymes regulate G4 formation and removal. We will describe what we currently know about mtG4 formation, biological impact, and tools being generated for their study. The hope is that mtG4 can be leveraged with targeting molecules to treat mitochondrial defects in genetic and idiopathic diseases.

0940-1000 **Inv. 34 - Mike Wendt, *University of Iowa***

Targeting G-Quadruplex to Eliminate Dormant Breast Cancer

Mike Wendt[1]

[1]*University of Iowa*

Despite recent therapeutic advances, metastatic breast cancer (MBC) remains an incurable disease with only 32% of patients surviving 5-years beyond diagnosis. An unmet need that contributes to the eventual progression of MBC is the lack of therapeutic strategies capable of eradicating disseminated dormant disease. Several recent studies from our group and others suggest that cellular plasticity is critical to drug persistence and tumor re-initiating capacity of MBC. The concept of phenotypic plasticity leading to cellular heterogeneity of metastatic tumors largely occurs at the epigenetic level and likely contributes to the inability of highly-specific targeted therapies to produce complete responses in MBC patients. Our lab has demonstrated that fibroblast growth factor receptor 1 (FGFR1) is upregulated in metastases and that this signaling axis plays critical roles in metastatic tumor growth. Targeted inhibition of FGFR kinase activity with FDA approved small molecules can regress active metastases, but the remaining dormant disease upregulates PDGFR to facilitate cell survival. We have demonstrated that the proximal promoter of FGFR1 contains G-quadruplex (G4) and pharmacologic stabilization of these structures limits expression of FGFR1 and PDGFR. In contrast to specific enzymatic targeting of FGFR1, G4-mediated normalization of its expression is capable of eliminating dormant disease. Ongoing studies are focused on understanding the complete epigenetic reprograming that takes place upon G4 stabilization reducing survival of dormant disease.

1000-1010 **BIO.1.3 – Not All G4 Binders Are the Same: New Insights into G4-Mediated Immune Gene Activation Through Proteomic Analysis of Micronuclei**

Giulia Miglietta[1], Monica Procacci[1], Marco Russo[1], M.P. Ximénez De Embún Cadarso [2], Javier Munoz[2], Giovanni Capranico[1]

[1]*University of Bologna,* [2]*Spanish National Cancer Research Centre*

G-Quadruplexes (G4) have fueled studies to selectively target DNA regions, driving the development of numerous high-affinity ligands. These G4 ligands have primarily been explored for their role in repressing oncogenes and inducing cell death. However, in our lab, we uncovered their potential as immunomodulatory agents. We show that G4 binders, at subcytotoxic concentrations, stimulate micronuclei (MNi) formation in cancer cells, activating an interferon-dependent response via the cGAS-STING pathway. To discover more effective ligands, we screened diverse G4 binders for their ability to trigger an innate immune response via MNi accumulation. Surprisingly, not all MNi induce the same cellular outcome. To investigate G4-mediated immune gene response, we isolate MNi and Nuclei of treated cells for proteomic analysis. Our findings reveal that MNi induced by pyridostatin (PDS) are enriched in autophagosomes and innate immune components, while those induced by RHPS4 contain mitochondrial proteins and do not trigger interferon signalling. Genome-wide profiling highlights that autophagy promotes MNi resolution, cGAS activation, and interferon expression, unlike mitophagy, seems to inhibit innate immunity. I will discuss unpublished data, providing new insights into the G4-mediated micronuclei response revealing unexpected pathways, which can be targeted to enhance a hot immunological phenotype in human cancers.

BIOLOGY & DISEASE 2: Tuesday, June 3, 2025 1040-1220

1040-1100 **Inv.4 – Robert Hansel-Hertsch, *University of Cologne***

G-quadruplex DNA in trained immunity and aging

Robert Hänsel-Hertsch[1]

[1]*University Hospital Cologne*

G-quadruplex (G4) DNA structures are dynamic epigenetic phenomena that form in nucleosome-depleted regions (NDRs) (e.g., promoters, enhancers). Active enhancers are marked by H3K27ac, crucial for gene regulation. Innate immune memory (trained immunity) is another epigenetic event in which immune cells (e.g., macrophages) recognize and "memorize" exogenous signals in their chromatin, leading to enhanced immune responsiveness in the future. We hypothesize that G4s, which predominate in NDRs and exclude nucleosomes, support long-lasting transcription of immune genes critical for trained immunity. Indeed, we observe persistent H3K27ac near G4-forming sites in monocyte-derived macrophages from human individuals who received ≥2 SARS-CoV-2 mRNA vaccinations, suggesting that G4 structures enhance immune memory. G4s can promote DNA double-strand breaks (DSBs) when exogenously stabilized by small molecules targeting G4s and are thought to cause mutations when not properly maintained by helicases. Previously, we used G4-mapping to reveal their increased formation in various cancer models, suggesting that their presence in regulatory and unstable regions may contribute to cancer development. I will present our efforts to quantify G4 levels in human and murine aging models and how the loss of sirtuin function may explain increased G4 levels, persistent DSBs, and senescence in aged human and murine cells as well as in aged tissues.

1100-1120 Inv.5 – Guangchao Sui, *Northeast Forest University*

G-Quadruplexes Promote Molecular Motility in MAZ Phase-Separated Condensates to Activate CCND1 Expression

Wenmeng Wang[1], Dangdang Li[1], Qingqing Xu[1], Jiahui Cheng[1], Guangyue Li[1], Zhiwei Yu[2], Guangchao Sui[1]

[1]Northeast Forestry University, [2]Harbin Medical University Cancer Hospital

G-quadruplexes (G4s) can recruit transcription factors to activate gene expression, but detailed mechanisms are not well characterized. We demonstrate that G4s in the CCND1 promoter promote the molecular motility in MAZ phase-separated condensates and subsequently activate CCND1 transcription. Zinc finger (ZF) 2 of MAZ is responsible for G4 binding, while ZF3-5, but not a highly disordered region, is critical for MAZ condensation. MAZ nuclear puncta overlaps with signals of G4s and various coactivators including BRD4, MED1, CDK9 and active RNA polymerase II, as well as gene activation histone markers. MAZ mutants lacking either G4 binding or phase separation ability did not form nuclear puncta, and showed deficiencies in promoting hepatocellular carcinoma cell proliferation and xenograft tumor formation. Overall, we unveiled a novel mechanism that G4s recruit MAZ to the CCND1 promoter and facilitate the motility in MAZ condensates that compartmentalize coactivators to activate CCND1 expression and subsequently exacerbate hepatocarcinogenesis.

1120-1140 Inv.6 – Kateryna D. Makova, *Penn State University*

Non-Canonical DNA in Human and Other Ape Telomere-To-Telomere Genomes: Computational Predictions, Experimental Validations, and Evolution

Kateryna Makova[1], Linnéa Smeds[1], Saswat Mohanty[1], Jacob Sieg[1], Huiqing Zeng[1], Hana Palova[1], Kaivan Kamali[1], Iva Kejnovská[2], Eduard Kejnovsky[3], Francesca Chiaromonte[1]

[1]Penn State University, [2]Czech Academy of Sciences, [3]Biophysics Institute

Non-canonical DNA structures regulate key cellular processes and serve as mutational hotspots, yet their precise detection and evolution have remained elusive due to incomplete genome sequences. Here, we analyze non-B DNA in the recently deciphered telomere-to-telomere (T2T) genomes of humans and great apes. First, we computationally predict motifs capable of forming non-B DNA. These motifs are enriched at the genomic regions added to T2T assemblies, including repetitive sequences, short arms of acrocentric chromosomes (where they may influence satellite dynamics), and centromeres (where they may contribute to centromere function). Second, we experimentally validate non-B DNA structure formation using Permanganate/S1 footprinting with Direct Adapter Ligation and sequencing (PDAL-seq). We show that clusters of different non-B DNA motifs–particularly direct repeats, G-quadruplexes (G4s), and Z-DNA–drive single-stranded DNA formation. PDAL-seq signal is enriched at promoters, enhancers, and 5′ UTRs, supporting a regulatory role for non-B DNA. Third, we investigate the evolution of G4s, identifying thousands of conserved and species-specific pG4s. The conserved pG4s are hypomethylated and linked to regulatory regions, while species-specific pG4s may contribute to adaptation and genome expansion. Thus, non-B DNA is unevenly distributed across ape genomes and might have novel functions in previously inaccessible genomic regions.

1150-1200 BIO.2.1 – Non-Canonical DNA in Human and Other Ape Telomere-To-Telomere Genomes

Linnéa Smeds[1], Kaivan Kamali[1], Iva Kejnovská[2], Eduard Kejnovský[2], Francesca Chiaromonte[1], Kateryna D. Makova[1]

[1]Penn State University, [2]Czech Academy of Sciences

Non-canonical (non-B) DNA structures—e.g., bent DNA, hairpins, G-Quadruplexes, Z-DNA, etc.—which form at certain sequence motifs (e.g., A-phased repeats, inverted repeats, etc.), have emerged as important regulators of cellular processes and drivers of genome evolution. Yet, they have been understudied due to their repetitive nature and potentially inaccurate sequences generated with short-read technologies. Here we comprehensively characterize such motifs in the long-read telomere-to-telomere (T2T) genomes of human, bonobo, chimpanzee, gorilla, Bornean orangutan, Sumatran orangutan, and siamang. Non-B DNA motifs are enriched at the genomic regions added to T2T assemblies, and occupy 9-15%, 9-11%, and12-38% of autosomes, and chromosomes X and Y, respectively. Functional regions (e.g., promoters and enhancers) and repetitive sequences are enriched in non-B DNA motifs. Non-B DNA motifs concentrate at short arms of acrocentric chromosomes in a pattern reflecting their satellite repeat content and might contribute to satellite dynamics in these regions. Most centromeres and/or their flanking regions are enriched in at least one non-B DNA motif type, consistent with a potential role of non-B structures in determining centromeres. Our results highlight the uneven distribution of predicted non-B DNA structures across ape genomes and suggest their novel functions in previously inaccessible genomic regions.

1200-1210 BIO.2.2 – RNA Capping and Quadruplexes

Lydia Hepburn[1]

[1]Cancer Research UK

Gene expression of RNA Pol II transcripts requires the addition of the methylated 5′ cap to protect the RNA from degradation and facilitate splicing, 3′end processing, nuclear export, and translation into protein. RNA Pol II directly interacts and recruits all capping enzymes but one, RNMT (RNA guanine-7 methyltransferase). We wanted to understand how RNMT is recruited to transcripts and why inhibition of the enzyme negatively impacts specific groups of transcripts in different cell states. To do so we explored RNMT-protein interactions by IP-Mass Spectrometry and RNMT-RNA interactions by CLIP-sequencing, both of which have pointed in the same direction – RNA Quadruplexes. We identify a direct RNMT-Quadruplex interaction and are looking at how this impacts enzymatic activity and RNMT regulated transcripts in different cell contexts.

1210-1220 BIO.2.3 – G-Quadruplexes in Viral Genomes: A Comparative Analysis

Vaclav Brázda[1], Jean-Louis Mergny [2]

[1]Czech Academy of Sciences, [2]Laboratoire d'Optique et Biosciences

G-Quadruplexes (G4s) are non-canonical nucleic acid structures with emerging roles in viral biology. We conducted a comprehensive bioinformatic analysis of G4-forming sequences (PQS) across 11,000 viral genomes encompassing 350 Mbp. PQS frequencies differ across evolutionary groups of viruses, and are enriched in repeats, replication origins, 5'UTRs and 3'UTRs. Importantly, PQS presence and localization is connected to viral lifecycles and corresponds to the type of viral infection rather than to nucleic acid type; while viruses routinely causing persistent infections are enriched for PQS, viruses causing acute infections are significantly depleted for PQS. Strikingly, we observed an asymmetric distribution of PQSs within retroviral genomes, with a significant enrichment on the negative (antisense) strand in most mammalian retroviruses, with notable exceptions like HIV-1. Interestingly, PQS density in modern HBV genomes is closer to that of the human host genome compared to ancient HBV strains, suggesting a potential evolutionary adaptation. Our findings highlight the diverse roles of G4s in viral biology, emphasizing their potential as targets for antiviral therapies. Moreover, the observed strand-specific distribution of PQSs in retroviruses suggests distinct regulatory mechanisms for the sense and antisense transcripts.

BIOLOGY & DISEASE 3: Tuesday, June 3, 2025 1345-1525

1345-1405 Inv.7 – Pavel Ivanov, *Harvard University*

Structural and Functional Diversity of Tetramolecular RNA G-Quadruplexes Derived from Endogenous tRNA Variants

Pavel Ivanov[1]

[1]Harvard Medical School

Transfer RNAs (tRNAs) are the key adaptor molecules essential for protein synthesis. Besides their canonical functions in mRNA translation, tRNAs are implicated in non-canonical functions unrelated to protein synthesis. tRNAs are rich source of small non-protein coding RNAs called tRNA-derived RNAs (tDRs), which include tRNA-derived stress-induced RNAs (tiRNAs) formed in response to cellular stress. Growing evidence suggests that several tDRs engage in translation regulation, with a subset of tiRNAs acting as translation silencers. Specifically, 5'-terminal oligopyrimidine (TOG) motif containing tiRNAs such as 5'tiRNAAla and 5'tiRNACys can form unique tetramolecular G-quadruplex structures (G4-tiRNAs). These G4-tiRNAs inhibit canonical (cap-dependent) translation initiation but do not target non-canonical Internal Ribosome Entry Site (IRES)-driven translation initiation. Furthermore, G4-tiRNAs also promote the assembly of Stress Granules (SGs), RNA-protein condensates with stress-adaptive pro-survival functions. Here, we expanded our analysis of G4-tiRNAs to incorporate the tiRNAs derived from tRNA isodecoders. Our findings suggest that, within a tRNA isodecoder pool, even a single nucleotide variation in TOG motif neighborhood results in the variability in the G4 structures. Importantly, such structural variation drives significant diversity in the biological functions of G4-tiRNAs.

1405-1425 Inv.8 – Norifumi Shioda, *Kumamoto University*

"G4 Prionoid" RNA G-Quadruplexes in Neuropathology

Norifumi Shioda[1]

[1]Kumamoto University

Synucleinopathies, including Parkinson's disease, are triggered by alpha-synuclein aggregation, triggering progressive neurodegeneration. We demonstrated that RNA G-quadruplex assembly forms scaffolds for alpha-synuclein aggregation, contributing to neurodegeneration. Purified alpha-synuclein binds RNA G-quadruplexes directly through the N terminus. RNA G-quadruplexes undergo Ca2+-induced phase separation and assembly, accelerating α-synuclein sol-gel phase transition. In alpha-synuclein preformed fibril-treated neurons, RNA G-quadruplex assembly comprising synaptic mRNAs co-aggregates with alpha-synuclein upon excess cytoplasmic Ca2+ influx, eliciting synaptic dysfunction. Forced RNA G-quadruplex assembly using an optogenetic approach evokes α-synuclein aggregation, causing neuronal dysfunction and neurodegeneration. The administration of 5-aminolevulinic acid, a protoporphyrin IX prodrug, prevents RNA G-quadruplex phase separation, thereby attenuating α-synuclein aggregation in alpha-synuclein preformed fibril-injected synucleinopathic mice (Cell. 2024). In this symposium, I will also present the relevance of RNA G-quadruplex to other neurodegenerative diseases.

1425-1445 Inv.9 – Nayun Kim, *University of Texas Austin*

G4 DNA and Zn Finger Transcription Factors

Nayun Kim[1]

[1]University of Texas at Austin

DNA secondary structure and topology are in dynamic flux. Important question is what is the functional significance of non-canonical DNA structures. Sequences containing runs of guanines and capable of forming stable G4 DNA are highly enriched at gene promoters suggesting their potential role in regulating transcription. Recently, human Zn-finger transcription factors such as Sp1 and YY1 were shown to be G4 DNA-binding proteins. Our preliminary experiments led to the novel discovery that yeast transcription factor Msn2 binds to G4 DNA in vivo and in vitro. And G4 DNA forming sequences (PQS) are present at many of Msn2-target gene promoters. Transcription at these genes are up-regulated by

G4 ligands. Here, we describe the kinetics and specificities of Msn2 interaction with G4 DNA and the effect of Msn2-G4 interaction in the regulation of transcription. We identified additional Zn-Finger transcription factors in both yeast and human that bind to G4 DNA with high affinity in support of a novel mode of transactivation in which G4 DNA becomes a docking site for multiple TFs including Msn2 to establish a hub of transactivation.

1455-1505 BIO.3.1 – RAD51 Accommodates G4 Within its Filamentous Structure and Promotes Gap-Filling by Template Switch

Michaela Pospíšilová[1], Lumir Krejci[1]

[1]Masaryk University

G-Quadruplexes (G4) are non-canonical DNA structures abundant in the human genome that can stall replication forks and induce replication stress. While RAD51 is well-known for its roles in double-strand break repair and stalled fork processing, its precise function in G4 metabolism has remained elusive. In this study, we reveal that RAD51 selectively binds parallel G4 structures, forming distinct nucleoprotein filaments with unique conformations compared to its canonical complexes with single-stranded DNA (ssDNA). Functional analyses in U2OS cells demonstrate that RAD51 depletion leads to an accumulation of G4 foci, underscoring its critical role in processing G4 structures. Stabilization of G4s in RAD51-deficient cells exacerbates replication stress, resulting in the formation of ssDNA gaps downstream of stalled replication forks. We propose that RAD51 protects G4-containing ssDNA gaps, promoting their processing through template switching facilitating accurate gap filling and ensuring the stability of G4-rich genomic regions. These findings uncover a novel function of RAD51 in safeguarding genome stability by resolving G4-induced replication stress, offering new insights into its broader roles in replication and repair pathways. This work highlights the interplay between RAD51 and G4 structures, with implications for understanding genome stability mechanisms and potential vulnerabilities in cancers with compromised HR function.

1505-1515 BIO.3.2 – Quadratlas: Decoding the Interplay of RG4s and RNA-Binding Proteins

Erik Dassi[1]

[1]University of Trento

RNA G-Quadruplexes (RG4s) are non-canonical regulators of gene expression whose function and role in disease are increasingly recognized. RG4 folding and unfolding is driven by RNA-binding proteins (RBPs) competing and cooperating to determine the fate of this process. Being able to explore transcriptome-wide RG4 formation and interaction with RBPs is thus crucial to understanding how RG4s are regulated. This knowledge would allow to exploit RG4s as innovative therapeutic targets. To make this type of analysis possible, we developed QUADRatlas, a database of RG4s and RG4-binding proteins in the human transcriptome. Experimentally-derived and computationally predicted RG4s are coupled with known interactions of RG4s with RBPs and an extensive RBP binding sites dataset. Users can intersect RG4s with RBPs that can be key to their function. We also provide analysis tools predicting if an RBP can bind RG4s, RG4 enrichment and de novo RG4 prediction. Furthermore, RG4s are enriched with function annotations and disease association. This user-friendly toolset allows formulating novel hypotheses on RG4 regulation, function, and pathogenicity. QUADRatlas (https://rg4db.cibio.unitn.it) is a significant step forward in our ability to understand the biology of RG4s. Its significant amount of data, coupled with its rich toolset, paves the way for RG4s exploitation as therapeutic targets, enabling the scientific community to start decoding the interplay between RG4s and RBPs.

1515-1525 BIO.3.3 – G-Quadruplex Structures in 16S rRNA and Thermal Adaptation in Prokaryotes

Qisheng Song[1], Bo Lyu[1], Deborah Anderson[1], Kangkang Niu[2], Qili Feng[2]

[1]University of Missouri, [2]South China Normal University

G-Quadruplex (G4) structures are specialized nucleic acid secondary structures formed in guanine-rich regions of DNA and RNA. These structures are involved in various biological processes, including gene regulation, genome stability, and adaptation to environmental stress. While prokaryotic organisms thriving in high-temperature environments exhibit unique genomic and structural adaptations, the role of G4 structures in their thermal adaptation remains underexplored. In this study, we investigated the correlation between G4 structures in the[1]6S rRNA encoding regions and the optimal growth temperatures (Topt) of prokaryotes. Our analysis revealed that while genomic G4 patterns were weakly correlated with Topt, the frequency and stability of G4 structures in[1]6S rRNA regions showed a strong positive correlation with Topt. We further explored evolutionary differences in G4 stability between Thermotoga and Pseudothermotoga species. Experimental validation via circular dichroism spectroscopy confirmed that stable G4 structures are more prevalent in hyperthermophilic Thermotoga species, suggesting selective pressures favoring G4 stability at elevated temperatures. Additionally, treatment with TMPyP4, a G4-stabilizing compound, inhibited bacterial growth and downregulated[1]6S rRNA gene expression, underscoring the functional importance of G4 structures in ribosomal activity and thermal adaptation. Collectively, this study provides new insights into the molecular mechanisms of thermal adaptation and opens avenues for exploring G4 structures as biomarkers for extreme environmental conditions.

YOUNG SCHOLARS: Tuesday, June 3, 2025 1555-1735

1555-1605 YS.1 – Premature Progerin-Driven Aging Promotes G-Quadruplex DNA and Interlinked Genome Instability

Anna Koch[1], Priscilla Piccirillo[1], Joana Frobel[1], Julia Popow[1], Linda Hannak[1], Sara Desideri[1], Olivia Van Ray[1], Pascal Hunold[1], Michaela Höhne[1], Magda Hamczyk[2], Miguel Araujo-Voces[2], Carlos López-Otín[2], Robert Hänsel-Hertsch[1]

[1]University Hospital Cologne, [2]Instituto Universitario de Oncología

G-Quadruplexes (G4s) can pose significant challenges for genome integrity and are associated to DNA double-strand breaks (DSBs). Deficiencies in G4-resolving enzymes, such as helicases, have been implicated in various pathologies with signs of premature aging. In this study we demonstrate that G4 structures accumulate in human and murine models of Hutchinson-Gilford progeria syndrome (HGPS), a premature aging disease characterized by detrimental genomic instability. In order to explore a direct link between G4 accumulation and genome instability, we developed Cleavage Under Targets, tagmentation and DNA double-strand break capture (CUT&Break). CUT&Break enables genome-wide detection of epigenetic features in close proximity to DSBs. We show that DSBs are significantly enriched at sites of G4 formation in livers of an HGPS mouse model. Moreover, we show that promoters with potential to form both R-loops and G4s - but not R-loops alone - are more susceptible to DSB formation. Our findings uncover a novel mechanism, in which progerin-driven degradation of SIRT7 leads to compromised activity of the helicase DDX21, resulting in G4 accumulation, persistent DSBs and senescence. In light of the data, we propose a model for aging in which a decrease in sirtuin levels leads to accumulation of compromised helicases that fail to maintain levels of secondary structures, thus promoting genome instability. This mechanism may contribute to age-related frailty and diseases in humans.

1605-1615 YS.2 – BCL2 Promoter Secondary Structures Facilitate AID Mutagenic Activity

Mason Mccrury[1], Rylie Mangold[1], Todd Spears[1], Samantha Kendrick[1]

[1]University of Arkansas

The apolipoprotein B mRNA editing catalytic polypeptide (APOBEC) family of nucleic acid deaminating enzymes is the second leading known cause of mutagenesis in cancer. The most ancient APOBEC, activation induced cytidine deaminase (AID), has a physiological role in mutating immunoglobulin DNA to diversify the antibody repertoire, though it diverges from its canonical substrate to mutate oncogenes including BCL2. However, the mechanisms of AID recruitment to oncogenes remain unclear. Despite extensive structural homology among members, AID is the only APOBEC known to require G-Quadruplexes (G4s) for its physiological function. We examined AID and the homologous APOBEC3C to determine how key amino acid variations might confer AID-G4 interaction in oncogenes. We discovered that relative to APOBEC3C, AID displayed a higher specificity for the BCL2 G4 and i-Motif (iM: an intercalated cytosine-rich structure complementary to the G4) over linear DNA. For the first time, we show AID prefers to deaminate cytosines in a non-immunoglobulin G4 and iM relative to linear DNA, lending credence to the hypothesis that AID initiates mutagenic events in BCL2 facilitated by secondary structure formation. However, the G4 and iM inhibited APOBEC3C activity, suggesting that discrete amino acid variations in AID relative to other APOBECs confer G4/iM substrate preference. These findings indicate a central role for DNA secondary structures in AID off-targeting and oncogenesis.

1615-1625 YS.3 – Guanine Quadruplex Structures Mediate Genome-Wide Regulation of Small Rnas Upon Ionizing Radiation Stress in Hela Cells

Shruti Mishra[1], Himani Tewari[1], Swathi Kota[1]

[1]Bhabha Atomic Research Centre

Guanine Quadruplex (G4) structures play a crucial role in essential genome functions such as replication, transcription, genome stability and epigenetic regulation. While recent studies have identified G4 structure motifs in certain small RNAs, their genome-wide significance remains unclear. Here, the role of G4 structure dynamics in the regulation of small RNAs under ionizing radiation stress was examined in HeLa cells. Cells were treated with G4 structure-stabilizing ligands to explore their effects on cellular responses to IR. Cell viability and DNA double-strand break repair kinetics were evaluated following exposure to ionizing radiation, combined with or without G4 dynamics stabilization. Further, small RNA sequencing was employed to identify differentially expressed small RNAs in response to G4 stabilization and ionizing radiation. Several known and novel microRNAs were identified to be differentially regulated. Cellular targets of microRNAs were also identified to be involved in transcription, chromatin organization etc. All these findings highlight the intricate interplay between G4 structural dynamics and small RNA regulation in cellular responses to ionizing radiation.

1625-1635 YS.4 – Understanding Sequence-Level Drivers of G-Quadruplex Stability, Ligand-Modulated Stabilization, and Protein Recognition

Justin Martyr[1], Bryan Guzman[1], Yue Hu[1], Alli Jimenez[1], Maria Aleman[1], Daniel Dominguez[1]

[1]University of North Carolina Chapel Hill

Regulation of RNA processing and downstream gene expression has increasingly been linked to structured RNA elements, including the RNA G-Quadruplex (rG4). The function of rG4s strongly depend on their stability, dynamics, and ability to interact with RNA-binding proteins (RBPs). While the underlying sequences for rG4s generally follow the canonical (G2-4N1-7)4 motif, sequence features which modulate rG4 stability and recognition by both RBPs and rG4 ligands remain largely unknown and difficult to study. To directly evaluate these sequence-level drivers of rG4 folding, we designed a library of ~3,000 synthetic rG4s with varied G-tract lengths, loop lengths, and loop compositions. Through RT-stop

followed by sequencing, we confirm known characteristics which increase rG4 stability, but further reveal loop composition preferences and other sequence features which similarly impact rG4 folding. We further selected 6 natural sequence rG4s which were deeply mutagenized, allowing us to pinpoint specific mutations that alone were able to drastically shift the rG4's stability, even when outside of the G-tracts. We further used this complex pool to perform massive scale binding assays with RBPs and small molecule ligands, identifying changes in recognition based on sequence variation. This novel massive scale approach offers deep insights into rG4 stability and interactions with RBPs and ligands, advancing our understanding of fundamental rG4 biology.

1635-1645 YS.5 – Small Molecule-Based Regulation of Gene Expression in Human Astrocytes Switching On and Off the G-Quadruplex Control Systems

Vijay Kumar M J Rao[1], Jérémie Mitteaux[2], Zi Wang[3], Ellery Wheeler[4], Nitin Tandon[4], Sung Yun Jung[5], Robert H E Hudson[3], David Monchaud[2], Andrey Tsvetkov [4]

[1]University of Texas Health Science Center at Houston, [2]Université de Bourgogne, [3]The University of Western Ontario, [4]The University of Texas, [5]Baylor College of Medicine

A great deal of attention is being paid to strategies seeking to uncover the biology of the four-stranded nucleic acid structure G-Quadruplex (G4) via their stabilization in cells with G4-specific ligands. The conventional definition of chemical biology implies that a complete assessment of G4 biology can only be achieved by implementing a complementary approach involving the destabilization of cellular G4s by ad hoc molecular effectors. We report here on an unprecedented comparison of the cellular consequences of G4 chemical stabilization by pyridostatin (PDS) and destabilization by phenylpyrrolocytosine (PhpC) at both transcriptome- and proteome-wide scales in patient-derived primary human astrocytes. Our results show that the stabilization of G4s by PDS triggers the dysregulation of many cellular circuitries, the most drastic effects originating in the downregulation of 354 transcripts and 158 proteins primarily involved in RNA transactions. In contrast, destabilization of G4s by PhpC modulates the G4 landscapes in a far more focused manner with upregulation of295 proteins, mostly involved in RNA transactions as well, thus mirroring the effects of PDS. Our study is the first of its kind to report the extent of G4-associated cellular circuitries in human cells by systematically pitting the effect of G4 stabilization against destabilization in a direct and unbiased manner.

1645-1655 YS.6 – Structure And Dynamics of the PDGFR-B Oncogene Promoter G-Quadruplex: Insights into Regulation and Targeting

Yichen Han[1], Jonathan Dickerhoff[1], Danzhou Yang[1]

[1]Purdue University

Overexpression of the PDGFR-β kinase plays a critical role in human cancers and cardiovascular diseases but specific kinase inhibition is challenging. The PDGFR-β promoter forms DNA G-Quadruplex that represses transcription and is an attractive drug target. Using truncated sequences, we showed that the PDGFR-β promoter can form a vacancy G-Quadruplex (vG4) filled-in by either an intramolecular guanine or external guanine derivatives such as cGMP, suggesting regulation by metabolites. The novel vacancy presents a unique binding site for small molecule targeting. However, how these fill-in vG4s form and interconvert in the extended promoter remains unknown. Herein, we report that the PDGFR-β promoter adopts a novel equilibrium of two broken-strand G-Quadruplexes with different intramolecular fill-in guanines. This equilibrium requires a vG4 intermediate that is readily filled-in by external guanine metabolites, providing crucial insights into PDGFR-β vG4 formation and regulation. We determined high-resolution NMR structures of the two broken-strand G-Quadruplexes. Both G-Quadruplexes adopt parallel folding and contain the same vG4 but are filled-in by different guanines of a$^{3'}$-snap-back loop, which forms a hairpin with a unique reverse-Hoogsteen G-G capping base pair. Our structures provide important information for structure-based drug design to specifically target PDGFR-β G-Quadruplex for human diseases.

1655-1705 YS.7 – Exploring B-MYB G-Quadruplex as a Therapeutic Target In Cancer: Small Molecule Interactions and Their Biological Effects

André Miranda[1], Anne Cucchiarini[2], Cyril Esnault[3], Jean-Christophe Andrau [3], Paula Oliveira[4], Jean-Louis Mergny[2], Carla Cruz[1]

[1]University of Beira Interior, [2]Laboratoire d'Optique et Biosciences, [3]University of Montpellier, [4]Centre for Research and Technology of Agro-Environmental and Biological Sciences

The B-MYB proto-oncogene encodes a transcription factor crucial for regulating the cell cycle and differentiation; however, abnormal B-MYB expression associated with poor prognosis is observed in various cancers. Gene promoter regions are enriched in guanines (G), enabling the formation of G-Quadruplexes (G4), that arise from the self-folding of four G into planar arrangements stabilized by Hoogsteen bonds. G4 can play regulatory roles in gene expression due to their prevalence near transcription start sites and represent promising anticancer targets. Small molecules can bind to promoter G4s and stabilize the structure, and this stabilization may suppress oncogene transcription and inhibit downstream pathways. This work aimed to identify and validate G4 structures in the B-MYB promoter as potential therapeutic targets. We demonstrated that the B-MYB promoter contains several G/C-rich motifs compatible with G4 formation. Using a combination of bioinformatics, biophysical, and biochemical techniques, we confirmed the existence of G4 structures in the promoter region. Additionally, we employed the G4access method to validate these G4 in a cellular context. Furthermore, we assessed the ability of G4 ligands to recognize and interact with the B-MYB G4, using spectroscopic techniques, and evaluated its biological outcomes (viability, migration and gene expression). These findings highlight the potential of B-MYB G4 as a potential target for developing innovative cancer therapies.

1705-1715 YS.8 – Guanine By Guanine: Decoding the BCL2 RNA G-Quadruplex Conformation Landscape

Carla Ferreira Rodrigues[1]

[1]*University of Zurich*

G-Quadruplexes (G4s) in the5′ untranslated region of mRNAs are known to play important roles in translational regulation. One such example is the BCL2 RNA G-Quadruplex, located in a highly conserved sequence and position 42 nucleotides upstream of the translation start site. This G4 has been linked to the repression of Bcl-2 protein expression. This anti-apoptotic protein is tightly regulated and aberrant regulation has been associated with cancer, Alzheimer's, and Parkinson's diseases. Therefore, understanding this structure and its dynamic properties is the first step in elucidating the mode of action and designing future drug treatments. The wild-type BCL2 G4 sequence, comprising 18 guanines, adopts multiple G4 structures in dynamic equilibrium. This project aims to understand the conformational landscape by investigating the role of each guanine. By reducing the number of guanines in the sequence, we forced the G-Quadruplex into reduced dynamics or even a single conformation. By systematically reintroducing the removed guanines, we assessed the impact of each guanine on the structural and dynamic properties of the G-Quadruplex. Additionally, using luciferase assays, we explored the biological impact of these mutants on the downstream regulation of protein expression. Understanding the effect of these mutations on the regulatory mechanisms and structure of the BCL2 RNA G-Quadruplex is a critical step in exploring its future therapeutic potential.

1715-1725 YS.9 – Tetraplexed Nucleic Acids Structures as Templating Platform for Proximity-Enhanced Photochemical Reaction: Applications in DNA Targeting and Aptamer Fabrication

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

[1]*Ghent University*

G-Quadruplexes (G4) and I-Motif (IM) DNA secondary structures are involved in key biological processes like gene expression and telomere maintenance. While G4s have been widely studied and targeted with various ligands, the role of IM remains less understood, and tools for studying them are still limited. To address this, our lab has focused on developing chemical-biological tools to specifically target these structures in complex environment. We used ligand-PNA constructs with a photo-reactive warhead that activates into an electrophile on demand, allowing precise targeting of G4 and IM sequences. G4 structures, in particular, exhibit intrinsic topology polymorphism, influenced by factors such as salt type, concentration, sequence, and the presence of binding molecules. The stability of a specific G4 topology is crucial for aptamer and decoy therapies, as it mediates protein recognition. By applying our photochemical tool, we engineered "stapled"-DNA structures, which were characterized by ORBITRAP-MS, NMR, and in silico analysis. Our results showed improved thermodynamic properties, including higher melting temperatures, increased metabolic stability with enhanced nuclease resistance, and topology stabilization. These structures retained biological activity in an ex-vivo model, providing strong proof-of-concept for their potential in therapeutic applications. These findings support the potential for engineered DNA structures in future therapeutic applications.

1725-1735 YS.10 – Unveiling the Kinetic Tango: Exploring G-Quadruplex Ligand Binding Dynamics and Transfer Mechanisms

Hariz Iskandar Mohd Nizal[1], Anthony Mittermaier[1]

[1]*McGill University*

Guanine Quadruplex (G4s)-containing genes have been implicated in oncogenesis, making them valuable targets for therapeutics. Small molecules can interact with G4s, modulating their stability and function. In this context, the binding kinetics are critical as it determines the bound lifetime of the complex. This governs how the ligand finds its in-cell target and the extent to which it becomes trapped in off-target complexes. In this study, we utilize NMR and SPR techniques to measure the binding kinetics of the cMYC G4 with the porphyrin ligand TMPyP4. We found that the kinetics were extremely slow, to the extent that the ligand is effectively trapped onto the first G4 structure encountered. However, these rates increased dramatically as the G4 concentration increased. This revealed a mechanism in which ligands are transferred directly between G4s via collisions in solution, mimicking facilitated diffusion in protein-DNA interactions. The same direct transfer effect was also observed when dsDNA was introduced to the system. This allows ligands to rapidly reach intended G4 targets by utilizing more readily available neighbouring dsDNA. This has potential implications for the development of G4-binding ligands which can capitalize on this direct transfer for enhanced therapeutic activity.

STRUCTURE & DYNAMICS 1: Wednesday, June 4, 2025 0830-1010

0830-0850 **Inv.16 – Harald Schwalbe, *University of Frankfurt***

Static and Time-Resolved NMR Studies to Study G4 Folding

Harald Schwalbe[1]

[1]*Goethe University*

Work in the group of the author focus on the development and application of NMR to study G4 structue, dynamics and folding. In the past, we have use light-induced folding induction to study the folding of spare-tire DNA and non-canonical G4 structures. In this contribution, three recent studies will be presented:

- Time-resolved NMR to study the effect of G4 binding ligands on G4 folding. This work has been done by I. Burkhart together with J. Plavez.
- Time-resolved NMR using photoreversible ligands to study G4 folding. This work has been done by J. Martins
- The effect of Zuo-1 protein on a metastable G4 forming sequence from yeast. This work has been done by I. Burkhart and M. Limmer together with K. Paeschkie and J. C. Penedo

0850-0910 **Inv.17 – Guang Zhu, *Hong Kong University of Science and Technology***

G-Quadruplex Structures Formed by C9orf72 DNA and RNA

Guang Zhu[1]

[1]*The Hong Kong University of Science and Technology*

We are interested in the structure-functional study of G-quadruplexes (G4s) in human diseases and DNA replication, and in search of specific stabilizers targeting these G4s. I will briefly present some results from our endeavor, including structures of a telomeric G4, and structures formed by G4C2 DNA and RNA repeats determined by NMR and x-ray crystallography. We showed that the parallel d(G4C2)2 G4 folds as a symmetric tetramer, while the antiparallel d(G4C2)2 adopts the topology of an asymmetric dimer. We have also identified three first-in-class marine natural products, chrexanthomycin A(cA), B(cB), and C(cC), with remarkable bioactivities. cA shows the highest permeability and lowest cytotoxicity to HEK293T cells. In silico analysis and NMR titration experiments we showed that cA, cB, and cC selectively bind to RNA and DNA G4C2 G4s. Additionally, we demonstrated that human hCdc6 could also interact with the G4 DNA. Mutagenesis and in vivo investigations confirm the highly specific nature of Cdc6 in recognition of G4s. This research sheds light on the intricate regulation of DNA replication processes.

0910-0930 **Inv.18 – Shuntaro Takahashi, *Konan University***

Physicochemical properties of tetraplexes for the dynamic regulation in biological functions

Shuntaro Takahashi[1]

[1]*Konan University*

Tetraplexes including G-quadruplex and i-motif play a crucial role in gene expression and diseases. As these tetraplexes are highly responsive to the environment changes in solution, molecular environment should have a main factor to control tetraplex formation and functions in cells. Here, we systematically investigated the stability and functions of i-motif DNAs by using various polyethylene glycols that mimicked diverse cellular crowding environments. The thermodynamic and molecular dynamics simulation revealed that the helicity of the i-motif dynamically changed depending on various physicochemical factors of the solution environment. Furthermore, cosolute-induced twisting dynamics controlled by different cosolutes changed the activation energy barrier of replication along the i-motif-forming DNAs. Our findings implied that the regulatory mechanism of the biological roles of i-motifs in different cellular phases does not rely on binding proteins. We will also discuss the function of G-quadruplex under various conditions in cells.

0940-0950 **STR.1.1 – Supramolecular Assembly of D(G4C2) and D(G4C2)4 Repeats Associated with ALS and FTD**

Melani Potrc[1], Elena Cokor[1], Irena Drevensek-Olenik[2], Lea Spindler[2]

[1]*University of Maribor,* [2]*Josef Stefan Institute*

Quadruplex formation of d(G4C2)n repeats is believed to be involved in some fatal neurological disorders, especially amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Nevertheless, their supramolecular assembly is still far from being fully understood, as their extensive structural polymorphism represents a big challenge to experimental investigations. We focused ond(G4C2) and d(G4C2)4 sequences, which are not only capable of forming G-Quadruplexes but are also prone to higher-order supramolecular assembly. Previous studies showed that the d(G4C2) sequence assembles into a tetrameric symmetric Quadruplex, while the d(G4C2)4 sequence folds into a unimolecular Quadruplex with three edge loops. By using dynamic light scattering we investigated solutions with concentrations ranging from 0.1 mM to 6 mM. We observed a surprisingly complex interplay between stacking interactions at lower and typical polyelectrolyte behavior at higher concentrations. Upon reaching a critical concentration, the G-assemblies ordered orientationally, which led to the formation of liquid crystalline (LC) phases for both sequences. Despitetheir apparent similarity, the two sequences exhibited very different LC textures. Moreover, these LC phases deviated significantly from those observed previously for short or long DNA molecules, which opens up new interesting paths for G-Quadruplex investigations under crowding conditions.

0950-1000 **STR.1.2 – G-Quadruplexes in Archaea**

Lionel Guittat[1], Anne Cucchiarini[2], Zackie Aktary[2], Kate Sorg[2], Guglielmo Vesco[3], Dorian Noury[2], Pierre Mahou[2], Daniela Verga[4], Vaclav Brázda[5], Nicolas Olivier[2], Marie Bouvier[6], Marta Kwapisz[6], Béatrice Clouet-D'orval[6], Thorsten Allers[7], Roxane Lestini[2], Jean-Louis Mergny [2]

[1]Ecole Polytechnique, [2]Laboratoire d'Optique et Biosciences, [3]University of Insubria, [4]PSL Research University, [5]Czech Academy of Sciences, [6]Center for Integrative Biology, [7]University of Nottingham

The archaeal domain encompasses a diverse group of microorganisms that inhabit a wide range of environments and represents the closest known relatives of eukaryotes. This evolutionary connection suggests that eukaryotes may have originated from an archaeal ancestor.G-Quadruplexes (G4) are essential DNA/RNA conformations involved in critical biological processes. Little is known, however, on the formation and roles of G4s in archaea. A bioinformatics analysis of the Haloferax volcanii genome, a halophilic archaeon, identified potential G4-forming sequences (PQS). Biophysical studies confirmed that these PQS adopt stable G4 structures in vitro under physiological conditions. Using the BG4 antibody and super-resolution microscopy, we detected G4 at the single-cell level in both DNA and RNA across different growth phases. Similarly, in the thermophilic archaeon Thermococcus barophilus, we identified G4 structures alongside helicases that may facilitate their unfolding. Furthermore, combining fluorescence in situ hybridization (FISH) with G4 detection allows for the simultaneous visualization of G4 alongside chromosomal organization. This approach provides an opportunity to investigate their dynamics in relation to cellular ploidy at the single-cell level. By establishing archaea as novel models for G4 studies, this research bridges bacterial and eukaryotic systems, offering new insights into the evolutionary conservation of G4 across the tree of life.

1000-1010 **STR.1.3 – G-Quadruplex-Driven Molecular Disassembly and Type I-To-Type II Photophysical Conversion of a Heavy-Atom-Free Photosensitizer For Site-Specific Oxidative Damage**

Marco Deiana[1]

[1]Wroclaw University of Science and Technology

Light-responsive, molecular-targeted therapies are redefining precision oncology by confining DNA damage to malignant cells while sparing healthy tissue. We introduce DBIPOE, an amphiphilic, heavyatomfree photosensitizer (PS) derived from sulfursubstituted dibenzothioxanthene imide and functionalized with a hydrophilic polyoxyethylene (POE) side chain. In aqueous solution, DBIPOE selfassembles into nanoaggregates that disassemble upon selective binding to GQuadruplex (G4) DNA, triggering a "turnon" of emissive properties, facilitating twophoton nearinfrared excitation, and reprogramming the PS's photophysical reactivity. In its aggregated state, DBIPOE favors a Type I pathway that produces superoxide radicals; however, G4 recognition switches it to a Type II mechanism that predominantly generates singlet oxygen. The singlet oxygen oxidizes guanine residues, triggering G4 unfolding—a phenomenon confirmed by biophysical assays and molecular dynamics simulations. Moreover, polymerase stop assays at singlebase pair resolution reveal that photoactivated DBIPOE causes precise, sitespecific oxidative lesions that stall DNA polymerase exclusively at G4 sites while sparing nonG4 regions. This unique combination of supramolecular disassembly, photophysical pathway switching, and G4selective oxidative damage underscores the high specificity and therapeutic potential of DBIPOE, paving the way for nextgeneration cancer treatments that target oncogenic DNA architectures.

STRUCTURE & DYNAMICS 2: Wednesday, June 4, 2025 1040-1220

1040-1100 **Inv.19 – Danzhou Yang, *Purdue University***

Oncogene DNA G-quadruplexes: Structures, Drug Targeting, and Protein Interactions

DNA G-quadruplex secondary structures formed in guanine-rich oncogene promoters are transcription regulators and attractive anticancer drug targets. I will present our progress on structural studies and drug targeting of promoter G-quadruplexes of oncogenes. I will also present structural and functional study of protein interactions of the MYC oncogene promoter G-quadruplex.

1100-1120 **Inv.20 – Sam Butcher, *University of Wisconsin-Madison***

Rules for the pUG Fold: An Unusual Quadruplex That Directs the Amplification of RNAi

Samuel Butcher[1]

[1]University of Wisconsin-Madison

The pUG fold is an unusual left-handed (Z-form) quadruplex that forms in poly(UG)-rich RNA sequences. We determined the high-resolution structure of the pUG fold and showed it is responsible for directing the amplification of RNA interference. We discovered that pUG folds mark mRNAs as vectors for the transgenerational epigenetic inheritance of gene silencing in vivo. We used several orthogonal methods, including atomic-level mutagenesis of reporter RNAs containing 7-deazaguanosine, to demonstrate this. The injection of atomically modified RNAs into live animals, accompanied by measurement of the silencing efficiency of GFP reporters, allowed us to provide direct evidence for functional importance of the pUG fold. I will describe the unusual biophysical properties of pUG RNAs, including their structure, dynamics and ability to form high-order multimeric interactions that enable efficient formation of condensates. I will also describe the "rules" that govern pUG folding and will show many sequences that are not poly(UG) can still adopt this unusual fold. These data lead to the identification of many thousands of potential pUG folds in regulatory regions

of human RNAs, and some of these are associated with disease. The rules for understanding pUG folding broaden our understanding of nucleic acid quadruplexes and will be important in the future for understanding when and where pUG folds form within transcriptomes.

1120-1140 Inv.21 – Kyeong Kyu Kim, Sungkyunkwan University

A Novel Strategy for Developing Antibiotics Targeting the G-Quadruplex

Kyeong Kyu Kim[1]

[1]Sungkyunkwan University

The rise of multidrug-resistant (MDR) Staphylococcus aureus and the limited availability of novel antibiotic targets underscore the urgent need for alternative therapeutics. This study explores G-quadruplex (G4)-binding ligands as potential antibiotics due to their ability to disrupt vital gene expression. Screening several G4-binding ligands against hypervirulent and MDR S. aureus USA300 identified N-methyl mesoporphyrin IX (NMM) as the most effective, with a minimum inhibitory concentration (MIC) of 5 μM. NMM disrupted cell division and cell wall formation by repressing genes within the division cell wall (dcw) gene cluster. Genome-wide bioinformatics analysis revealed a G4 motif in the promoter region of mraZ, a master regulator of the dcw cluster. Spectroscopic techniques confirmed the formation of a parallel G4 structure in the mraZ promoter and its interaction with NMM. In vitro transcription/translation assays further validated that NMM targets the mraZ G4 motif to exert its antibacterial effect against both gram-positive and gram-negative bacteria. In vivo studies using RAW264.7 cells and Galleria mellonella models demonstrated NMM's superior antibacterial activity over established antibiotics without cytotoxicity. Overall, this study identifies NMM as a promising broad-spectrum antibiotic targeting G4 motifs to inhibit bacterial growth.

1150-1200 STR.2.1 – From Meme to Model: How Cryo-EM Helped Resolve a Misconception in the G-Quadruplex Field

Robert Monsen[1], Eugene Chua[2], Jesse Hopkins[3], Jonathan Chaires[1], John Trent[1]

[1]University of Louisville, [2]National Center for Cryo-EM Access and Training, [3]The Biophysics Collaborative Access Team

Genomic regions with high guanine content have the capacity to fold into four-stranded non-B DNA structures known as G-Quadruplexes (G4s). Extensive bioinformatic inquiries have revealed that G4 motifs are conserved and non-randomly distributed throughout the genome. G4s are epigenetic features that act as transcription factor hubs which can regulate gene expression in temporal, spatial, cell-type, and cell-state-dependent ways. No high-resolution structural information exists for G4s in their natural genomic environment because of the difficulty in studying such long heterogenous DNAs by X-ray diffraction and NMR methods. Here we present an investigation of a[2]8.5 kDa duplex-G4-duplex ("DGD") model promoter system using the integration of cryo-EM, molecular dynamics, and small-angle X-ray scattering (SAXS). The nominal resolution of the cryo-EM maps range from 6.8-8.2 Å and offer adequate secondary structure information for model refinement. The integration of SAXS and molecular dynamics with models derived from cryo-EM show that in the context of a duplex bubble the G4 preferentially stacks against one of the two duplex "handles" to form an asymmetric particle with 49-67° bend. This model goes against the common belief in the G4 field that G4s (and their C-rich complements) protrude away from the duplex axis and are fully accessible to binding ligands. Instead, we find that the G4 moiety is relatively occluded by the adjacent duplex and loop features.

1200-1210 STR.2.2 – Post Transcriptional Regulation of Kras Gene Via 5UTRr RNA G-Quadruplexes and Long Noncoding RNA

Zahraa Othman[1], Ylenia Cortelezzis[2], Francesca Agostini[2], Gilmar Salgado[1], Luigi Xodo[2], Eros Di Giorgio[2]

[1]Bordeaux University, [2]University of Udine

In this work, we aim to elucidate the role of the hnRNPA1 protein in regulating both RNA and DNA G-Quadruplexes (G4s) as a checkpoint modulator of KRAS gene expression. It is established, though not fully understood, that G4 structures in the KRAS promoter may facilitate transcription factor recruitment and also block histone modifiers that make it accessible to the transcription initiation site. On the other hand, rG4 structures within the 5′ untranslated region (5′ UTR) of KRAS mRNA may act as steric barriers that regulate translation initiation while also maintaining the overall 5′ UTR structure and stability required for translation. To investigate these mechanisms, we employed ChIP-seq and RIP-seq experiments, which confirmed the presence of folded G4 structures in vivo. Furthermore, genome-editing using CRISPR/Cas9 allowed us to disrupt G4 structures, resulting in a significant increase in KRAS mRNA levels. Using biophysical techniques such as biolayer interferometry and NMR, we characterized the interaction of hnRNPA1 with both DNA and RNA G4s, in order to decipher its role in stabilizing their respective secondary structures. Our findings suggest that hnRNPA1 functions as a dual regulator, controlling two critical checkpoints in KRAS expression: transcriptional regulation via DNA G4s and post-transcriptional mRNA stability through rG4 formation in the 5′ UTR.

1210-1220 STR.2.3 – Gene Transcription Regulation by G-Quadruplex

Lijun Xiang[1], Kangkang Niu[1], Qili Feng[1]

[1]South China Normal University

DNA advanced structures such as G4 and i-motif, have been found to involve in multiple biological processes. To study the roles of G-Quadruplex (G4) structures in regulation of gene transcription, we analyzed genomic DNA G4 and gene transcription in silkworm by using CUT&Tag and ChIP-seq methods in combination with RNA-seq and ATAC-seq. Intersection analysis of genomic G4s and transcriptome indicated that genes that contained G4s were mostly in an active transcription state, whereas the genes that did not contain G4 structures were in an active transcription state. Transcription level is positively related with G4 CUT&Tag signals. Intersection analysis of G4 signals and chromatin accessibility signals

revealed that most of G4 signal positions overlapped with chromatin accessibility signal positions, suggesting that G4 structures may more easily form in the chromatin accessible regions of the genome. Intersection analysis of histone modification and G4 structures revealed that G4s was co-localized with active unmethylated histone marks, but not with transcriptional repression mark H3K9me3. There was a significant difference in the formed G4 structures and gene expression patterns in the fat body and brain. Differential G4s patterns in various organs may result in distinct active transcription of tissue-specific genes. This study is supported by grants from the Chinese National Natural Science Foundation (32250710148 and 31930102).

NANOTECH & BIOTECH 1: Thursday, June 5, 2025 0830-1010

0830-0850 Inv.13 – Hanbin Mao, *Kent State University*

Understanding the Role of Tandem G-Quadruplex Structures in the Liquid-Liquid Phase Separation (LLPS)

Hanbin Mao[1]

[1]*Kent State University*

Liquid-liquid phase separation (LLPS) occurred inside cells has attributed to modulating a multitude of cellular functions. These LLPS condensates are often formed between positively charged peptides and negatively charged nucleic acids. In our research, we have investigated the LLPS condensation between peptides and single-molecule nucleic acid templates anchored between two optically trapped beads in a laser tweezers instrument. By monitoring the mechanical tension of the nucleic acid templates upon addition of positively charged poly-l-lysine (PLL), we revealed a multistage LLPS process mediated by the long-range interactions between nucleic acids and polyelectrolytes. We found that compared to random nucleic acid sequences, single-stranded DNA templates containing tandem G-quadruplex units are most difficult to form LLPS condensates. This is likely because poly-G-quadruplex has the propensity to form rigid nucleic acid–PLL complexes, reducing the condensate formation during the LLPS process. In addition, we also discovered that LLPS processes follow matched chirality between poly-lysine and G-quadruplex. Poly-l-lysine and poly-d-lysine are easier to form condensates with left- and right-handed G-quadruplexes, respectively. We anticipate that these results can provide new strategies to interfere with biological functions of LLPS condensates involving nucleic acids with secondary structures, such as tandem G-quadruplex, which occurs frequently inside cells.

0850-0910 NANO.2 – Using Parallel-Type G4 as a Scaffold for Delivery of Immunostimulatory Cpg Oligodeoxynucleotides to Immune Cells

Tomohiko Yamazaki[1]

[1]*National Institute for Materials Science*

Oligodeoxynucleotides (ODNs) with unmethylated cytosine-phosphate-guanine (CpG) motifs are effective as vaccine adjuvants due to their ability to trigger immune responses via toll-like receptor 9. However, linear CpG ODNs with phosphodiester (PD) backbones degrade quickly due to nucleases, limiting their usefulness in clinical settings. Modified CpG ODNs with phosphorothioate show better nuclease resistance but have negative side effects like prolonged coagulation and toxicity. To overcome the limitations, we developed PD-backbone-based CpG ODNs with high nuclease resistance using the guanine-Quadruplex (G4) structure as scaffold. The insertion of CpG sequences into the loop region of G4 enhanced nuclease resistance and cellular uptake of CpG ODNs, resulting in the high immunostimulatory activity of G4-CpG ODNs in immune cells. In this study, we investigate immunomodulatory properties of different topologies of G4. The parallel-type G4-CpG ODN demonstrated the highest serum stability and cellular uptake, resulting in the strongest immune response from macrophage cells amongst all three topologies (parallel, antiparallel, and hybrid) of G4 CpG ODNs. These findings provide valuable insights into the development of CpG ODN-based vaccine adjuvants.

0910-0930 NANO.3 – I-Motif Kinetics as a Unique Opportunity in Sensor Design: A Case Of Molecular Calorimeters

Irina Nesterova[1]

[1]*Northern Illinois University*

Hemiprotonated cytosine-cytosine bonding mediates i-motif folding. Engaging proton imposes a substantial entropic penalty on the folding transition; the penalty increases with pH as proton activity drops. As a result, i-motif folding rates at pHs close to neutral are very slow. The kinetics enables the engineering of molecular sensors that operate as calorimeters: i-motifs switch conformation (unfold) in response to a released heat but do not fold back when the heat trigger dissipates. As a result, the i-motif scaffolds can function as calorimeters by integrating small heats released over time. As a part of the rational design of molecular calorimeters, we systematically investigate the structure/kinetic properties correlation in i-motifs, derive folding mechanisms, assess kinetic trapping, and evaluate the effect of the environment on the i-motif's folding/unfolding rates. I-motif-based molecular calorimetry empowers measuring heat changes in small open systems and is especially suitable for biochemical systems. Most biochemical processes release/absorb heat slowly but equilibrate with surroundings quickly limiting conventional calorimetry's usefulness. I-motif-based calorimeters address a range of situations that are currently inaccessible such as measuring heat in very small volumes and/or small heats released over long periods, and/or achieving a spatial resolution in heat measurements.

0940-0950 NANO.1 – Single Molecule Studies on Interactions of Telomeric Overhangs and Telomere-Specific Proteins

Hamza Balci[1], Ahmet Yildiz[2], Sajad Shiekh[1], Amanda Jack[2]

[1]*Kent State University,* [2]*University of California, Berkeley*

We present single-molecule fluorescence studies—including FRET, FRET-PAINT, PIFE, and mass photometry—that investigate the folding patterns and accessibility of telomeric overhangs with physiologically relevant lengths (up to 28 GGGTTA repeats). Specifically, we examine the impact of the shelterin complex, POT1, small molecules, and molecular crowders on the accessibility and protection of these vulnerable genomic regions. Our findings reveal length-dependent periodic accessibility patterns and demonstrate how POT1 and the shelterin complex influence these patterns. Notably, our studies show that the junction between single- and double-stranded telomeres is highly accessible in the absence of shelterin. We further explore this junction region to identify the minimum requirements for effective protection by shelter in or POT1 and assess the effects of 5'-phosphorylation of the junction on the protection of this region. Finally, we present

mass photometry experiments that directly probe the binding interactions of POT1, RPA, and the shelterin complex with long telomeric overhangs and the inhibition presented by G-Quadruplex studies on these binding interactions.

0950-1000 **Inv.14 – James Vesenka, *University of New England***

Persistence lengths of extended Tet1.5 and G10 G-wire DNA

James Vesenka[1], Mayuri Gilhooly[1], Eva Balog[1], Thomas Marsh[2]

[1]University of New England, [2]St. Thomas University

We explore G-wire DNA structure as part of a larger effort illuminate their growth kinematics. The primary tool used to analyze the DNA was atomic force microscopy imaged in air and liquid. AFM images were analyzed to confirm orientation correlations on untreated mica using ultra-high resolution atomic force microscopy. Persistence lengths measurement were taken from numerous samples on mica treated with poly-L-ornithine. Preliminary studies were undertaken to explore growth rates on gold nanoparticle terminated DNA and surface plasmon resonance shifts based on the length of the terminated G-wires. Many elements of this research are ideal suited for undergraduate students because of the ease of sample construction and imaging.

1000-1010 **Inv.15 – Thomas Marsh, *St. Thomas University***

Use of G4 for the Construction of Supramolecular Scaffold Materials

Thomas Marsh[1]

[1]St. Thomas University

Guanosine and Guanine-rich nucleic acids have the well-known property of self-assembling into supramolecular materials in a variety of conditions. We have investigated the potential for use of G-rich oligonucleotides (GROs) as molecular scaffolds to harness the robust assembly and stability of the supramolecular GRO termed G-wires. G-wires readily self-assemble in the presence of specific monovalent and divalent cations to produce stable polydisperse linear structures. G-wires have been used as a scaffold for aligning gold nanoparticle though the potential biological activity is unknown. Initial studies with the transfection of mammalian cells show G-wires are taken and persist within the cell for days in various cellular locations. However, the polydispersity of G-wires and confirming retention of G4 structure present in cells present a challenge for characterization of their impact on cell metabolism. Here we summarize work investigates a strategy for limiting the polydispersity of simple G-wire forming GROs for controlling self-assembly. Specifically, the G-DNA specific fluorescent dye, N-methylmesoporphoryn (NMM) can attenuate the extent of G-wire self-assembly and potentially serve as probe to monitor retention of G4 in cells transfected with G-wires.

STRUCTURE & DYNAMICS 3: Thursday, June 5, 2025 1040-1220

1040-1100 **Inv.24 – Janez Plavec, *National Institute of Chemistry***

Dynamic NMR structures of quadruplex DNA tuned by C-methylation

Janez Plavec[1]

[1]National Institute of Chemistry

The polymorphism of DNA, which allows the adoption of multiple structures beyond the classical double helix, plays a key role in its functions. Alternative structures such as G-quadruplexes (G4) arise due to sequence composition, environmental conditions and small molecules such as heterocyclic ligands. Methylation of cytosine, an epigenetic modification, raises questions about its influence on G4 formation and stability. We have studied the impact of 5-methylcytosine (Cm) on the bcl2Mid G4 structure formed by the GC-rich region upstream of the P1 promoter, a site that regulates BCL2 gene expression. Using NMR and biophysical methods, we found that Cm has a sequence-specific effect on the folding kinetics of bcl2Mid G4. The substitution of cytosine by Cm slows down folding and alters the equilibrium between major and minor structures in the presence of K+ ions. The major G4 adopts a 3+1 hybrid topology (D. Yang, et al., Nucleic Acids Res. 2006, 34, 5133), while the minor G4 has parallel strands and a snapback element that fills a G-quartet vacancy.

1100-1120 **Inv.23 – Gary Parkinson, *University College London***

I-motifs from Human Telomeric Sequences as a Molecular Target: Combining Crystallographic and Single Molecule FRET to Understand Topology and Stability

Gary Parkinson[1], Zoë Waller[1], Shahed Al Olimat[2], Wenqian Chen[2]

[1]University College London, [2]UCL School of Pharmacy

Investigations into i-motifs in telomeric regions have shown their ability fold into tetrahelical i-motif structures in vitro however little has been revealed structurally to understand their drugability, possibility due to the limited understanding in their role in human telomere biology. Recent data has shown that telomeric i-motifs can inhibit the activity and processivity of telomerase extension, highlighting a possible therapeutic role. Here we report on the use of single based FRET studies on telomeric i-motifs within ssDNA and dsDNA constructs. Sequences were labelled allowing us to follow pH dependent folding/unfolding pathways, both temporal and spatially. The constructs were also designed to show the contribution of the presents of dsDNA, both at the 3' end and internally. Our results show impact on i-motif stability when in the presence of

dsDNA. Crystallisations were also undertaken on these model constructs leading to the successful crystallisations of two of these constructs. Using SAD methods, we have now determined the crystallographic structure of the intramolecular i-motif from the human telomeric sequence. Our overall vision is to reveal the detail in iM structures and their ligand binding sites, enabling the potential of iM structures as therapeutic molecular targets for rational drug design.

1120-1140 Inv.35 – Shozeb Haider, *University College London*

Deep Learning of G-Quadruplexes

Shozeb Haider[1]

[1]*University College London*

G-quadruplexes (G4) are widely distributed higher-order structures in nucleic acids. Their potential involvement in various biological processes have attracted enormous interest. Due to G4 polymorphism, it is challenging to predict topologies of potential G4-forming sequences (pG4 or PQS) based on strand geometry and the conformation of guanine residues in quartets. Besides, G4 structures are highly stable, so the core dynamics is undifferentiated between different polymorphisms. A deep neural network, Convolutional Variational Autoencoder (CVAE), is applied to the grouping of G4 structures to investigate the similarities between their dynamics characterized by ligand, sequence, and topology. The CVAE method captures characteristics of the investigated G4 dynamics and compresses them into a low-dimensional latent space in a discrete manner.

1150-1200 STR.3.1 – First Atomic Resolution Structure of an Intramolecular Higher-Order Five Tetrad G-Quadruplex

Thomas Sabo[1], John Trent[1], Jonathan Chaires[1], Robert Monsen[1]

[1]*University of Louisville*

ZEB1 (Zinc finger E-box binding homeobox[1]) is a member of the homeobox transcription factor family and plays a key role in regulating the epithelial mesenchymal transition (EMT) in carcinoma cells. Direct inhibition of ZEB1 with small molecule therapeutics has not yet been achieved. Another avenue for regulating ZEB1 is through the stabilization of DNA G-Quadruplexes (G4s) found within the ZEB1 promoter with small molecules. G4-small molecule binding can influence the transcription of adjacent genes, particularly cancer-related protein promoters like ZEB1, by repressing proteins that are difficult to target or considered “undruggable”. Here we have identified and structurally characterized with solution nuclear magnetic resonance spectroscopy (NMR) a unique and first of its kind, 5-tetrad, interlocked, intramolecular higher-order G4 fold using a thymidine scanning of the ZEB1 promoter. The two-stack and the three-stack G4 systems are essentially adopting a 5’-5’ stacking interface, which is predicted as the most stable form of G4 dimerization. The high thermodynamic stability is confirmed using CD melting analysis. The interlocking of nucleotide G21 into the three stacked G4 domain is shown to be essential for its formation. The unique folding of the ZEB1 G4 offers a unique targetable landscape for the indirect inhibition of ZEB1 in carcinoma, specifically at the junction of the two G4 units that forms a pocket with the interlocking G21.

1200-1210 STR.3.2 – Structural Basis for Nucleolin Recognition of MYC Promoter G-Quadruplex

Luying Chen[1], Jonathan Dickerhoff[1], Ke-Wei Zheng[2], Guanhui Wu[1], Saburo Sakai[1], Danzhou Yang[1]

[1]*Purdue University,* [2]*Hunan University*

Protein interactions are important for G-Quadruplex (G4) functions. However, the few known G4-protein structures all involve processive helicases that stack onto external G-tetrads. This mode can’t explain protein recognition of genomic G4 which has limited G-tetrad accessibility. The MYC oncogene promoter NHE III1 forms a G-Quadruplex (MycG4), and nucleolin was identified in 2009 as a major MycG4-binding protein and transcriptional repressor. However, structural information of the MycG4-nucleolin complex is unknown. Unlike the binding of its known substrate NRE-RNA which only needs two RBDs, we found that all four nucleolin RBDs are essential for high-affinity MycG4 binding. We determined the 2.6 Å crystal structure of the 1:1 nucleolin-MycG4 complex. In the crystal structure, the parallel MycG4 is very well defined with two K+ ions between three G-tetrads, which resembles the free MycG4 structure in solution. Nucleolin uses all 4 RBDs to wrap around the globular MycG4, interacting with its multiple loops and flanking regions. These multivalent interactions lead to specific and high-affinity binding. Furthermore, CUT&Tag sequencing demonstrates nucleolin binding to the MycG4-forming promoter region in cells. This is the first high-resolution structure of a G4 in complex with a regulatory protein and the first example of a G4 conformation-based protein recognition. Our findings suggest G4-mediated epigenetic transcriptional regulation and aid G4-targeted drug discovery.

1210-1220 STR.3.3 – G-Quadruplex Topologies Determine the Functional Outcome of Guanine-Rich Bioactive Oligonucleotides

Prakash Kharel[1], Pavel Ivanov[2]

[1]*University of Kansas Medical Center,* [2]*Harvard Medical School*

Guanine-rich nucleic acid sequences can exert sequence and/or structure specific activities to influence biological and pathobiological cellular processes. As such, it has been reported that different G-rich oligonucleotides (both DNA and RNA) can have cytotoxic as well as cytoprotective effects to the cells. However, the mechanisms of such a biological outcome are unclear. In this report, we report that G-rich DNA oligonucleotides (ODNs) that can form four stranded secondary structures called G-Quadruplexes (G4s) can have topology-dependent biological outcome. Using different biochemical, biophysical, and cellular approaches, we demonstrate that only the parallel topology G4-forming ODNs can repress eukaryotic translation by directly interacting with eukaryotic translation initiation protein[1] (EIF4G1), while the antiparallel topology G4s do not have inhibitory effect on mRNA translation To the best of our knowledge, this is the first report to directly

connect the G4 topological differences with differential functional biological impacts. Our study provides the foundation for the rational design of G-rich oligonucleotides for a desired therapeutic outcome.

STRUCTURE & DYNAMICS 4: Thursday, June 5, 2025 1345-1525

1345-1405 Inv.25 – Masato Katahira, *Kyoto University*

Quadruplex RNA That Inhibits the Interaction Between Aβ and its Receptor, Prion Protein, and In-Cell NMR Studies of Nucleic Acids Involving Quadruplex DNA

Masato Katahira[1]

[1]Kyoto University

Prion protein (PrP) causes prion diseases. PrP also functions as a receptor of Aβ protein to transmit the pathological signal of Aβ into cells, resulting in the repression of long-term potentiation (LTP) (Lauren et al., Nature, 2009). We isolated an RNA aptamer, that forms quadruplex, against the PrP, and determined the structure in complex with PrP. This clarified a mechanism how the aptamer exerts high affinity (Mashima et al., NAR, 2013). We also demonstrated spectroscopically that this aptamer inhibits the interaction between Aβ and its receptor, PrP, through tight binding to PrP (Iida et al., FEBS J., 2019). Recently, we have revealed electrophysiologically that the aptamer rescues the LTP repressed by Aβ, through the inhibition of the Aβ-receptor (PrP) interaction (Nakao, et al., in preparation). We succeeded in observing the in-cell NMR signals of nucleic acids in living human cells for the first time. Then, we found that a G:G base pair of the quadruplex DNA opens more frequently in living cells than in vitro (Yamaoki et al., Nature Commun., 2022; Eladl et al., Chem. Commun., 2022; Eladl et al., Int. J. Mol. Sci., 2023; Eladl et al., submitted).

1405-1425 Inv.26 – Liliya Yatsunyk, *Swarthmore College*

DNA sequences with the potential to form five-tetrad G-quadruplexes

Liliya Yatsunyk[1], Liliane Mouawad[2], Jason Hu[1], Joseph Eyiolowope[1], Eric Xing[1], Kailey Martin[1], Nick Kaplinsky[1], Robert Monsen[3]

[1]Swarthmore College, [2]Chemistry and Modelling for Biology of Cancer, [3]University of Louisville

G-quadruplexes (GQs) are noncanonical DNA secondary structures composed of stacks of G-tetrads. They play important biological roles and have therapeutic potential as drug targets. Although many 2-4 G-tetrad GQs have been well characterized, monomolecular sequences with the potential to form 5 or more G-tetrads have not. Here, we investigate sequences with four stretches of 5Gs connected by loops of differing length and capped by 0-4T (labeled LM). Biophysical characterization of 34 LM variants indicates that they fold into predominantly antiparallel GQs—only five variants display significant hybrid/parallel character. PAGE revealed that LM variants are monomolecular and relatively homogeneous. All LM variants display high stability. Increase in the number of 5′-T or loop length diminishes the antiparallel fold and decreases stability of the GQs, with loop length having stronger effect. 3′-T have less effect on the fold and stability. We designed mutants where each 5G stretch was trimmed to 4G – these mutants displayed significantly lower thermal stability (by > 6 °C) suggesting that LM variants indeed contain 5-tetrads. We solved a crystal structure of one of the variants which displayed an interlaced dimer of 5-tetrad hybrid GQs. One G from each monomer participates in the formation of the first G-tetrad in the symmetry generated partner. The 5-tetrad GQs can be great therapeutic targets, as their rarity would lead to a greater selectivity of drugs that target them.

1425-1445 Inv.27 – Dengguo Wei, *Huazhong Agricultural University*

Functional Investigation of G-Quadruplexes in Viral Genomes and the Rational Design of G-Quadruplex-Targeted Antiviral Therapeutics

Research and development of drugs targeting G-quadruplexes are still in the developmental phase. To propel advancements in this field, we have conducted a study on the potential G-quadruplex-forming sequences within the genomes of agricultural viruses and evaluated the antiviral efficacy of G-quadruplex ligands. In the 3′ untranslated region (3′ UTR) of the IE180 gene of Pseudorabies virus (PRV), the formation of G-quadruplexes is conducive to the expression of IE180, potentially offering a new approach for modulating the latency and reactivation of PRV. We have identified that small molecules capable of disrupting the stability of G-quadruplexes effectively inhibit viral proliferation. Furthermore, in the genome of Tobacco Mosaic Virus (TMV), the folding of G-quadruplexes inhibits viral propagation, while photosensitive G-quadruplex ligands inhibited viral replication by cleaving the G-quadruplex structure. In the case of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), G-quadruplexes in the negative strand of its genome also inhibit viral proliferation and interact with helicases to modulate the replication process. This investigation elucidates that G-quadruplexes within the genomes of diverse viruses can regulate viral replication through mechanisms such as folding, unwinding, and interaction with helicases. Small molecule compounds demonstrate the potential to inhibit viral proliferation by stabilizing, disrupting, or photolytically cleaving G-quadruplex structures. These discoveries offer novel insights and strategies for comprehending the role of G-quadruplexes and for the development of G-quadruplex-targeted antiviral therapeutics.

1455-1505 STR.4.1 – Best Method to Determine DNA G-Quadruplex Folding: The 1H-13C HSQC NMR Experiment

Jonathan Dickerhoff[1], Danzhou Yang[1]

[1]Purdue University

G-Quadruplexes (G4s) are four-stranded secondary structures that are enriched in promoter regions of oncogenes, telomeres, and replication initiation sites. They are functionally important and attractive drug targets. In contrast to duplex B-DNA, which is a uniform antiparallel double-helix with all anti glycosidic confomers, G-Quadruplexes exhibit great folding diversity and can involve parallel and antiparallel oriented strands and both anti and syn guanine conformers. Therefore, determining the correct folding of a G4 is a crucial task and an experimental method that can clearly and unambiguously identify syn and anti tetrad-Gs is highly desirable. CD spectroscopy is commonly used to determine G4 folding, however, it is low-resolution and can be misleading. 2D NOESY NMR experiments can determine glycosidic conformation and G4 folding, but the analysis is very tedious and can be ambiguous. Here, we present the 1H-13C HSQC NMR experiment at natural abundance as best method to unambiguously determine nucleotide glycosidic conformation and G-Quadruplex folding. We use several examples to demonstrate the clear and straightforward determination of guanine glycosidic conformations and G-Quadruplex folding structures. Moreover, the 1H-13C HSQC can help to define loops and capping conformations, especially for unusual syn conformers. Therefore, we believe that the 1H-13C HSQC should be a standard experiment for any NMR structural study of G-Quadruplexes.

1505-1515 STR.4.2 – Probing Residual Water in G-Quadruplex Structures through Molecular Vibrations

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

[1]University of Perugia, [2]Università Politecnica delle Marche, [3]Elettra Sincrotrone Trieste, [4]Istituto Officina dei Materiali

The inherent polymorphism of G-Quadruplexes (GQs) makes their structure highly sensitive to environmental factors, with solvation being a key determinant of their folding and stability. Here, we employ a dual-spectroscopy approach to study GQ dilute solutions during thermal unfolding. UltraViolet Resonance Raman (UVRR) scattering enhances the vibrational features of GQs, providing a method previously applied to other biosystems but novel for GQs in probing solute-solvent interactions. By analyzing the OH stretching vibrational band, which reflects the hydrogen-bonded water network, we reveal how residual water molecules interact with two distinct GQ conformers—hybrid and parallel. We demonstrate that coupling UVRR with circular dichroism spectroscopy allows for the correlation of vibrational properties with secondary structural features, even in spectral regions dominated by solvent contributions. Expanding this approach to other GQs may offer deeper insights into the critical role of solvation in GQ stability and function. Notably, the subtraction method used to calculate residual water in UVRR spectra was recently applied to cells, paving the way for characterizing structural water in G-Quadruplexes, even in crowded environments.

1515-1525 STR.4.3 – Unraveling the Thermodynamics of I-Motif Formation

San Hadzi[1], Mojca Pevec[1], Neza Zerjav[1], Uros Zavrtanik[1], Primoz Sket[2], Natasa Medved[3], Janez Plavec[3], Jurij Lah[1]

[1]University of Ljubljana, [2]Chemical Institute Ljubljana, [3]National Institute of Chemistry

Although DNA is most stable in the duplex form, it can adopt other structures that occur in certain contexts. Widely known are G-Quadruplexes, which are formed from guanine-rich sequences, and i-motifs, which arise from the cytosine-rich complementary strands. In i-motif structures, two parallel duplexes are intercalated in an antiparallel manner and stabilized by three hydrogen bonds between unprotonated and protonated cytosine (Nucleic Acids Res. 2002, 30, 4618). Although i-motif formation in vitro is favored in slightly acidic solutions, some studies suggest that this conformation can also occur in vivo (Nat. Chem. 2018, 10, 631). The formation of i-motif structures from cytosine-rich DNA fragments in vitro is usually considered a two-state process. Thus, the formation of intermediates along the folding pathway is not well understood. The present work addresses this issue by thermodynamic analysis of isothermal titration calorimetry data describing the conformational stability phase space of cytosine-rich DNA in terms of phase diagrams. The observed phase space is of comparable complexity to that of G-Quadruplexes (Angew. Chem. Int. Ed. 2016, 55, 10340). The predicted species are consistent with those observed by NMR spectroscopy, linking the thermodynamic driving forces of their formation to the structural features. Overall, we will present the thermodynamic principles by which cytosine-rich DNA sequences can fold into different structures depending on pH and temperature.

BIOLOGY & DISEASE 4: Thursday, June 5, 2025 1555-1735

1555-1615 Inv.10 – Sua Myong, *Harvard University*

Opposing Roles of Two R-loop Associated G-Quadruplexes in Tuning Transcription Activity

Sua Myong[1]

[1]Boston Children's Hospital

Guanine (G)-rich sequences in nucleic acids can form noncanonical secondary structures such as R-loop and G-quadruplex (G4) during transcription. The R-loop formed on the template strand promotes and stabilizes G4 in the non-template strand. However, the precise role of G4/R-loop-forming sequences on transcription remains poorly understood.
In this study, we investigated the effect of different potential G4-forming sequences (PQS) on G4/R-loop formation and

transcription dynamics. We employed gel-based assays and single-molecule fluorescence resonance energy transfer (FRET) to measure RNA synthesis and concomitant formation of G4 and R-loop during transcription by T7 RNA polymerase. We reveal two types of R-loop that form successively; an R-loop with an intramolecular DNA G4 (IG4) initially forms during transcription, followed by an R-loop with an intermolecular DNA: RNA hybrid G4 (HG4). We found that IG4 R-loops inhibit whereas HG4 R-loops enhance transcription. We identified that an HG4/IG4 ratio highly correlates with transcriptional activity. PQS with short linkers favors IG4, reducing transcription, while PQS with long linkers that induce loosely folding PQS favor HG4, increasing transcription. Since IG4 formation precedes HG4, tightly folded PQS forms IG4 quickly and stably, slowing its conversion to HG4 and reducing transcriptional enhancement.

1615-1635 Inv.11 – Katrin Paeschke, *University Hospital Bonn*

Viral Hijacking of hnRNPH1 Unveils a G-Quadruplex Driven Mechanism of Stress Sontrol

Katrin Paeschke[1]

[1]University Hospital Bonn

A common feature of most viruses is their dependence on regulatory RNA elements. Recent research suggested that non-canonical structures called G-quadruplexes (G4s) are overrepresented in viral genomes and have emerged as promising anti-viral targets. Using yellow fever virus (YFV) as a model system, we characterized the formation and the biological consequences of a conserved G4 within the genomes of the Flaviviridae family. We determined that this G4 is essential to promote viral replication and suppress the host cell stress response pathway. In subsequent mechanistic analyses, we pinpoint that this unique G4 function is associated with the nuclear host protein hnRNPH1. Specifically, G4 interaction leads to the retention of hnRNPH1 in the cytoplasm, causing an impaired stress response and alleviation of the anti-viral effects of stress-induced G4-forming tRNA fragments (tiRNAs). In conclusion, our data reveal a unique interplay of hnRNPH1 with host and viral G4 targets, controlling the integrated stress response and viral infection.

1635-1655 Inv.12 – Yinsheng Wang, *University of California, Riverside*

Chemistry and Biology of G-Quadruplex-Binding Proteins

Yinsheng Wang[1]

[1]University of California Riverside

Recent studies documented the widespread presence of guanine quadruplex (G4) in the human genome and transcriptome. Identifying cellular proteins involved in the recognition of G4 structures is important for understanding the biological functions of G4. In this presentation, I will discuss our recent experimental and bioinformatic approaches toward identifying novel DNA and RNA G4-binding proteins in vitro and in live cells. Affinity pull-down, photo-crosslinking and proximity labeling, followed by quantitative proteomic analysis, allowed us to identify a large number of candidate DNA and RNA G4-binding proteins. Bioinformatic analysis of publicly available ChIP-seq and CLIP-seq data also resulted in the identification of candidate G4-binding proteins. We also validated the abilities of some of these proteins in binding directly and selectively with G4 DNA and RNA. Moreover, we illustrated the functions of these newly identified G4-binding proteins in modulating various important biological processes, including long-range DNA looping, mRNA splicing, alternative polyadenylation, and telomere maintenance. Together, our integrative chemical biology approach provided a better understanding about the biological functions of DNA and RNA G4.

1705-1715 BIO.4.1 – The Changing Shape of DNA in the Insulin-Linked Polymorphic Region

Zoë Waller[1], Dilek Guneri[1], Shozeb Haider[1], Gary Parkinson[2], Kamel El Omari[3], Zuzana Dvorakova[4], Effrosyni Alexandrou[2], Christopher Morris[2], Chris Waudby[2], Rupesh Chikhale[5], Daniel Pike[2]

[1]University College London, [2]UCL School of Pharmacy, [3]Diamond, [4]Institute of Biophysics, [5]CCDC

The insulin-linked polymorphic region is a variable number of tandem repeats region of DNA in the promoter of the insulin gene that regulates transcription of insulin[1]. This region is known to form i-motifs and G-Quadruplexes[2]. Individuals have different sequence variants of tandem repeats and although previous work investigated the effects of some variants on G-Quadruplex formation[3], there was not a clear picture of the relationship between the sequence diversity, the DNA structures formed, and the functional effects on the insulin promoter. We recently showed that different sequence variants of the insulin linked polymorphic region form different DNA structures in vitro[4]. Additionally, reporter genes *in cellulo* indicated that insulin expression may change depending on which DNA structures form. Here we describe our observations and insights into mutants and variants of the ILPR sequences and our working hypothesis in the relationship between formation of G-Quadruplex versus i-motif and hairpin structures in the ILPR and their effects on insulin promoter expression.

1715-1725 BIO.4.2 – Designing an Expression Cassette to Study G-Quadruplex Formation in Bacillus Subtilis

Polina Marchenko[1], Maria Vittoria Cottini[1], Sidra Ishrat[1], Pavol Vadovic[1], Jan Jamroskovic[1]

[1]Slovak Academy of Sciences

G-Quadruplex (G4) structures are well-studied in eukaryotic systems, particularly in humans and yeasts, where they play critical roles in gene regulation and genome stability. Their relevance in cancer research has led to the development of numerous G4-targeting drugs. However, the function of G4 structures in bacteria remains largely unexplored. While previously considered insignificant in bacterial genomes, recent studies in Escherichia coli, Pseudomonas putida, and Mycoplasma have demonstrated the roles of G4 structures in gene regulation and virulence. In our study, we investigate G4 structures in Bacillus subtilis, a model organism for Gram-positive bacteria, widely used in basic research and industrial biotechnology. Through bioinformatics analysis, we identified G4-forming sequences enriched near transcription start sites,

suggesting their potential in regulating gene expression. To experimentally assess their formation in vivo, we developed an expression cassette controlled by inserted G4 structures, which can be integrated into the B. subtilis genome. G4 formation at the DNA or RNA level depends on whether the G4-forming sequence is located on the template or non-template strand, and thus its positioning can differentially influence gene expression. As In situ G4 detection remains challenging, typically requiring BG4 antibody-based ChIP assays, we propose that our system provides a valuable tool for studying G4 formation and functionality in bacteria.

1725-1735 BIO.4.3 – The Role of BAZ2-Dependent Chromatin Remodeling in Suppressing G4 DNA Structures and Associated Genomic Instability

Adrianna Vandeuren[1], Kierney O'dare[2], Rosemary Wilson[2], Patrick Van Eijk[2], Lindsay Julio[1], Shannon Macleod[1], Ella Chee[1], Annika Salpukas[1], Emma Kriz[1], George Lantz[1], Shellaina Gordon[1], Simon Elsässer[3], Simon Reed[2], Tovah Day[1]

[1]Northeastern University, [2]Cardiff University, [3]Ming Wai Lau Centre for Reparative Medicine

DNA G-Quadruplexes (G4s) are secondary structures playing a key role in genomic regulation and stability. While their dysregulation has been implicated in various diseases and genomic instability, the mechanisms driving these effects are not fully understood. The observation that the same genomic sequences yield distinct patterns of G4 formation in different cellular contexts implicates the chromatin context in their regulation. We screened a library of chromatin modifying enzymes and identified nine potential suppressors of G4 formation, including seven that were novel. Among these, we further investigated the BAZ2 family of chromatin remodelers, which includes BAZ2A and BAZ2B, as suppressors of G4 DNA. Depletion of either unit led to increased G4 formation, particularly at enhancers, promoters and active transcription start sites. While some G4s were regulated by both BAZ2 complexes, a subset was unique to each, suggesting that they exhibit both overlapping and distinct roles. Genome-wide BAZ2B occupancy analysis revealed significant overlap with G4 peaks arising upon BAZ2B depletion. Additionally, depletion of these units also led to modest but significant increases in double-strand breaks (DSBs) which were further increased upon treatment with BRACO19, a G4-stabilizing ligand. Our study establishes BAZ2 chromatin remodeling complexes as suppressors of G4 formation and provide new insights into G4-dependent genome instability.

TARGETING 1: Friday, June 6, 2025 0830-1010

0830-0850 **Inv.28 – Tracy Brooks, *Binghamton University***

Optimized Targeting of the MYC Promoter G4 with DNAi

Tracy Brooks[1]

[1]*Binghamton University*

Selective stabilization of individual promoter G-quadruplexes (G4s) offers therapeutic potential for cancer, neurological disorders, and Fragile X syndrome. While achieving high selectivity with small molecules remains challenging, oligonucleotide (ODN) therapeutics can overcome this selectivity hurdle, though they face difficulties with cellular delivery and uptake. Our group pioneered a clamp-based oligonucleotide approach (termed DNAi) to stabilize the MYC promoter G4 by binding flanking DNA with a limited linker. We optimized this DNAi for cost-effectiveness and biological activity in lymphoma. To address limitations in cellular delivery and uptake, this presentation will discuss modifications to the DNAi backbone, coupling to cell-penetrating peptides, and targeted delivery with antibodies. These advances were undertaken in order to develop the ODN therapeutically for mantle cell lymphomas and breast cancer, where we will discuss cellular stability, localization, and biological effects. The DNAi technology has potential applications for a range of therapeutically tractable G4s in oncological, inflammatory, and other critical disease states.

0850-0910 **Inv.29 – Scott Horowitz, *Denver University***

G4s Dictating Protein Folding, Misfolding, and Neurodegeneration

Scott Horowitz[1]

[1]*University of Denver*

Protein homeostasis governs many diseases, including most neurodegenerative diseases. RNA has long been suspected to directly impact these processes, and it is imperative to determine which RNAs are particularly important in protein folding and aggregation, and their specific effects in biology and pathology. Through a combination of in vitro, cellular, and C. elegans approaches, we determined that G-quadruplexes are powerful modulators of protein folding and aggregation. For example, we found that G-quadruplexes can catalyze protein folding, but under chronic stress, G-quadruplexes can seed protein oligomerization and are a common mechanistic link between disparate neurodegenerative diseases, and that modulating G-quadruplexes is a promising general therapeutic approach for neurodegeneration.

0910-0930 **Inv.30 – Antonio Randazzo, *University of Naples***

Enhancing Chemo-Sensitivity and Overcoming Drug Resistance in Cancer with G4 Ligands

Antonio Randazzo[1]

[1]*University of Naples Federico II*

Cancer stands as a pervasive global health challenge and chemotherapy stands as the prevailing approach to treat it. While traditional chemotherapeutic agents have proven effective in cancer treatment, the consequential impact on the physical and psychological well-being of patients remains notably severe. Furthermore, the adaptability of tumor cells to develop resistance against a wide array of chemotherapeutic drugs poses a significant obstacle, ultimately resulting in treatment failures. In this context, chemo-sensitization has gained attention as a strategy using small molecules to enhance cancer cells' sensitivity to conventional drugs. This approach aims to tackle chemoresistance mechanisms, reduce chemotherapy-induced side effects, and improve clinical outcomes. This presentation explores the growing relevance of G-quadruplex (G4) structures as promising anti-cancer targets. The goal is to boost the antitumor efficacy of standard chemotherapeutics by leveraging G4-interacting molecules, enabling synergistic effects with conventional drugs while minimizing toxicity in healthy cells and overcome drug resistance.

0940-0950 **TAR.2.2 – Investigating the Interactions of Carbazole Ligands with I-Motif DNA**

Anna Dembska[1], Klaudia Kmiecik[1], Agata Głuszyńska[1]

[1]*Adam Mickiewicz University*

The increasing number of i-motif-interacting agents has provided new tools for research on its biological roles and recognition in the nuclei of human cell. The studies on development of i-motif ligands are often conducting with the already known G-Quadruplexes (G4)-interacting agents and their analogues. So far, many carbazole derivatives were identified as potent G4 stabilizers. Therefore, we have decided to investigate the interactions of i-motifs formed by the DNA sequence derived from proto-oncogenes (i.e. c-MYC, Bcl-2) or by the telomeric sequence with two carbazole derivatives, which possess single cationic charge on benzothiazolium moiety and different substituent attached to the nitrogen atom of carbazole ring. The binding interactions of compounds 1–2 with i-motifs were investigated by UV–vis spectrophotometry, fluorescence and CD spectroscopy. The results have shown that both ligands have ability to interact with the various intramolecular i-motifs with the very comparable binding affinities in the order of 105 M−1. Moreover, the spectral changes observed during the spectrophotometric and spectrofluorimetric titration are analogous as for studies with G4.

0950-1000 **TAR.1.2 – Harnessing G-Quadruplex Modulation as a Therapeutic Strategy in Neurodegenerative Diseases**

Valentina Pirota[1], Stephana Carelli[2], Emmanuele Crespan[3], Mauro Freccero[1]

[1]University of Pavia, [2]University of Milan, [3]Institute of Molecular Genetics IGM-CNR

Being key regulators of gene expression and genomic stability, G4s represent fascinating targets in neurodegenerative diseases. Here, it will be presented how dynamic G4-folding modulation correlates with pathogenic pathways of Parkinson's and Alzheimer's disease (PD, AD), CANVAS syndrome, and amyotrophic lateral sclerosis (ALS), highlighting its therapeutic potential. Specifically, selective small molecules and antisense oligonucleotide derivatives were used for the precise control over the stabilization or destabilization of newly identified G4s. In PD, G4-ligands reduced α-synuclein expression by stabilizing G4s within SNCA mRNA, while peptide nucleic acids selectively disrupted pSNCA-G4 at the transcription start site (TSS), leading to ~70% reduction in SNCA transcription and protein expression. For AD, modulation of the highly stable pApoE-G4 located in a critical transcription factor binding region near APOE gene TSS provided a robust framework to finely tune ApoE protein expression. In ALS, phosphorodiamidate morpholino oligomer successfully unfolded toxic G4s formed by C9orf72 repeat expansions, offering new insights into therapeutic G4 disruption. Finally, in CANVAS syndrome, G4-folding was evidenced in pathogenic RFC1 repeats, unlike normal sequences, emphasizing their relevance in repeat expansion disorders. Altogether, these findings show how precise G4-folding modulation can drive innovative and personalized therapies for neurodegenerative disorders.

1000-1010 **TAR.1.3 – Hemopeptides Conjugates as Versatile Ligands for the Selective Binding of G-Quadruplexes**

Leen Massalha[1], Adiel Richter-Levin[1], Nurit Adiram-Filiba[1], Eyal Golub[1]

[1]Bar-Ilan University

The design of topology- and sequence-specific GQ ligands has greatly benefited from conjugating multiple binding elements targeting distinct structural motifs. A particularly effective approach involves conjugating aromatic molecules with short peptide chains, where the former binds external tetrads and the latter interacts with loops/grooves.
We repurposed microperoxidase-11 (MP-11), a semi-natural hemopeptide with an 11-mer peptide tethered to a heme group, as a versatile GQ ligand. Similar to heme, MP-11 preferentially binds parallel GQs with minimal dsDNA interaction. MP-11's subcomponents synergistically enhance GQ binding: the heme moiety directs specificity to parallel GQs, while the peptide chain increases affinity via non-specific interactions. This enables MP-11 to achieve sequence specificity through unique mechanisms: (i) selectively uncaging a GQ sequence in dsDNA encoding c-MYC, and (ii) binding hybrid GQs and converting them to parallel topologies, favoring less stable sequences. Accordingly, MP-11 represents an elaborate platform for exploring specificity mechanisms for GQs by designed ligands. Moreover, MP-11 is a versatile ligand that can be adapted to a specific purpose as it can be further manipulated by either varying the metal ion at the center of the porphyrin ring or mutating the residues at the GQ sequence.

TARGETING 2: Friday, June 6, 2025 1040-1220

1040-1100 **Inv.31 – Aaron Engelhart, *University of Minnesota***

Worth their salt and then some: G4s have unique stability and catalytic functions in saline solutions

G-quadruplexes are unique among nucleic acid structures in their interactions with salt. Specific divalent-phosphate interactions are ubiquitous in nucleic acid folding, but monovalent ions typically play a general charge screening role in folding of most nucleic acid structures. G4s invert this paradigm, with exquisitely specific monovalent recognition based on the interplay between ionic radius, coordination energy, and hydration energy. A range of extremophiles exhibit high frequencies of G4-forming sequences in their genome, including halophiles such as Haloferax volcanii, salt-tolerant organisms such as the Hadesarchaea, as well as numerous thermophiles. In the past ten years, high concentrations of oxychlorine salts (0.5% w/w in regolith/soil, and multiple molar in brines) have been identified on Mars - thought to be one of the likeliest environments for extraterrestrial life. Motivated by these observations, we have investigated the stability of G4-forming sequences in a range of high-salt conditions. We have observed that G4s in high salt are uniquely tolerant of the presence of denaturants - either classical denaturants like urea, or intrinsically denaturing salts, such as chaotropic Hofmeister salts. We have observed that this phenomenon can be used to switch a G-rich/C-rich Watson-Crick duplex between quadruplex and duplex forms. Additionally, even in high concentrations of chaotropic salt, G4-hemin-based peroxidases can catalyze electron transfer reactions. We also have found that rG4-hemin holoenzymes, in addition to their well-known capacity to catalyze electron and oxygen transfer chemistry, can employ an oxychlorine anion (chlorite) as both an electron acceptor and chlorine donor. By doing so, these catalysts can perform chlorination chemistry on organic substrates - extending the range of chemistry that ribozymes can perform.

1100-1120 **Inv.32 – Claudia Sissi, *University of Padova***

Combination of DNA Foldings at a Single Site

Claudia Sissi[1], Davide Auricchio[1]

[1]University of Padova

Nucleic acids targeting agents are widely used in clinic to treat various pathologies. Their main limitation is generally related to their poor selectivity for selected nucleic acids sites. To overcome this drawback, several approaches have been developed, i.e. the design of agents working at protein-nucleic acids interface, to take advantage of the protein

selectivity in terms of localization or functions, or the use of oligonucleotides-related therapeutics that act by pairing to complementary DNA or RNA strands. Alternatively, the search for systems able to recognize selected sequences based on their "non-canonical" folding has been extensively exploited. The advantages of this approach rests on the limited abundance of these nucleic acid sites and on their time- and space-controlled intracellular occurrence. Nevertheless, this promising approach is moving smoothly. Herein we will discuss some issues that might have led to this poor output, starting from the selection of the best secondary structure models to be used along screening protocols. As a main issue, we will discuss the diverse structural behavior of short DNA domains when analyzed as isolated single stranded elements or embedded in a more complex genomic context that covers flanking ends and, more intriguingly, a complementary strand. The description of the equilibria behind these higher order systems is expected to represent an innovative support for the rational development of novel targeted drug projects.

1120-1140 **Inv.33 – Lisa Prevette, *St. Thomas University***

Binding Thermodynamics of G-wires with Cellular Delivery Agents

Lisa Prevette[1]

[1]*St. Thomas University*

Guanine-rich oligonucleotides (GRO) can self-assemble in the presence of monovalent cations to form varied lengths of G-quadruplex DNA strands known as G-wires. If delivered to cells, these G-wires may compete with intracellular G-quadruplex DNA to impact cell division and gene expression and, thus, could be used as therapeutics. However, like any foreign nucleic acid, G-wire cell uptake is improved with use of a delivery agent that can package the DNA, protect it from degradation, and bind to cell surface receptors triggering uptake. Cationic polymers and liposomes are popular DNA delivery agents due to their ability to electrostatically bind to the DNA phosphate backbone, as well as anionic cell surface glycosaminoglycans. Although these agents have been widely used to deliver double- and single-stranded nucleic acids, their use for G-quadruplex DNA is understudied. Here, we examined polycation-G-wire binding thermodynamics for three common agents, HIV-1 TAT peptide, generation 5 polyamidoamine (PAMAM) dendrimer and linear polyethyleneimine (PEI), through isothermal titration calorimetry (ITC), dynamic light scattering (DLS) and electrophoretic mobility shift assays (EMSA) to gain insight into the properties of the resulting complexes and how polycation structure affects the strength and stoichiometry of binding. This information not only aids in understanding the cellular transfection process but also structure-function relationships, which can lead to improved design of future G-quadruplex DNA delivery vectors.

1150-1200 **TAR.2.3 – Interactions of Simple BODIPY Dyes with Various DNA G-Quadruplexes**

Jakub Żubertowski[1], Magdalena Rapp[1], Jan Dolichter[2], Błażej Rubiś[2], Anna Dembska[1]

[1]*Adam Mickiewicz University,* [2]*Poznan University*

A class of compound with a skeleton origin of 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) is a vital and diverse group of dyes, which hence to their attractive optical properties have found the application in sensing, bioimaging, bio-labeling, photodynamic therapy or in drug delivery systems. Several reports have been made on BODIPY derivatives ability to recognize and bind with DNA G-Quadruplexes (G4). Recently we have synthesized a series of simple derivatives, bearing modified or unmodified phenyl ring substituted at the meso position of BODIPY core, aiming to elucidate the potential of their utilization as GQuadruplex ligands. However the emissive properties of studied compounds were found out to be highly-dependent on the experimental condition (mainly due to their tendency for aggregates formation), we managed to select two derivatives showing clear preference towards DNA GQuadruplexes of different topologies, over single or double stranded DNA. Results of spectroscopic investigations (UV-vis absorbance, fluorescence and circular dichroism spectroscopy) will be presented to elucidate the mode of observed interactions, together with the thermodynamic analysis of melting profiles and the results of G4/hemin peroxidase inhibition assay. The evaluation of tested compounds cytotoxicity has also been made and the results obtained from the MTT assays will be presented.

Poster Abstracts

The poster numbers are divided first by session, then by theme, and finally with a unique number.
Session – Theme – Board Number (ex. P1.BIO.1)

BIO = Biology & Disease **NANO = Nanotech & Biotech**
STR = Structure & Dynamics **TAR = Targeting**

POSTER SESSION 1

Tuesday, June 3, 2025 1900-2100

P1.BIO.1 – Integrative Analysis of Stress-Induced G4 DNA Reveals G4-Binding Transcription Factor Feedback Mechanism

Lindsay Julio[1], Diana Turrieta[1], Annika Salpukas[1], Ella Chee[1], Elizabeth Crane[1], Shannon Macleod[1], Adrianna Vandeuren[1], Mynaja Ferguson[2], Rachel Muriph[2], Justin Crane[1], Jason Evans[2], Tovah Day[1]

[1]Northeastern University, [2]University of Massachusetts

DNA G-Quadruplexes (G4s) are secondary structures with critical roles in regulating genome function. G4s in regulatory regions are important for controlling gene expression, while their aberrant formation has been linked to genomic instability and human disease. Despite their importance and inherent danger, the dynamic formation of DNA G4s remains incompletely understood. Here, we integrate genomic, transcriptional, and proteomic analyses to define the multi-omic landscape of stress-induced G4s and reveal new mechanistic insights into the interplay between genotoxic stress responses and the regulation of non-canonical DNA structures. Stress-induced G4s are enriched at regulatory regions and exhibit distinct biophysical properties, suggesting unique formation dynamics. Functional studies and computationally-derived evidence demonstrate a mechanism by which novel G4-binding transcription factors are required for induction of G4s under conditions of genotoxic stress. We identify G4-binding transcription factors which regulate pathways related to DNA repair, apoptosis, autophagy, and protein complex formation, highlighting the biological relevance of G4s that form under those conditions. The mechanism by which G4s form, are bound, and regulate related pathways after genotoxic stress underscores the role of DNA G4s at regulatory regions of genes involved in the cellular stress response and provides a foundation for future exploration of G4-mediated genome instability and disease.

P1.BIO.2 – Oral G-Quadruplex Stabilizer Drug Entering Phase I for Advanced Solid Cancers

Hong Xu[1]

[1]Hexin Biotech

The effort of pushing G-Quadruplex targeting drugs in clinics has lasted for decades, with the best example entering into phase II clinical trials but then withdrawing. Recently, two G4 targeting drugs, CX5461 and QN302 in phase I and phase 1b clinical trial have shown early signs of success. With growing research progress, now is the time to invest more in G4-targeting drugs and apply them in clinics. We invented a G4 stabilizer drug XY003 that will enter phase I clinical trial soon. Distinct from other G4 clinical drugs that are IV-delivered, XY003 will be orally delivered. Inducing DNA damage at G4 loci XY003 specifically kills cancer cells with DNA damage deficiency, including homologous recombination deficiency (HRD) and non-homologous end joining (NHEJ). XY003 has demonstrated strong in vitro and in vivo anti-cancer efficacy with no observed adverse events at effective and several-fold higher doses. It shows no drug-drug interactions, as evidenced by the absence of CYP450 enzyme induction or inhibition in hepatocytes, making it an ideal candidate for combination therapy. Preclinical GLP toxicity and safety studies confirm a favorable safety profile. Notably, XY003 efficiently crosses the blood-brain barrier (BBB), offering potential for treating brain metastases and primary brain tumors.

P1.BIO.3 – DNA G-Quadruplex Profiling Reveals Functional and Mechanistic Role of G-Quadruplexes in Skeletal Muscle Stem Cells

Feng Yang[1], Suyang Zhang[1], Xiaona Chen[1], Huating Wang[1], Xiaofan Guo[2], Jieyu Zhao[3], Yulong Qiao[1], Liangqiang He[1], Yang Li[1], Qin Zhou[1], Michael Ong[1], Chun Kit Kwok[3], Hao Sun[1]

[1]The Chinese University of Hong Kong, [2]The University of Hong Kong, [3]City University of Hong Kong

DNA G-Quadruplexes (G4s) are non-canonical secondary structures formed in guanine-rich DNA sequences and play important roles in modulating biological processes. Emerging G4 profiling permits global mapping of endogenous G4 formation. Here in this study, we map the G4 landscapes in adult skeletal muscle stem cells (MuSCs) which are essential for injury induced muscle regeneration. Throughout the myogenic lineage progression of MuSCs from quiescent to activated and further differentiated cells, we uncover dynamic endogenous G4 formation with a pronounced G4 induction when MuSCs become activated and proliferating. We further demonstrate that the G4 induction promotes MuSC activation thus the regeneration process. Mechanistically, we found that promoter associated G4s regulate gene transcription through facilitating chromatin looping. Furthermore, we found that G4 sites are enriched for transcription factor (TF) binding events in activated MuSCs; MAX binds to G4 structures to synergistically facilitate chromatin looping and gene transcription thus promoting MuSC activation and regeneration. The above uncovered global regulatory functions/ mechanisms are further dissected on the paradigm of Ccne1 promoter, demonstrating Ccne1 is a bona fide G4/MAX regulatory target

in activated MuSCs. Altogether, we demonstrate the prevalent and dynamic formation of G4s in adult MuSCs and the mechanistic role of G4s in modulating gene expression and MuSC activation/proliferation.

P1.BIO.4 – Pirh2-Dependent DNA Damage in Neurons Induced by the G-Quadruplex Ligand Pyridostatin

Rocio Diaz Escarcega[1], Abhijeet A. Patil[1], Jose F. Moruno-Manchon[1], Akihiko Urayama[1], Sean P. Marrelli[1], Nayun Kim[1], David Monchaud[2], Louise D. Mccullough Mccullough[1], Andrey Tsvetkov[3]

[1]The University of Texas McGovern Medical School at Houston, [2]Université de Bourgogne, [3]The University of Texas

The G-Quadruplex (G4) is a four-stranded nucleic acid structure that folds from single-stranded guanine (G)-rich DNA or RNA sequences. In cancer cells, G4-DNA regulates multiple DNA-dependent processes, including transcription and replication. How G4s function in neurons is not clear. Here, we performed a genome-wide gene expression analysis (RNA-Seq) to identify genes modulated by a G4 ligand, pyridostatin (PDS), in primary neurons. PDS promotes stabilization of G4 structures, thus allowing us to define genes directly or indirectly responsive to G4 regulation. We found that 901 genes were differentially expressed in neurons treated with PDS out of a total of 18,745 genes with measured expression. Of these, 505 genes were downregulated, and 396 genes were upregulated and included gene networks regulating p53 signaling, the immune response, learning and memory, and cellular senescence. Within the p53 network, the E3 ubiquitin ligase Pirh2 (Rchy1), a modulator of DNA damage responses, was upregulated by PDS. Ectopically overexpressing Pirh2 promoted the formation of DNA double-strand breaks, suggesting a new DNA damage mechanism in neurons regulated by G4 stabilization. Pirh2 downregulated DDX21, an RNA helicase that unfolds G4-RNA and R-loops. Finally, we demonstrated that Pirh2 increased G4-DNA levels in the neuronal nucleolus. Our data reveal the genes responsive to PDS treatment and suggest similar transcriptional regulation by endogenous G4-DNA ligands.

P1.BIO.5 – Structural Characterization of RFC1 Repeat Nucleic Acids in the Neurological Disorder CANVAS

Kenta Kudo[1], Karin Hori[1], Norifumi Shioda[1]

[1]Kumamoto University

Short tandem repeats are inherently unstable during DNA replication depending on repeat length, and the expansion of the repeat length in the human genome is responsible for repeat expansion disorders. Pentanucleotide AAGGG and ACAGG repeat expansions in intron 2 of the gene encoding replication factor C subunit 1 (RFC1) cause cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS) and other phenotypes of late-onset cerebellar ataxia. We revealed the structural polymorphism of the RFC1 repeats associated with CANVAS in vitro. Single-stranded AAGGG repeat DNA formed a hybrid-type G-Quadruplex, whereas its RNA formed a parallel-type G-Quadruplex with three layers. The RNA of the ACAGG repeat formed hairpin structure comprising C-G and G-C base pairs with A:A and GA:AG mismatched repeats. Furthermore, both pathogenic repeat RNAs formed more rigid structures than those of the nonpathogenic repeat RNAs. These findings provide novel insights into the structural polymorphism of the RFC1 repeats, which may be closely related to the disease mechanism of CANVAS (Kudo et al., J Biol Chem. 2024). Furthermore, fluorescence recovery after photobleaching (FRAP) assays revealed that pathogenic repeat RNAs exhibit aggregation properties. These results suggest that neurotoxicity caused by pathogenic repeat RNAs may be involved in the pathogenesis. We are currently investigating the mechanism of neuronal dysfunction caused by pathogenic repeat RNAs.

P1.BIO.6 – Evolutionary Dynamics of G-Quadruplexes in Human and Other Great Ape Telomere-To-Telomere Genomes

Saswat Mohanty[1], Francesca Chiaromonte[1], Kateryna D. Makova[1]

[1]Penn State University

G-Quadruplexes (G4s) are non-canonical DNA structures that facilitate genomic instability by increasing point mutations and structural variation. Numerous G4s participate in telomere maintenance and regulating transcription and replication, and evolve under purifying selection. Despite these important functions, G4s have remained understudied in human and ape genomes due to incomplete assemblies. Here, we conducted a comprehensive analysis of predicted G4s (pG4s) in the recently released, telomere-to-telomere (T2T) genomes of human and other great apes—bonobo, chimpanzee, gorilla, Bornean orangutan, and Sumatran orangutan. We annotated 41,236–147,753 new pG4s in these T2T compared to previous ape genome assemblies (5%–18% increase). Analyzing inter-species whole-genome alignments, we identified pG4s shared across apes and thousands of species-specific pG4s. pG4s accumulated and diverged at rates consistent with divergence times between species, following molecular clock. pG4 shared across apes were enriched and hypomethylated at regulatory regions—enhancers, promoters, UTRs, and origins of replication—suggesting their conserved formation and functions. Species-specific pG4s were located in regulatory regions, potentially contributing to adaptations, and in repeats, likely driving genome expansions. Our findings illuminate the evolutionary dynamics of G4s, conservation of their role in gene regulation, and their contributions to ape genome evolution utilizing T2T genomes.

P1.BIO.7 – Exploring the Structural Diversity of Conserved West Nile Virus Genomic RNA Quadruplexes

Jessica Siemer[1], Thao Le[1], Ananya Paul[1], David Boykin[1], Margo Brinton[1], W. David Wilson[1], Markus Germann[1]

[1]Georgia State University

Despite the significant global human disease caused by arthropod-borne orthoflaviviruses, no antivirals and few vaccines exist for these RNA viruses, which include West Nile virus (WNV), dengue virus, yellow fever virus and Zika virus. G-Quadruplexes are complex nucleic acid structures composed of stacked guanine tetrads with functional roles in cells that have been implicated in a variety of diseases. There are <100 solved RNA G-Quadruplex structures in the Protein Data Bank which limits the success of the current computational tools for their identification and structure prediction. Seven highly-conserved potential G-Quadruplex sequences were predicted in the genomes of >56 orthoflaviviruses. While the high level of conservation among members of the genus suggests that these structures are functionally important, the roles of RNA Quadruplexes in the viral lifecycle remain unknown. The conserved potential Quadruplex sites in the West Nile virus genome were selected for further study. Each sequence was analyzed to determine nucleotide consensus across a

set of genomic sequences deposited in the National Center for Biotechnology Information virus database. Biophysical experiments were performed to confirm the Quadruplex formation in vitro. These data support the formation of both intermolecular or intramolecular Quadruplexes suggesting that some genomic Quadruplex sequences may require long-range RNA interactions to form.

P1.BIO.8 – G-Quadruplexes in Ultra-Short Cell-Free DNA

Martin Gajarský[1], Robert Hänsel-Hertsch[2]

[1]Center for Molecular Medicine Cologne, [2]University Hospital Cologne

Recently, advanced DNA capture methods coupled with ssDNA library preparation revealed a novel population of ultra-short cell-free DNA centered at ~50nt. The US-cfDNA is predominantly found upstream of blood-related, nucleosome-depleted, accessible transcription start sites. These gene promoter regulatory regions are only enriched for US-cfDNA when they contain the potential to adopt non-canonical DNA secondary structures, namely G-Quadruplex (G4). Astonishingly, a substantial difference in the prevalence of US-cfDNA in plasma of healthy donors versus cancer patients was observed, with the latter containing a significantly smaller fraction of US-cfDNA. We hypothesize that the pool of US-cfDNAs, consisting of G-rich ssDNA sequences, forms secondary structures (G-Quadruplex) and that these structural characteristics of US cfDNA are important for blood biology and can be rationally exploited for cancer diagnostics and therapy.

In our project we employed a combination of high-resolution spectroscopic tools, secondary structure specific antibodies and small molecule ligands to uncover the structural characteristics of US cfDNA. We show, to the best of our knowledge, the first experimental proofs of G-Quadruplex presence in human blood plasma. Using targeted ssDNA library preparation method, we aim to reveal precise G4 landscapes from blood plasma US-cfDNA and to find critical DNA fragments potentially inferring status, biology and vulnerabilities of cancer from blood samples.

P1.BIO.9 – Probing Direct Effects of G4 Structures on the Nucleosome

Leman Simpson[1], Lu Bai[2]

[1]Penn State University, [2]Penn State University, Center for Eukaryotic Gene Regulation

G4 structures have been identified in CG-rich promoters, including many housekeeping gene promoters, which have high chromatin accessibility and transcription levels. Importantly, G4 structures are associated with nucleosome-depletion by ATAC/MNase-seq implying a mutual exclusivity in cells. However, there is little direct data on the compatibility of G4s and chromatin. Using chromatin molecular biology and DNA library approaches, we seek to understand if G4s can form on a nucleosome, if G4 stability affects chromatin, and the regulatory roles of TFs in maintaining nucleosome-depleted regions near G4s. This work involves building a molecular toolkit for studying dsDNA G4's in the context of the nucleosome, low-throughput chromatin experiments (positioning and reconstitution efficiency), and the use of a high-throughput DNA library approach for determining the sequence characteristics affecting G4/chromatin interplay.

P1.BIO.10 – CBN-Binding DNA Aptamer Cross Reactivity and Structure Analyzed by Circular Dichroism Spectroscopy

Forrest Empey-Kohl[1], Satoko Suzuki[1], Ai Yamane[1], Taiji Oyama[1], Ken-Ichi Akao[1], Takehiko Wada[2]

[1]Jasco Inc., [2]Tohoku University

A potential route for targeted treatments of disease and illness are DNA aptamers, short single-stranded DNA oligonucleotides. Depending on their tertiary structure, aptamers bind to specific ligands allowing for selective treatment. THC and CBN are two of the 100's of natural chemical components called cannabinoids found in the cannabis plant. CBN is a mildly psychoactive component and is gaining popularity for its potential benefits for sleep and pain management. Here, structural changes in a CBN-binding DNA aptamer were monitored based on the Circular dichroism (CD) spectrum. CD is a technique sensitive to higher-order structure (HOS) of oligonucleotides making it valuable for studying conformational changes in solution-state. In the far-UV region, CD signals produced by the nucleotide backbone are used to observe changes in oligonucleotide HOS. Using CD spectroscopy, the HOS and homogeneity before and after a change can be analyzed. PCA analysis was performed to evaluate structural changes induced through CBN binding. The potential for the aptamer to bind to other types of cannabinoid, cannabidiol (CBD) and cannabigerol (CBG), was also evaluated. High throughput CD was determined to be highly suitable for early-stage screening of DNA aptamer candidates.

P1.BIO.11 – Remodeling Ca2+ Dynamics by Targeting a Promising E-Box Containing G-Quadruplex At ORAI1 Promoter in Triple-Negative Breast Cancer

Suman Panda[1]

[1]Institut Polytechnique de Paris

ORAI1 is an intrinsic component of store-operated calcium entry (SOCE) that strictly regulates Ca2+ influx in most non-excitable cells. ORAI1 is overexpressed in a wide variety of cancers, and its signal transduction has been associated with chemotherapy resistance. There is extensive proteomic interaction of ORAI1 with other channels and effectors, resulting in various altered phenotypes. However, the transcription regulation of ORAI1 is not well understood. We have found a putative G-Quadruplex (G4) motif, ORAI1-Pu, in the upstream promoter region of the gene, having regulatory functions. High-resolution 3-D NMR structure elucidation suggests that ORAI1-Pu is a stable parallel-stranded G4, having a long 8-nt loop imparting dynamics without affecting the structural stability. The protruded loop further houses an E-box motif that provides a docking site for transcription factors like Zeb1. The G4 structure was also endogenously observed using Chromatin Immunoprecipitation (ChIP) with anti-G4 antibody (BG4) in the MDA-MB-231 cell line overexpressing ORAI1. Ligand-mediated stabilization suggested that the stabilized G4 represses transcription in cancer cell line MDA-MB-231. Down regulation of transcription further led to decreased Ca2+entry by the SOCE pathway, as observed by live-cell Fura-2 Ca2+imaging.

P1.BIO.13 – 1,8-Naphthalimide-Nucleobase Derivatives as Potent Human Telomeric G-Quadruplex DNA Stabilizers and Anticancer Agents

Alaa Salem[1], Chahlaa Nassab[1]

[1]UAE University

Cancer remains the second cause of death worldwide, with one in every five people at risk of developing cancer. Therefore, development of novel potent, selective and less toxic anticancer agents remains an urgent need. 1,8-Naphthalimides are known for their anticancer properties, acting through DNA binding, and p53, lysosomal, and mitochondrial pathways. Some of these compounds have progressed to clinical trials. Consequently, research on developing potent and selective 1,8-naphthalimide derivatives is actively ongoing. In this work, we designed, synthesized, and characterized several 1,8-naphthalimide derivatives functionalized with adenine, cytosine, and guanine nucleobases. Stabilization of human telomeric G-Quadruplex DNA by these compounds were evaluated using UV-Vis, fluorescence, and circular dichroism (CD) spectroscopies. The results confirmed the formation of 1:1 and 1:2 (drug:G-Quadruplex DNA) complexes. Melting temperature showed stabilization effects by 4°C, 2°C, and 6°C for adenine, cytosine, and guanine derivatives, respectively. Molecular docking revealed favorable exothermic binding, with affinity scores of -9.2, -8.9, and -10.7 kcal/mol, respectively. Furthermore, toxicity estimation using ADMET predicted low to moderate toxicity and carcinogenicity, with no mutagenicity. These findings suggested modified 1,8-naphthalimides as promising G-Quadruplex DNA stabilizers, warranting further in vitro and in vivo anticancer studies in cellular and animal models.

P1.BIO.14 – G-Quadruplexes Within the Influenza a Virus Genome Interacting With G4-Specific Ligands - Potential Antiviral Targets?

Maria Nalewaj[1], Karolina Zielińska[1], Ryszard Kierzek[1],
Elżbieta Kierzek[1], Marta Szabat[1]

[1]Institute of Bioorganic Chemistry Polish Academy of Sciences

Influenza A virus (IAV) causing pandemic outbreaks is a significant research subject. Despite high genome variability, the viral RNA (vRNA) secondary structure retains consistent features across strains, and its role in the viral life cycle has been described. Among the vRNA structures, G-Quadruplexes (G4s) have been confirmed to have function during viral replication and thus are investigated as potential drug targets. We searched the IAV genome for potential Quadruplex-forming sequences (PQSs), studied their ability to fold into G4s, and their role in the viral life cycle. We identified PQS twelve motifs within the IAV vRNA. Then we determined their propensity to form G4s using spectroscopic methods (UV, 1H NMR, and CD), and native PAGE. Next, we examined via RT stop assay, if selected motifs interact with G4-specific ligands. Studies in cell cultures allowed us to observe the influence of G4-specific ligands on IAV replication. We conducted our research using two models - the IAV minireplicon system and IAV infection. Our results revealed that three PQSs form stable G4s within protein-coding segments and that G4-specific ligands interact with the selected G4s. Moreover, G4-specific ligands inhibit IAV minireplicon replication and have an impact on IAV infection. We concluded that G4s are present within the IAV genome and can be targeted by ligands leading to viral replication inhibition. Our findings suggest that PQS motifs can serve as potential antiviral targets.

P1.BIO.15 – Discovery of G-Quadruplex and i-Motif formation in Leishmania parasites

Ying-Zhi Xu[1], Hayden Hilton Roys[1], Tiffany Weinkopff[1], Samantha Kendrick[1]

[1]University of Arkansas

Leishmaniasis is a serious public health threat characterized by destructive skin lesions or infection of visceral organs affecting millions of patients worldwide. Current treatments are toxic with many side effects and the disease can be fatal if untreated. Together with frequent drug resistance, there is an urgent need to identify novel targets and develop new treatments. Using immunofluorescence, we show Leishmania parasites readily form RNA and DNA G-Quadruplex (G4) structures on a global level. Importantly, Leishmania harbor these genomic sequences at a four-fold times higher frequency than humans, indicating a therapeutic window due to the potential dependency on these structures for parasite survival and positions G4s as promising therapeutic targets. While G4-forming DNA was previously identified in sequencing studies, we demonstrate for the first time both RNA and DNA form bonafide G4 structures in genes are associated with Leishmania virulence factors (e.g. LPG and GP63) and in strand-switch regions responsible for initiating transcription. We further discovered these G4s are stabilized with the pan-G4 ligand PDS, which in-turn decreased parasite viability. Lastly, we also found the complementary C-rich sequences can adopt stable i-Motif (iM) structures. These studies set the foundation for continued efforts to define mechanisms for G4/iM transcriptional control and posit these structures as therapeutic targets for leishmaniasis.

P1.NANO.16 – Selective Targeting of a B-MYB G-Quadruplex Motif with an Acridine-Based Nucleic Acid Probe

Pedro Lourenço[1], André Miranda[1], Maria Paulo Cabral Campello[2], António Paulo[2], Jean-Louis Mergny [3], Carla Cruz[1]

[1]University of Beira Interior, [2]Universidade de Lisboa, [3]Laboratoire d'Optique et Biosciences

The B-MYB gene encodes a transcription factor (B-Myb) that regulates cell growth and survival. Abnormal expression of B-Myb is frequently observed in lung cancer and poses challenges for targeted drug therapy. Oncogenes often contain DNA structures called G-Quadruplexes (G4s) in their promoter regions, and B-MYB is no exception. These G4s play roles in genetic regulation and are potential cancer treatment targets. In this study, a probe was designed to specifically identify a G4 within the promoter region of the B-MYB gene. This probe combines an acridine derivative ligand with a DNA segment complementary to the target sequence, enabling it to hybridize with the adjacent sequence of the G4 being investigated. Biophysical studies demonstrated that the acridine derivative ligands C5NH2 and C8NH2 not only effectively stabilized the G4 structure but also exhibited moderate affinity. They were capable of altering the G4 topology and exhibited enhanced fluorescence emission in the presence of this Quadruplex. Additionally, these ligands increased the number of G4s observed in cellular studies. The probe was synthesized with good yields, and subsequent cellular studies validated its co-localization with the target sequence.

P1.NANO.17 – Effect Of Chirality On The Binding Between Ligands and G-Quadruplex Structures

Grinsun Sharma[1], Akm Kafi[1], Pratiksha Chaudhary[1], Masayuki Tera[2], Yuri Shimasawa[2], Kazuo Nagasawa[2], Hanbin Mao[1]

[1]Kent State University, [2]Tokyo University of Agriculture and Technology

G-Quadruplexes (GQs) play significant roles in telomere maintenance and genomic stability. GQs have become attractive therapeutic targets, including cancer treatment. The interaction between GQs and ligands is influenced by various factors, with chirality playing a key factor in binding specificity and affinity. In this study, we corelated the in vitro and in vivo binding between diastereomeric ligands with left- and right-handed GQs. We determined the Kd of these bindings through an innovative cyclic voltammetry method exploiting the newly discovered chirality induced spin selectivity phenomenon. We also constructed two dual luciferase plasmids (Plasmid pC-KIT1) by respectively cloning the left- and right-handed GQ forming sequences into the promoter region of Renilla luciferase gene, whereas the promoter of Firefly luciferase gene HSV TK remained unchanged. The relative abundances of these two compounds were measured to reflect the inhibitory effect of the GQ structure formed in the Renilla gene promoter with and without diastereomer ligands. We found that the binding affinities estimated by the in vivo and the in vitro approaches were in accord with each other, which demonstrated that the chirality played a critical role to determine the binding affinity between ligand diastereomers and GQs. These results provide a guideline in the design of better ligands to specifically recognize GQs in gene promoters.

P1.STR.18 – Identification of G4 Structures in Industrial Bacterium Bacillus Subtilis

Maria Vittoria Cottini[1], Sidra Ishrat[1], Polina Marchenko[1], Jan Jamroskovic[1]

[1]Slovak Academy of Sciences

G-Quadruplexes (G4) have been extensively studied mainly in human model systems, where they have been shown to play a role in basic cellular processes. Despite ongoing research, their presence and function in bacteria remain poorly understood. Our research aims to fill this gap by identifying, characterizing, and studying the function of G4 structures in the gram-positive bacterium Bacillus subtilis, a key organism in biotechnology, industry, and environmental science. As 60 % of the global enzyme market is produced by genetically modified Bacillus strains, a better understanding of its genome regulation is crucial for industrial applications. Using bioinformatics tools, we identified potential G4-forming sequences within the B. subtilis genome. Further analysis revealed their enrichment near transcription start sites, which suggests their possible role in gene regulation. To study the folding and stability of these structures, we conducted in vitro experiments, such as primer extension assay, qPCR stop assay, and circular dichroism. Additionally, the binding of selected structures by the BG4 antibody was assessed by employing the immuno-dot blot assay. The current research focuses on the identification of folded G4 structures in vivo in B. subtilis cells using immuno-detection methods.

P1.STR.19 – AFM Analysis of G-Wire DNA Structure and Nanoparticle Decoration

James Vesenka[1], Mayuri Gilhooly[1], Eva Balog[1], Thomas Marsh[2], Marybeth Vesenka[1], Katya Podlesnaia[3], Wolfgang Fritzsche[32]

[1]University of New England, [2]University of St. Thomas, [3]Leibniz Institute of Photonic Technology

We explore G-wire DNA structure as part of a larger effort illuminate their growth kinematics. The primary tool used to analyze the DNA was atomic force microscopy imaged in air and liquid. AFM images were analyzed to confirm orientation correlations on untreated mica using ultra-high resolution atomic force microscopy. Persistence lengths measurement were taken from numerous samples on mica treated with poly-L-ornithine. Preliminary studies were undertaken to explore growth rates on gold nanoparticle terminated DNA and surface plasmon resonance shifts based on the length of the terminated G-wires. Many elements of this research are ideal suited for undergraduate students because of the ease of sample construction and imaging.

P1.STR.20 – Structure of a Human Telomeric Anti-Parallel G-Quadruplex with Terminal Overhang at a ds-ss DNA Junction

Constanza Avendano Avila[1], Brahim Heddi[1]

[1]Centre national de la recherche scientifique

Human telomeric DNA G-Quadruplexes exhibit high polymorphism, with their structure being strongly influenced by the flanking sequence. In this study, we investigated the G-Quadruplex formation at a double-stranded/single-stranded (ds-ss) telomeric junction, where POT1 was recently shown to preferentially bind when the first G-tract is split at the ds-ss junction within the single-stranded overhang. We showed that a single G-Quadruplex conformation can be favored at this junction, compared to sequences with different flanking ends. The high-resolution structure of the single-strand sequence was solved, revealing a two-layered, anti-parallel G-Quadruplex capped by two triads, with an unstructured 5' tail.

P1.STR.21 – Recognition of PIM1 Quadruplex-Duplex Hybrids by Bis-Quinolinium Ligands: In Vitro and In-Cell NMR Studies

Martina Lenarcic Zivkovic[1], Anirban Ghosh[2], Jakub Harnoš[2], Petr Stadlbauer[3], Jiri Sponer[3], Lukas Trantirek[2]

[1]National Institute of Chemistry, [2]Masaryk University, [3]Czech Academy of Sciences

Quadruplex-duplex hybrids (QDHs) are distinctive structural motifs with both biological relevance and technological potential, yet their structural dynamics and ligand recognition remain poorly understood. The PIM1 oncogene, overexpressed in triple-negative breast cancer, forms both hybrid and antiparallel QDHs in vitro. Here we show that in Xenopus laevis oocytes, the intracellular environment strongly favors the antiparallel form. Additionally, we investigate the binding and stabilization of PIM1 QDHs by bis-quinolinium ligands Phen-DC3 and 360A using solution and in-cell NMR spectroscopy. Both ligands selectively recognize and stabilize the hybrid QDH conformation, effectively counteracting the cellular preference. High-resolution NMR structures reveal distinct binding dynamics at the Quadruplex-duplex (Q-D) junction: Phen-DC3 binds rigidly, whereas 360A exhibits dynamic reorientation. Additionally, we introduce an in-cell ^{19}F NMR approach using 2'-FANA-modified DNA, enabling direct detection of ligand-QDH interactions in live cells. This method provides a high-throughput platform for screening QDH-targeting ligands, advancing therapeutic development.

P1.STR.22 – Effects of Flanking Regions on DNA i-Motif Folding And Stability

Chuyang Zhang[1], Hariz Iskandar Mohd Nizal[1], Christopher Hennecker[1], Anthony Mittermaier[1]

[1]McGill University

i-Motifs (iMs) are four-stranded non-canonical nucleic acid secondary structures that are formed by cytosine-rich sequences. Putative iM forming sequences are concentrated in human promoter and telomeric regions, suggesting possible biological roles. However, many iMs do not readily fold at neutral pH, sparking interest in factors that may stabilize them. We performed a systematic study on how the nucleotides flanking iMs affect their stabilities and folding kinetics. We found that the mere presence of flanking nucleotides led to dramatically slower folding and lower stability, compared to isolated iMs. Conversely, complementary flanking nucleotides that comprise an inverted repeat and form a hairpin with the iM in the loop led to greater stability and faster folding than the iM on its own. A bioinformatic analysis of human promoter regions showed that the flanking regions are more likely than average to be complementary to each other, suggesting that this stabilization might be biologically relevant. We analyzed several naturally occurring iM sequences and found that complementary flanking regions substantially stabilized the structures (up to 53-fold faster folding and 16 °C more stable). Our results show that the regions of DNA flanking iMs are an important and hitherto overlooked factor in iM folding and stability.

P1.STR.23 – Unraveling the Molecular Mechanisms of Vimentin Interaction with G-Quadruplex Repeats

Marta Cozzaglio[1], Nicolò Dal Ponte[1], Martina Rotondo[2], Barbara Biondi[3], Lukas Trantirek[4], Riccardo Rigo[1], Barbara Spolaore[1], Claudia Sissi[1]

[1]University of Padova, [2]University of Napoli, [3]Institute of Biomolecular Chemistry, [4]Masaryk University

Vimentin is a cytoskeletal protein that exists as either soluble tetramers or filaments. Its overexpression is associated with epithelial-to-mesenchymal transition and cancer progression. While mainly localized in the cytoplasm, vimentin is also present in the nuclear matrix of cancer cells. We identified vimentin as a high-affinity binder of DNA G4-repeats, non-canonical structures containing two or more adjacent G-Quadruplex units. To uncover the molecular basis of this interaction, we employed an integrated structural biology approach, combining techniques such as HDX-mass spectrometry and electron-microscopy. Mapping the structural rearrangements of vimentin upon G4-repeat binding revealed unique perturbations specific to the vimentin-DNA complex, as well as additional changes common to both G4-repeat binding and vimentin polymerization. Furthermore, a peptide from the N-terminal domain of vimentin was identified as a specific G-Quadruplex binding element, directly contributing to the stabilization of the vimentin-DNA complex. Overall, these findings establish a detailed molecular framework for vimentin selective recognition of G4-repeats and indicate the mechanism through which DNA structures regulate vimentin polymerization.

P1.STR.24 – Physiological Recognition of the MYC G-Quadruplex by Berberine

Jinho Jang[1], Jonathan Dickerhoff[1], Nicole Brundridge[1], Scott Mcluckey[1], Danzhou Yang[1]

[1]Purdue University

The medicinal natural product berberine shows an anti-cancer effect that has been correlated with its tendency to bind G-Quadruplexes. A prominent G-Quadruplex is located in the MYC promoter region (MycG4). Targeting this MycG4 with small molecules leads to the down-regulation of MYC expression, which can have devastating effects on cancer cells. Despite many attempts to develop berberine derivatives with improved selectivity and potency, no interesting candidate for further studies has yet been identified. The rational design of drug candidates is often guided by high-resolution structures of ligand-Quadruplex complexes. Many previous design attempts of berberine derivatives have been driven by a berberine-Quadruplex crystal structure that showed the binding of berberine dimers to each binding site. Remarkably, our mass spectrometry and NMR study reveals that berberine binds substantially differently under physiologically relevant solution conditions. The monomeric binding of berberine is preferred, and the berberine orientation, as well as the ligand-DNA interface, deviates from the crystal structure. In conclusion, we present the physiologically relevant basis for a structure-based rational design of berberine derivatives as MycG4-targeting cancer drugs. Moreover, our results emphasize that crystallization of the dynamic Quadruplexes and their drug complexes can be prone to artifacts, which potentially mislead subsequent studies.

P1.STR.25 – DNA Secondary Structures and Their Chirality Induced Spin Selectivity

Pravin Pokhrel[1], Akm Kafi[1], Hao Shen[1], Hanbin Mao[1]

[1]Kent State University

Given the inherent chirality of DNA molecules, their structural asymmetry influences electron transport, leading to spin-dependent electron trajectories. This phenomenon, termed Chiral-Induced Spin Selectivity (CISS), emerges from the interplay between an electron's spin and the helical chirality of the molecular system, resulting in preferential transmission of one spin orientation while the opposite spin state undergoes backscattering. In this study, we employed an electrochemical approach under controlled polarized magnetic fields to quantitatively assess the CISS efficiency of various DNA secondary structures through spin polarization measurements. Our results indicate that spin polarization is most pronounced in highly stable DNA conformations, such as G-Quadruplexes and triplexes, followed by double-stranded (dsDNA) and single-stranded (ssDNA) configurations. This trend correlates with the extent of base stacking within the DNA structure, where triplex and G-Quadruplex DNA structures exhibits greater π-π stacking interactions than duplex and single-stranded DNA, thereby enhancing spin-selective electron transport. These findings underscore the potential of DNA-based chiral molecules as effective spin-filtering agents under polarized magnetic fields, with promising applications in spintronic devices and DNA-based biosensing technologies.

P1.STR.26 – G-Quadruplex DNA Inhibits Unwinding Activity But Promotes Liquid-Liquid Phase Separation by the DEAD-Box Helicase Ded1p

Jun Gao[1], Zhaofeng Gao[2], Andrea Putnam[2], Alicia Byrd[1], Sarah Venus[2], John Marecki[1], Andrea Edwards[1], Haley Lowe[1], Eckhard Jankowsky[2], Kevin Raney[1]

[1]University of Arkansas, [2]Case Western Reserve University

G-Quadruplexes (G4) structures play important roles in gene expression, DNA replication, and telomere maintenance. In addition, G4 structures have been linked to genomic instability and human diseases, including cancer and neurodegenerative disorders. DEAD-box RNA helicases, characterized by a highly conserved "Asp-Glu-Ala-Asp" (DEAD) motif, are found in all eukaryotes, as well as in many bacteria and archaea. These enzymes are involved in most aspects of cellular RNA metabolism and in the formation of stress granules and P-bodies, cellular RNA-protein condensates that form by liquid-liquid phase separation (LLPS). Many DEAD-box helicases have been linked to diseases, including cancer, neurodegeneration and viral infections. The S. cerevisiae DEAD-box RNA helicase Ded1p, an ortholog of human DDX3X, is involved in translation initiation and localizes to stress granules and P-bodies. We found that G4DNA inhibits RNA unwinding activity of Ded1p. We observed G4DNA promotes LLPS of Cy5-labelled Ded1p in vitro by microscopy. Phen-DC3 (a well-characterized G4 stabilizer) promotes Ded1-GFP to form a non-membrane organelle-like structure at the outside of the nucleus in the DED1-GFP yeast cell. This indicates that Phen-DC3 promotes LLPS of Ded1-GFP in cells. In summary, we demonstrated that G4DNA inhibits RNA unwinding activity of DEAD-box helicase Ded1p, and promotes LLPS of Ded1p. Our findings suggest possible new roles for G4 structures and DEAD-box RNA helicases.

P1.STR.27 – Insights Into The Determinants of Vimentin-RNA Interaction

Riccardo Rigo[1], Marta Cozzaglio[1], Ernesto Mucenji[1], Claudia Sissi[1]

[1]University of Padova

Vimentin is a cytoskeletal protein belonging to the type III intermediate filaments, playing a crucial role in maintaining cell shape and nuclear integrity. It contributes to cellular processes such as migration, adhesion, and signal transduction, and is essential for the mechanical resilience of mesenchymal cells. Beyond these structural functions, vimentin has been recently implicated in the regulation of RNA metabolism. Moreover, it has been observed that vimentin recognizes genomic DNA in a structure-dependent manner. Here, we present a study on the interaction between vimentin and RNA. By employing biophysical and molecular biology techniques, we characterized the determinants of this interaction from both the protein and nucleic acid perspectives. Our findings reveal that vimentin is specifically recruited by G-Quadruplex RNA and this interaction appears to promote liquid-liquid phase separation (LLPS). These results broaden our understanding of vimentin's functions, extending its role beyond cytoskeletal organization.

P1.STR.28 – Indenoisoquinolines Strongly Bind and Stabilize the MYC Promoter G-Quadruplex And Downregulate MYC

Mercedes Demoss[1], Rio Ohtake[1], Kaibo Wang[2], Guanhui Wu[1], Mark Cushman[1], Danzhou Yang[1]

[1]Purdue University, [2]China Pharmaceutical University

Indenoisoquinolines are clinically tested anticancer drugs initially designed as human topoisomerase I inhibitors. However, certain indenoisoquinolines with potent anticancer activity do not exhibit strong Topo I inhibition, indicating an alternative mechanism of action. MYC is a central protein for tumorigenesis and is overexpressed in many human cancers, making it an attractive anti-cancer target, but MYC protein has been deemed 'undruggable'. The G-Quadruplex in the MYC promoter (MycG4) represses MYC transcription. G-Quadruplexes are noncanonical, globular, four-stranded DNA secondary structures targetable by small molecules and have emerged as novel therapeutic targets. Intriguingly, we discovered indenoisoquinolines bind MycG4 and downregulate MYC. Herein, we show strong binding of indenoisoquinolines to MycG4 in vitro using fluorescence, CD, and NMR spectroscopy. Furthermore, we show potent reduction of both MYC mRNA and protein levels in cells. Structure-activity-relationships of MycG4 recognition by indenoisoquinoline are investigated to understand structural requirements for binding. We work on understanding the molecular level recognition of MycG4 by indenoisoquinoline using high-field NMR spectroscopy. The structural information of MycG4 in complex with the drug will help understand the exceptional activity and guide structure-based rational design of indenoisoquinoline MycG4-targetedanticancer drugs.

P1.STR.29 – Structure and Dynamics of a Duplex-Embedded G-Quadruplex System Resolved to 7.4 Å Resolution by cryo-EM And SAXS

Robert Monsen[1]

[1]University of Louisville

Genomic regions with high guanine content fold into non-B form DNA four-stranded structures known as G-Quadruplexes (G4s). Extensive in vivo investigations have revealed that promoter G4s are transcriptional regulators. Little structural information exists for these G4s embedded within duplexes, their presumed genomic environment. Here we report the structure and dynamics of a 28 kDa duplex-G4-duplex (DGD) model system using cryo-EM, molecular dynamics, and small-angle X-ray scattering (SAXS) studies, resolved to 7.4 Å. The DGD structural ensemble features a 49-67° bend induced by a stacked duplex-G4 interaction at the 5′ G-tetrad interface with a persistently unstacked 3′ duplex and poly dT surrogate-complement loop. Structural analysis shows that the DGD model is quantifiably more druggable than the monomeric G4 structure alone and represents a new structural drug target. Our results illustrate how the integration of cryo-EM, MD, and SAXS can reveal complementary detailed static and dynamic structural information on DNA G4 systems.

P1.STR.30 – Structural Insights Into PDGFR-B Vacancy G-Quadruplex and Fill-In Mechanism

Rongxue Zhang[2], Kaibo Wang[1], Jonathan Dickerhoff[2], Danzhou Yang[2]

[1]China Pharmaceutical University, [2]Purdue University

PDGFR-β overexpression drives various human diseases including cancer and atherosclerosis. The proximal PDGFR-β promoter forms G-Quadruplex that functions as a transcription silencer and is an attractive drug target. The PDGFR-β promoter contains seven G-runs and the major G-Quadruplex is formed in the 5′-mid region, which can be stabilized by G4-ligands to downregulate PDGFR-β gene. We determine the folding structure of the major 5′-mid G-Quadruplex using NMR in combination with DMS footprinting and mutational analysis. It adopts a novel "broken-strand" structure with a 2+1 discontinuity in K+ solution. The broken-strand structure can be considered as an intramolecular fill-in vacancy G-Quadruplex (vG4) with an incomplete G-tetrad. Using NMR in combination with biophysical and biochemical methods, we show that the 5′-mid broken-strand G-Quadruplex forms two equilibrating vG4s. Intriguingly, the PDGFR-β vG4 can be readily filled-in and stabilized by external guanine-derivatives and we determined the structure of the dGMP-fill-in-vG4 in K+ solution. Furthermore, we discovered that the small-molecule medicinal compound berberine binds and stabilizes the dGMP-fill-in-vG4, and we determined the ligand-bound ternary complex structure by NMR. Collectively, our findings provide a structural basis for the potential PDGFR-β transcriptional regulation by guanine metabolites and rational design of drugs targeting PDGFR-β G-Quadruplex.

P1.STR.31 – Molecular Recognition of G-Quadruplex DNA by Novel Thymoquinone Derivatives: Insights on Their G-Quadruplex Stabilization and Anticancer Effects

Alaa Salem[1]

[1]UAE University

Thymoquinone (TQ) is a biologically active compound with weak anticancer properties, exhibiting selectivity towards cancer cells over normal cells. Attempts have been made to enhance its potency through the synthesis of novel derivatives. In this study, eight novel TQ derivatives were synthesized in a one-pot reaction via nucleophilic substitution using various aryl or alkyl amines. Structural characterization confirmed mono substitution at carbon-3 of the TQ ring. The presence of an aliphatic spacer in the alkyl or aryl amine was found essential for progression of the synthetic reaction. Synthesized compounds exhibited mild to moderate cytotoxic effects against human lung cancer (A549), breast cancer (MDA-MB-231), and colorectal cancer (HT29) cell lines, with IC50 of 30.01, 45.14, and 55.81 μM, respectively. Stabilization of human telomeric G-Quadruplex DNA by synthesized TQs was evaluated using UV-Vis spectroscopy, fluorescence spectroscopy, circular dichroism, and melting temperature analysis. Binding affinities ranged in 7.76x104-1.33x107 M-1 and melting temperature shifts (ΔTm) ranged in 7.0- 21.0 °C were obtained. Synthesized compounds also demonstrated good selectivity for G-Quadruplex DNA over duplex DNA. The findings suggested novel TQ derivatives, particularly TQ8, as potential G-Quadruplex DNA stabilizers and anticancer agents.

P1.TAR.32 – Analysis of Quadruplex Propensity of Aptamer Sequences

Anne Cucchiarini[1], Michaela Dobrovolná[2], Vaclav Brázda[2], Jean-Louis Mergny [1]

[1]Laboratoire d'Optique et Biosciences, [2]Czech Academy of Sciences

Aptamers are short DNA or RNA sequences that can fold into unique three-dimensional structures capable of binding specifically to target molecules with high affinity, similar to antibodies. A unique feature of a number of aptamers is their ability to adopt a G-Quadruplex (G4) fold, a four-stranded structure formed by sequences rich in guanine. While G4 formation has already been proposed or demonstrated for some aptamers, we wanted to investigate how frequently a Quadruplex-prone motif would emerge from a SELEX process. To this aim, we investigated Quadruplex candidate sequences found in the UTexas Aptamer Database, which includes over 1400 aptamer sequences extracted from 400 publications spanning several decades. We analyzed G-Quadruplex and i-motif propensity of these 1400 sequences. While no likely i-motif forming candidate motif was found, nearly 1/4 and 1/6 of all DNA and RNA aptamers, respectively, were predicted to form G4 structures. Interestingly, we found many motifs for which G4 formation was not reported or suspected: out of 309 sequences containing a potential stable G4 motif, the word "Quadruplex" appeared only for 53 of them (17%), and we experimentally confirmed G4 formation for all sequences experimentally tested. These observations argue for a significant reevaluation of G4 propensity among aptamer sequences.

P1.TAR.33 – Targeting G-Quadruplex DNA in Pathogenic Bacteria as Novel Antimicrobial Treatment

Daniele Comi[1], Rubén Cebrián[2], Efres Belmonete-Reche[3], Valentina Pirota[1], Anne De Jong[2], Juan Carlos Morales[4], Mauro Freccero[1], Filippo Doria[1], Oscar P. Kuipers[2]

[1]University of Pavia, [2]University of Groningen, [3]International Iberian Nanotechnology Laboratory, [4]Consejo Superio de Investigaciones Cientificas

The potential of G-Quadruplex (G4s) DNA structures as antimicrobial targets remains largely unexplored. Hence, we evaluated a library of G4-ligands based on naphthalene diimides (NDIs) against Gram-positive and Gram-negative bacteria. We identified NDI-10 as the most promising compound, describing a different action mechanism for both Gram-positive or negative bacteria. In antimicrobial susceptibility tests, NDI-10 shows bacteriostatic effects against Gram-positive bacteria and bactericidal effects against Gram-negatives: this could be related to the different prevalence of putative G4 sequences in each group, higher in Gram-negatives compared to Gram-positives, as highlighted by in silico genome-wide analysis. Through transcriptomic analysis, NDI-10 exhibits significant repression of metabolic, transcriptional, and signaling pathways in Gram-negative bacteria, while Gram-positives activate replication and repair genes as a response to the stress. As expected, the biophysical assay confirmed high G4 binding and stabilization, whereas permeability studies indicated that Gram-negative outer membrane limits uptake. Finally, in vivo tests in Galleria mellonella confirmed antimicrobial efficacy with low toxicity. In this study, we demonstrated that targeting bacterial G4s could be a feasible strategy for antimicrobial development and with the necessary optimizations, G4-ligands may lead to novel antibiotics able to overcome resistance mechanisms.

P1.TAR.34 – Exploring the BODIPY Phenyl Derivatives as I-Motifs Ligands

Jakub Żubertowski[1], Daria Praska[1], Magdalena Rapp[1], Anna Dembska[1]

[1]*Adam Mickiewicz University*

Compounds with the skeleton origin of 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) are widely recognized and, thanks to their attractive optical properties, are already in use for bio-imaging, bio-labeling or as a photosensitizers in photodynamic therapy. BODIPY dyes have recently been recognized as another group of potential ligands interacting with DNA G-Quadruplexes. We decided to examine if those compounds could also interact with DNA i-motifs, altering their structure or stability. Therefore, we synthesized a series of derivatives bearing modified or unmodified phenyl moiety located at the meso position of BODIPY core and used them in studies on a several cytosine-rich oligonucleotides, known to fold into i-motif structure under acidic pH. Indeed, obtained results have confirmed that a few from considered compounds have a significant impact on the stability of tested tetraplexes. We would like to presents our conclusions, sharing the results of spectroscopic investigations (UV-vis absorbance, fluorescence and circular dichroism spectroscopy), detailed with the information obtained from the analysis of thermal denaturation studies.

P1.TAR.36 – Insights into the Natural Product Cepharanthine as a G-Quadruplex Ligand and New Therapeutic for Lymphoma

Kennith Swafford[1], Samantha Kendrick[1]

[1]*University of Arkansas*

Diffuse large B cell lymphoma (DLBCL) is the most common form of non-Hodgkin lymphoma with a refractory/relapsing rate of 40%. The poor patient prognosis is primarily due to genetic heterogeneity and has led to the search for alternative targeted therapies. A promising target is the scaffolding protein, CARD11, that constitutes a vital signalosome in the pro-survival pathways in DLBCL. We previously uncovered a G-Quadruplex (G4) within the promoter region of CARD11 as a targetable element of the oncogene. Stabilization of the G4 secondary structure prevents transcription and decreases the expression of CARD11. In search of potential stabilizers for the CARD11 G4, a 752 natural compound library was screened, revealing 97 interactive small molecules. Several of these compounds were further characterized for their selectivity towards the CARD11 G4, ability to affect CARD11 expression, and cytotoxicity to DLBCL cell lines to evaluate their therapeutic potential in DLBCL. Of these compounds, cepharanthine, a bisbenzylisoquinoline alkaloid, proved the most potent stabilizer of the CARD11 G4 increasing the melting temperature by 15°C, lowered CARD11 mRNA by 20%, and displayed the highest cytotoxicity towards DLBCL cell lines. Furthermore, cepharanthine was found to be non-interactive to canonical DNA structures and exhibited selectivity towards G4 structures. This study uncovers a new mechanism of action for cepharanthine and highlights its potential for use in treatment of DLBCL.

P1.TAR.37 – Indenoisoquinolines Potently Downregulate MYC and Induce Cancer Cell Death

Yichen Han[1], Kaibo Wang[1], Jun Wan[1], Mark Cushman[1], Danzhou Yang[1]

[1]*Purdue University*

The MYC promoter G-quadruplex (MycG4) is a transcription repressor. Indenoisoquinolines were designed as topoisomerase I inhibitors with three compounds in phase I/II clinical trials. Recently, we found that indenoisoquinolines strongly bind and stabilize MycG4 and potently downregulate MYC expression in cancer cells, whereas CPT topo1 inhibitor shows little binding to MycG4. We assessed global changes in gene expression by RNA sequencing in the MYC-dependent B-lymphoma Raji cells. The RNA-seq results indicate that the MYC pathway is the major cellular response of the clinical LMP compounds. We further examined the treatment-induced suppression of MYC-regulated genes and observed significant and durable inhibition of MYC-regulated gene pathways. Notably, we found that the clinical LMPs, especially LMP400, exhibited significantly stronger MYC suppression compared to other G4-ligands. Furthermore, they showed excellent anticancer activity in MYC-dependent tumor xenograft models. Thus, dual MYC-targeting and topoisomerase I inhibition is critical to the exceptional MYC inhibition and anticancer efficacy of indenoisoquinolines. In conclusion, we demonstrate that the clinical indenoisoquinolines effectively and persistently inhibit the global MYC transcriptional pathway, providing critical mechanistic understanding and therapeutic applications of indenoisoquinolines in MYC-dependent cancer.

POSTER SESSION 2

Thursday, June 5, 2025 1900-2100

P2.BIO.40 – The Impact of Guanine Quadruplexes on Mitochondrial DNA Stability in Yeast

Shelby Rheinschmidt[1], Nayun Kim[1]

[1]University of Texas at Austin

Guanine Quadruplexes (G4s) are non-canonical DNA structures that can function as key regulatory elements. G4s are also implicated in stalling replication, inducing DNA breaks, and thus elevated genome instability. Studies evaluating the mitochondrial DNA (mtDNA) in patients with mtDNA maintenance defects found that areas of frequent deletions occur near G4-forming sequences, yet the mechanisms underlying this association remain unclear. I will investigate the role of G4s on mtDNA instability by identifying G4 ligands that bind and stabilize G4s in mtDNA and further by determining molecular mechanisms involved with the regulation of G4s in mtDNA. To do this, the model organism Saccharomyces cerevisiae will be utilized for its ability to grow without functioning mitochondria, allowing changes to mtDNA stability to be monitored. The G4 ligands PhenDC3, RHPS4, TMPYP4, CX5461, and CX3543 will be evaluated along with a panel of yeast strains knocked out for genes with a potential link to G4-related functions including NUC1, OGG1, TOP1, and RRM3. Changes to mitochondrial dysfunction will be measured through petite formation assays and mtDNA copy number analysis with ddPCR. Additionally, mtDNA mutations will be identified with NGS and nanopore sequencing. This research will provide critical insights into the factors driving G4-associated mtDNA instability, with potential implications for understanding mitochondrial disorders and developing therapeutic strategies.

P2.BIO.41 – G-Quadruplexes are Sites of Differential Methylation and Alternative Promoter Usage in Metabolic Disorders and Cancer

Angelika Lahnsteiner[1], Victoria Ellmer[1], Esther Schoenauer[1], Angela Risch[1]

[1]University of Salzburg

The global rise in obesity and its association with metabolic diseases (MetDs) as well as their elevated cancer risk pose them a major health challenge. While the mechanisms driving MetD progression remain unclear, both genetic and environmental factors, including DNA methylation, play key roles. Emerging evidence suggests that deregulated DNA methylation and G-Quadruplexes (G4s) contribute to the observed deregulated transcription patterns. To investigate their impact, we analyzed DNA methylation in 160 MetD patients versus controls, focusing on overlaps with G4 formation and alternative promoter usage. Illumina MethylationEPICv2 arrays (~900,000 CpGs) identified significant differential methylation in regulatory regions enriched for predicted G4 motifs. These regions overlapped with
differentially methylated sites and deregulated alternative promoters in cancer. Using permanganate/S1 nuclease footprinting with direct adapter ligation (PDAL-Seq) and circular dichroism spectroscopy, we confirmed G4 formation in hypomethylated regions and identified a transcription factor and epigenetic regulator associated with these sites. Targeted in vitro studies revealed that loss of methylation promotes G4 formation and activates alternative promoters in regulatory elements, whereas methylated regions lack these functions.

Our findings provide new insights into the mechanistic link between MetDs and cancer, highlighting non-B DNA structures as key contributors to disease progression.

P2.BIO.42 – Permanganate/S1 Footprinting Reveals Evolutionary Dynamics of G-Quadruplex and Other Non-Canonical DNA Structures in Telomere-To-Telomere Ape Genomes

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DNA loci that do not adopt the canonical, B-form secondary structure ('non-B DNA') have emerged as functional genomic elements, drivers of evolution, and disease contributors. Recent complete Telomere-to-Telomere genomes for humans and other apes have revealed extensive potential for the formation of non-B structures, including G-Quadruplexes (G4s), cruciforms, and Z-DNA. However, it is not known which loci form non-B DNA structures in cells. Here, we applied permanganate/S1 footprinting with direct adapter ligation and sequencing (PDAL-seq) to determine single-stranded DNA (ssDNA) profiles for cell lines from humans and 5 other apes. The statistical analysis and modeling of these ssDNA structure profiles unraveled the driving forces and functional impact of non-B structures across the ape phylogeny. PDAL-seq reads were mapped to computational non-B DNA motifs predictions genome-wide. Our analyses indicated that ssDNA formation in cells is driven by clusters of diverse non-B DNA motifs, with the largest contributions from direct repeats, G4, and Z-DNA motifs. Likewise, regulatory elements such as promoters, enhancers, and 5'-untranslated regions were enriched in PDAL-seq reads, supporting regulatory functions for non-B structures. Interestingly, some PDAL-Seq reads are mapped to species-specific satellite arrays, suggesting novel functions of non-B DNA. Future work will identify evolutionarily significant non-B DNA-forming loci.

P2.BIO.43 – G Registry Exchange, the Spare Tire, and the Complementary Strand Sequence Influence G-Quadruplex Folding in a Duplex-G-Quadruplex-Duplex Context

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In the genome, promoter G4 folding is influenced by the flanking duplex regions, the complementary strand, DNA-interacting proteins, and torsional forces on the helical DNA. Previously, we demonstrated that the VEGF gene promoter-potential G4 sequence in the duplex state is sensitive to oxidative modification of a G nucleotide, which initiates the DNA repair to transform the strand back to its original state. Thus, we studied the impact of DNA damage on G4 folding in a genomic model that includes flanking duplexes, and a poly-T complementary strand, referred to as a duplex-G-Quadruplex-duplex (DGD). The NMR VEGF G4 with only the essential G nucleotides needed for folding was studied. The presence of the oxidation product 8-oxoguanine and its downstream DNA repair products in the DGD model were studied to find damage increased the folding rate. Next, we investigated additional factors that could influence folding: (1) The addition of native G nucleotides beyond those required for a G4 to examine how G registry exchange affects folding. (2) The native fifth G-run, or "spare tire," on the 3' side of the sequence in influencing folding. (3) Changes to the complementary strand to more closely mirror the true sequence. Our results find DNA damage accelerates G4 folding in the DGD context and these additional factors influence the folding.

P2.BIO.44 – Visualizing Dynamic Phase Transitions of Ribonucleoprotein Condensates

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Liquid-liquid phase condensation governs diverse protein-protein and protein-RNA interactions, leading to the formation of membraneless compartments such as stress granules and the nucleolus. While multivalency and protein disorder are known to drive phase transitions, a detailed structural understanding remains limited due to the complexity of key condensate-forming components. Here, we investigate the small human SERF2 protein and its role in stress granule formation. Using NMR spectroscopy, we resolved the structure of SERF2, identifying key disordered regions and multivalent interaction sites. Our findings demonstrate that SERF2 specifically binds G-Quadruplex (G4) RNA, a non-canonical tetrahelical structure previously linked to stress granule assembly. We show that this interaction enhances SERF2's recruitment into phase-separated condensates, highlighting a direct link between protein disorder and RNA-induced phase transitions. The biophysical tractability of SERF2 and G4 RNA enabled high-resolution visualization of their multivalent interactions, allowing us to map molecular contacts, characterize structural dynamics, and determine how these interactions modulate early-stage ribonucleoprotein condensate formation. Our results provide fundamental insights into the mechanisms governing liquid-liquid phase transitions and reveal a previously uncharacterized role of SERF2 in stress granule regulation.

P2.BIO.45 – G-Quadruplex Structures in Dysregulated Long Non-Coding RNA of Ovarian Cancer and their Binding Interactions with Human Serum Albumin

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Ovarian carcinoma (OC) is a leading cause of cancer-related deaths among women, particularly in resource-constrained regions. Dysregulated long non-coding RNAs (lncRNAs) play pivotal roles in OC progression, impacting drug resistance, metastasis, and other cellular processes. This study investigates the potential of these lncRNAs to form G-Quadruplex (G4) structures, unique RNA secondary structures that influence stability, protein interactions, and function. Through in-silico predictions and experimental techniques—circular dichroism spectroscopy, Thioflavin T fluorescence assays, dot blot, and reverse transcriptase stop assays—we identified five OC-associated lncRNAs (ERLNC1, DLX6-AS1, LINC01127, FMNL1-DT, and LINP1) capable of forming stable G4 structures. Competitive DNA-binding assays revealed key G-tracts essential for G4 integrity. Monovalent cations and ligands showed differential effects on G4 stability. Human serum albumin (HSA), a major circulatory protein, interacted with these G4 structures, modulating their stability and conformation. This highlights their potential as biomarkers for early diagnosis and as therapeutic targets, offering novel avenues for OC management.

P2.BIO.46 – Biophysical Studies Of Triplex-Protein Interactions From MALAT1 & METTL16

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LncRNAs have the ability to form triple helices (triplexes) either with double stranded DNA or intramolecular resulting in a RNA:RNA•RNA triplex. The formation of these unusual structural elements has potential roles in vivo for cellular functions, like transcriptional regulation, post transcriptional RNA processing or chromatin modification. Therefore, lncRNAs and their respective structures gained more and more attention over the past years due to their potential of being drug targets of interest. The biological relevance was further underlined by the findings of certain proteins with the ability of recognizing triplex structures. We set out to investigate triplex-protein interactions employing biophysical methods for structural elucidation. We utilized electrophoretic mobility shift assays (EMSA), circular dichroism (CD) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy to investigate the interaction between the intramolecular triplex forming metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) and methyltransferase-like protein 16 (METTL16). METTL16 binds to MALAT1 in vitro, but until now, the exact binding domain is still elusive. We set out to further investigate the interaction and the structural elucidation of both the protein and the triple helical RNA in order to gain biologically relevant information, which will lead to a better mechanistic understanding of the RNA-protein (RNP) interaction.

P2.BIO.47 – ALTering Cancer by Triggering Telomere Replication Stress Through the Stabilization of Promoter G-Quadruplex in SMARCAL1

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Most of the human cancers are dependent on telomerase to extend the telomeres. But 10% of all cancers use a telomerase-independent, homologous recombination (HR) based pathway called alternative lengthening of telomeres (ALT). Due to the poor prognosis, ALT status is not being considered yet in cancer diagnosis. No specific treatment is available to date for ALT+ cancers. ALT+ cancers are dependent on replication stress to deploy DNA repair pathways to the telomeres to execute HR mediated telomere extension. SMARCAL1 resolves the replication stress and provides telomere stability in ALT telomeres, thus making it a suitable therapeutic target for the treatment of ALT+ cancers. Here, we found a significant downregulation of SMARCAL1 expression by stabilizing the G-Quadruplex (G4) motif found in the SMARCAL1 promoter by potent G4 stabilizers, which increased the localization of PML (promyelocytic leukemia) bodies in ALT telomeres and triggered the formation of APBs (ALT-associated promyelocytic leukemia bodies) in ALT+ cell lines, increasing telomere replication stress and DNA damage. Induction of replication stress and hyper-recombinogenic phenotype in ALT+ cells mediated by G4 stabilizing molecules already highlighted their possible application as a new therapeutic window to target ALT+ tumors. Moreover, our study will also provide a valuable insight toward the development of G4-based ALT therapeutics targeting SMARCAL1.

P2.BIO.48 – Exploring Specific Recognition of DNA G-Quadruplexes by Polyaromatic Heterocyclic Amidines

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Targeting human G-Quadruplex (G4) nucleic acids with small molecules is a promising strategy for anti-cancer therapeutics. The binding of small molecules to G4 structures linked to oncogenes can effectively modulate transcription. Despite the diverse topologies exhibited by G4 structures, the presence of G-quartets as a common structural element poses challenges for the design-specific ligands. It is essential to understand structural variations in the loops and grooves of G4 structures and binding mechanisms that utilize an end-stacking mode. We designed and synthesized a systematic series of new G-Quadruplex-stabilizing agents based on polyaromatic heterocyclic diamidine compounds. These ligands demonstrate specific binding and stabilization of promoter G4s with parallel topology over other G4 topologies (hybrid or antiparallel) and duplex DNAs. Circular dichroism melting studies reveal that these ligands can provide significant stability to c-MYC promoter G4s, for example, exhibiting an increase of up to 21 °C in ΔTm compared to telomeric and duplex DNAs (ΔTm ≤ 2.5 °C). The biacore-SPR binding results also indicate that the ligands possess a high binding affinity (KD ≈ 100 nM) for c-MYC G4. Molecular dynamics studies provide insight into structure-specific G4 interactions with amidines. The selectivity of these heterocyclic diamidines for G4 structures represents promising characteristics for developing novel probes for targeting Quadruplexes in vivo.

P2.BIO.49 – G-Quadruplex Mediated Preferential Downregulation of c-myc: A Unique Pathway of the Anticancer Action of Immunomodulator Drugs

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Immunomodulatory drugs, hydroxychloroquine (HCQ) and chloroquine (CQ) are in the preclinical investigation for cancer therapy, along with their extensive application in autoimmune and parasitic diseases. A hallmark of cancer cells is the elevated expression of oncogenes that drive tumor progression, often regulated by G-Quadruplex (G4) DNA structures located within their upstream promoter regions. This study elucidates that HCQ stabilizes the cellular G4 landscape most efficiently compared to other quinoline-based immunomodulatory drugs within oncogenic DNA, particularly the c-myc oncogene, a pivotal regulator of cancer progression. The drug-induced stabilization of c-myc G4 correlates with significant suppression of its transcriptional activity, culminating in a reduction of invasion and migration of TNBC cells. Mechanistically, the strong electrostatic interaction between the G4 phosphate backbone and the drug's charged side chain, anchors its quinoline group to enhance stacking with loop and quartet regions, stabilizing the G4. The in vivo investigation unveils the HCQ's capacity to potentiate the efficacy of conventional chemotherapeutic agents, representing it as a plausible candidate for adjunctive therapy. This study depicts an unconventional anticancer mechanism of immunomodulator drugs, wherein it exerts preferential transcriptional repression of the c-myc oncogene through G4-dependent stabilization, unveiling a novel strategy in oncological intervention.

P2.BIO.50 – Conserved G-Quadruplex Structures in Dengue Virus Genomes Are Potential Small Molecule Targets

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The four serotypes of dengue virus are globally endemic with over five million human infection cases reported in 2023. Despite the rising transmission of dengue virus, vaccination against dengue remains under development due to complications arising from immunoenhancement occurring after a secondary infection with a different dengue serotype. Development of antiviral therapies targeting conserved features in all four DENV serotypes will provide additional routes for treatment of dengue virus infection. Bioinformatic analyses have identified several conserved potential Quadruplex sites in the RNA genomes of members of the genus Orthoflavivirus. Biophysical characterization of NS5-B Quadruplex sequences obtained from viruses of each dengue serotype confirmed the formation of Quadruplex structures in vitro. Binding studies performed in the presence of Quadruplex-specific small molecules demonstrated their ability to stabilize the Quadruplexes from all four dengue serotypes. These results suggest that orthoflavivirus genomic Quadruplexes are promising targets for the development of novel therapeutics.

P2.BIO.51 – Transcriptome-Wide Analyses With G4-Ligands in Breast Cancer Identified a Multiple G4s Regulated Key Tumor Suppressor Gene CYLD

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G-Quadruplex (G4) and G4-ligand (G4L) mediated stabilization/destabilization of G4 structures is considered as promising cancer therapeutic strategy. However, genome-wide off-target interactions of G4Ls pose significant challenges to identify the key oncotarget. Therefore, effects of G4Ls on transcriptome followed by multiome-wide association study (MWAS) are necessary to select key oncotarget with therapeutic potential. Herein, RNA-sequencing analyses evaluated the genome-wide effects of two representative G4Ls, Pyridostatin and Quarfloxin in MCF7 breast cancer (BRCA) cells. Differentially expressed genes cross-referenced with TCGA BRCA datasets showed upregulation of 26 and 19 tumor suppressor genes (TSGs) and the downregulation of 22 and 14 oncogenes in Pyridostatin and Quarfloxin treatments, respectively. Further, the MWAS-based harmonizome data integration platform identified CYLD as a key TSG upregulated in both G4Ls. Among various G4Ls, BRACO-19 was identified as a potent inducer of CYLD which was further validated by diverse analyses in MDA-MB-231cells. Biophysical studies confirmed four stable and parallel G4s in CYLD promoter; however, the detailed molecular mechanism yet to be elucidated. Consistently, BRACO-19 upregulates CYLD expression resulting in significant reduction in cancer cell progression. Collectively, we identified CYLD as a key TSG, and its inducer BRACO-19 to develop scaffold-based drug-like derivatives for future BRCA therapies.

P2.BIO.52 – Microperoxidase-11 and its Derivatives as Selective Ligands for the Binding of G-Quadruplexes

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My research focuses on developing next-generation ligands for non-canonical DNA nanostructures of guanine-Quadruplexes (GQs). These structures are abundant in the human genome at strategic sites, such as telomere edges and oncogene promoters, making them key therapeutic targets. I study the binding properties of a novel hybrid GQ ligand, microperoxidase-11 (MP-11), an 11-mer peptide conjugated to a heme cofactor. Its versatility lies in elucidating various binding mechanisms instrumental for ligand development. MP-11 is also amenable to modifications enhancing specificity and affinity for GQ sequences. GQs are highly flexible, existing in dynamic equilibria between GQ and dsDNA forms. In my first study (Chem. Commun.), we investigated MP-11's interactions with GQs and dsDNA. Using UV-Vis spectroscopy, we showed that MP-11 exhibits distinct binding modes, selectively extracting GQ sequences embedded in dsDNA. This enabled specificity toward the oncogenic c-MYC GQ over other sequences, broadening ligand targeting. In follow-up work, we explored MP-11's effect on flexible GQs, observing its ability to convert non-parallel and hybrid topologies into a parallel form. Kinetic analysis revealed a linear relationship between GQ thermal stability and activation energy. This establishes flexible GQs as predictable nanostructures with applications in DNA nanobiotechnology.

P2.NANO.53 – Marginal Stability Mediated G-Quadruplex-Based Dnazyme Activity

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[1]Kent State University

G-Quadruplex (GQ) based DNAzymes have been widely applied in biocatalysis and molecular sensing. Given their significant potential, understanding their catalytic mechanisms and optimizing their performance remain critical research challenges. Factors such as GQ conformations and flanking sequences influence catalytic activities; however, no universal rule currently governs the catalytic properties of GQ-hemin DNAzymes. To address this, we employed single-molecule fluorescent MT-HILO (Magnetic Tweezers coupled with Highly Inclined and Laminated Optical sheet) microscopy on a DNAzyme using human telomere GQ (Tel-4G) as a skeleton. By applying tensile forces to individual DNAzymes, we evaluated their catalytic activities as a function of structural stabilities. We found that there existed an optimized catalytic activity within a certain range of forces, suggesting marginal stability plays a role in catalytic activity of the DNAzymes. When we varied stabilities of Tel-4G structures by using 8-oxoguanine (8-oxoG)-modified sequences for example, we observed that GQ structures with marginal stabilities had highest catalytic activities, a common feature demonstrated in natural enzymes. Our finding offers a novel approach to modulate DNAzyme activities by controlling the stabilities of DNA structures using various conditions such as mechanical force, temperature, and solvent conditions.

P2.STR.55 – Oxidative Lesions As Transcriptional Roadblocks In G-Rich Regions

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Many DNA-processing enzymes slide along the double helix or single DNA strands, but stable secondary structures like G-Quadruplexes can hinder their progression. We used a polymerase stop assay to examine which features of human telomeric and BCL2 promoter G-Quadruplexes stall the Klenow fragment. Primer extension profiles showed that G-quartets form strong roadblocks, while auxiliary base pairs are easily bypassed. To assess the impact of oxidative damage in G-rich regions, we introduced 8-oxoguanine to mimic such lesions. Although oxidative lesions occasionally decreased the efficiency of G-Quadruplex bypass, they generally destabilized G-Quadruplex structures, thereby facilitating enzyme progression. While this study focused on the Klenow fragment, the observed trends in enzyme bypass are likely applicable to other G-Quadruplex-forming sequences and DNA-processing enzymes that rely on a clamp-like mechanism for sliding along DNA.

P2.STR.56 – Effects of Single and Double Mutations on the MYC Promoter G-Quadruplex Using a Custom DNA Microarray

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Parallel DNA G-quadruplexes are key regulators of oncogene transcription. The prominent parallel G-quadruplex formed in the MYC promoter (MycG4) is a promising anticancer drug target. Guanine redundancy in promoter sequences is important for G-quadruplex formation and function. However, folding principles and effects of DNA damage or mutations on the conformational landscape are unknown. Permutation of G4-forming sequences creates a vast sequence space that is challenging for most experimental methods. We used a G4-DNA microarray to analyze all 2,145 single and double mutations of the MYC promoter G-quadruplex, allowing systematic analysis of the mutational effects on G-quadruplex formation and MycG4 conformational landscape. No single or even double mutation completely prevents G-quadruplex formation. Mutated MycG4 sequences can form G-quadruplexes with vacancies or bulges without replacing the damaged G-runs. Vacancies in one or even two tetrads are surprisingly tolerable. Both length and position of a G-run determine its resilience against mutations, and nearby redundant G-residues can efficiently compensate mutations. The most disruptive double mutations in non-adjacent G-runs cannot be compensated by one G-tract but may be by nearby redundant Gs. The results illustrate how mutations modulate G-quadruplex structures, highlighting their complex conformational landscape and a potential compensation mechanism involving nearby redundant G-residues.

P2.STR.57 – Double-Headed Nucleotides in G-Quadruplexes

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We have previously shown that one double-headed nucleotide can replace two consecutive nucleotides in a DNA double helix without compromising stability or overall topology. This approach condenses the genetic information in DNA, reducing molecular weight and charge density. We hypothesize that a similar strategy can be applied to G-Quadruplex (G4)-forming oligonucleotides to enhance their drug-like properties.

In this study, we investigate how incorporation of GG, a double-headed nucleotide with two guanines, affects G4 formation, topology, thermal stability, and association kinetics. Using a combination of circular dichroism, UV-based measurements, 1H-NMR, native PAGE, and fluorescence assays, together with molecular dynamics simulations, we have systematically characterized a range of oligonucleotides containing GG, covering different G4 model systems. These include tetramolecular and bimolecular oligonucleotides, derived from the Oxytricha nova telomeric sequence, along with the unimolecular thrombin binding aptamer (TBA). Our results show that certain GG-containing oligonucleotides successfully form G-Quadruplexes, with some exhibiting increased thermal stability and faster association kinetics. These findings have allowed us to establish some preliminary design principles for using GG double-headed nucleotides in G4-based applications and suggest their potential as powerful tools in development of G4-based aptamers.

P2.STR.58 – Cytosine Methylation Modulates the Stability and Folding Kinetics of the Bcl2mid G-Quadruplex

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DNA cytosine methylation is a well-characterized epigenetic modification that regulates gene expression without altering the DNA sequence and raises questions about its influence on the formation and stability of G-Quadruplex (G4) structures. We investigated how 5-methylcytosine (C^m) affects the bcl2Mid G4 structure which forms within the GC-rich region upstream of the BCL2 P1 promoter. Using NMR spectroscopy and biophysical techniques, we have observed sequence-specific effects of Cm on the folding kinetics of bcl2Mid G4. The substitution of cytosine by C^m slows down folding and shifts the equilibrium between major and minor structures in the presence of K^+ ions. While the major G4 structure adopts a 3+1 hybrid topology (D. Yang et al., *Nucleic Acids Res*. 2006, 34, 5133), the minor G4 structure features parallel strands with a snapback element that fills a G-quartet vacancy. These results show that Cm modulates G4 polymorphism, affects folding pathways and impacts thermodynamic stability, suggesting a potential role in G4-mediated regulation of *BCL2* expression. Our findings contribute to the growing understanding of how epigenetic modifications reshape the landscape of G4 structures, with implications for gene regulation and disease states.

P2.STR.59 – Influence Of Dangling Ends on a G-Quadruplex of Klebsiella Pneumoniae Structures

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In the last few years, the attention has raised on bacteria G-Quadruplexes (G4s), a secondary and noncanonical DNA and/or RNA structural motifs formed in guanine-rich sequences playing critical and pivotal biological functions in bacteria. Currently, structural and spectroscopic studies on G4s have been mainly focused on the G4-core, without considering extension of nucleotides sequences at the 5' and 3' ends, also known as dangling ends. As a result, G4s structural information landscape in their physiological environment is not properly accounted for. Indeed, bacterial G4 motifs are enclosed in longer sequences with extended single strand at 5' and 3' ends. Very few studies are reported on dangling ends in human G4s, confirming their effect on G4 structures. On the contrary, no studies have been reported on the structural effects on bacterial G4s bearing dangling ends. In this context, combining molecular dynamics simulations and spectroscopy tools we investigated structural features of two reported G4 structures of Klebsiella pneumoniae as G4-core sequence, namely with no dangling ends and known form, with four dangling nucleotides.

P2.STR.60 – Exploring the G-Quadruplex/I-Motif Co-Localization Within a B-DNA Context

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DNA can fold into variable arrangements which are expected to exploit regulatory functions. Genome sites enriched in guanines are prone to fold into G-Quadruplexes (G4) while the complementary strands fit with i-Motifs (iM) formation. Genome-wide analysis confirmed their formation although with a differential distribution along the cell-cycle stages, in accordance with a model that indicates steric hindrance as the main factor to prevent their simultaneous presence at one site. However, iM have been reported to inhibit G4 extension by telomerase, thus pointing to a functional interplay between these two structures. In this work, we started with a double-stranded construct where two lateral duplexes flank a central G/C rich region. Here, the non-canonical structures can form only when the duplex is destabilized. While in cell this physiologically occurs, for our in vitro studies we designed two not fully complementary G4 and iM forming sequences. Through an extensive biophysical characterization of different constructs, we proved that G4 and iM structures can be accommodated at the same time. A complete thermodynamic characterization of our construct unravelled the complex equilibria connected to the simultaneous folding in G4/iM of a genomic domain. Our findings shed light on an unripe issue, suggesting that the concomitant formation may play a role in fine-tuning complex biological mechanisms.

P2.STR.61 – A Systematic Analysis Of Topological Preferences of Two-Tetrad G-Quadruplexes

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Most of G-Quadruplex structure research is focused on three-layer motifs since two-quartet G4s are less stable and were long believed to be biologically irrelevant. However, more recent studies reveal that two-layer structures can play important roles in cellular processes. Therefore, there is an urgent need for more research aimed at determining the factors influencing two-tetrad G4s folding, stability, and structural variability. To systematically study the relationship between loop length and conformational preferences, we have investigated a complete set of 64 two-tetrad G4s containing thymidine–only loops of 1-4 nucleotide length ($GGT_{1\text{-}4}GGT_{1\text{-}4}GGT_{1\text{-}4}GG$). By using a combination of biophysical tools (NMR, CD, UV-Vis spectroscopies, and native PAGE) we were able to describe the conformational landscape of each studied molecule and identify those adopting a single conformation in solution. The combined use of informationprovided by several techniques also allowed us to correlate the observed trends in G4 electrophoretic mobilities with specific topologies that they assume. Importantly, we were also able to confirm the molecularity of each structure, using a combination of imino proton-to-deuteron exchange NMR experiments and gel mobility measurements. In consequence, we were able to obtain a curated subset of only unimolecular two-tetrad folds that allowed us to make more relevant assessments regarding the sequence-topology relationship in this kind of system.

P2.STR.63 – Characterizing the G4 Landscape Across Development in C. elegans

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G-Quadruplexes (G4s) are noncanonical secondary structures found in guanine-rich regions of DNA. They are enriched at promoters, transcription start sites (TSSs), and telomeres in human cells, with roles in regulation of DNA replication, gene expression, and telomere homeostasis. In silico and in vitro experiments show that motifs with G4-forming potential exist in genomes ranging from bacterial to mammalian. However, to date, no in vivo model has been used to study these structures at a multicellular organismal level. We begin to address this deficit by establishing the landscape of structured G4s across development in the nematode Caenorhabditis elegans. We report the profiles of G4s at two developmental stages – the first larval (L1) and the reproductive adult (RA) – using G4 CUT&Tag with the structure-specific antibody BG4. We identify 2,619 and 3,250 G4 peaks at the L1 and RA stages, respectively. Notably, only ~16% of G4 peaks in L1s overlap with those in RAs, compared to ~70% of L1 H3K4me3 peaks. This suggests that G4s are more dynamic between these stages than H3K4me3, a histone marker remodeled during the maternal-to-embryo transition. RAs exhibit enrichment of G4s at promoters and TSSs, while L1s are relatively depleted of G4s at these same genomic elements. These findings indicate a major shift in the G4 landscape during C. elegans development, raising the idea that G4s could have a functional role in driving progression from one developmental phase to the next.

P2.STR.64 – Custom G4 DNA Microarray Enables Broad and Unbiased Evaluation of G4-Targeted Small Molecules and Proteins

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G-quadruplexes (G4s) are biologically important globular DNA secondary structures that have emerged as promising therapeutic targets. Among them, the MycG4 functions as a transcriptional silencer and represents a compelling target for anticancer drug development. We developed a custom G4-DNA microarray platform containing over 25,000 oncogenic G4 sequences and mutants, enabling systematic, high-throughput, and unbiased analysis of binding affinity and selectivity for G4-targeting ligands, proteins, and antibodies. Using this platform, we assessed the binding properties of several G4-binding proteins, including nucleolin, FANCJ, PIF1, BLM, DHX36, WRN, IGF2, and CNBP, as well as the G4-specific antibody BG4. Notably, we identified specific sequences with high-affinity binding to nucleolin, which facilitates mechanistic and structural studies. Importantly, the microarray enabled quantitative assessment of small molecules targeting the MycG4, including BMVC—the first fluorescent probe developed to detect G4 structures in cells. Although initially designed for telomeric G4s, our results show that BMVC preferentially binds parallel G4s, especially MycG4. Furthermore, we identified a strong sequence selectivity of BMVC for a 3' flanking thymine, consistent with our NMR binding data. In summary, our custom G4-DNA microarray provides a powerful platform for large-scale, unbiased evaluation of G4-binding molecules and proteins, advancing drug discovery and mechanistic insights into G4 protein recognition.

P2.STR.65 – Exploring How Fluorescent Luminarosine Derivatives Interact With G-Quadruplex DNA - Fluorescence, CD, and NMR Studies

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Guanosine-rich sequences have the remarkable ability to form non-canonical DNA structures known as G-Quadruplexes (G4s). The formation of G4s can occur in various regions of the genome, including telomeres, oncogene promoters, and untranslated regions, making them highly relevant to biological processes and disease mechanisms. Many studies have focused on identifying and designing small-molecule ligands capable of interacting with G4s. These ligands can induce, stabilize, or disrupt G4 formation, offering promising strategies for targeted therapeutic interventions, especially in oncology. A significant portion of recent studies has been dedicated to improving the selectivity of ligands for specific G4targets. For example, tricyclic 9-methoxyluminarine (9-MeLM) can serve as a selective turn-off ligand for parallel G4 DNA. The fluorescence quenching is caused by a π-π interaction between the ligand and the exposed guanine tetrad of the parallel G4 structure. Herein, we decided to examine the interaction of the nucleoside of 9-MeLM, namely 2′,3′,5′-tri-O-acetyl-9-methoxyluminarosine chloride, as well as its parent derivative, 2′,3′,5′-tri-O-acetylluminarosine, with G4s formed by sequences corresponding to human telomeric DNA and the human proto-oncogene c-MYC. To gain a deeper understanding of these interactions, we conducted fluorescence, circular dichroism, and nuclear magnetic resonance spectroscopy measurements.

P2.STR.66 – Structural Recognition of the MYC Promoter G-Quadruplex by the Quinoline Derivative PEQ: Insights into Molecular Targeting of Parallel G-Quadruplexes

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The G-quadruplex (MycG4) formed in the oncogene MYC promoter acts as a transcriptional repressor and is a promising anti-cancer therapeutics target. Through screening of an NCI molecule library, we identified PEQ as a potent small molecule MycG4 binder. Different from most G-quadruplex ligands, PEQ has a drug-like flexible structure consisting of two aromatic rings connected via an ethenyl linker. Herein, we determined the high-resolution structures of PEQ-MycG4 2:1 complex using NMR spectroscopy. PEQ rearranges 5′ and 3′ flanking residues to form a specific binding pocket. To further understand small molecule binding to MycG4, we performed systematic analysis of all reported MycG4-small molecule complex structures and discovered a conserved recognition mode. In all reported MycG4-small molecule complexes, flexible flanking residues are recruited to form ligand-base plane covering external tetrads, and their position and orientation are conserved across different ligand scaffolds, suggesting a sequence-specific binding pocket for selective small molecule bindings. Additionally, we determined the solution structure of PEQ in complex with MycG4 bearing wild-type 3′ flanking. A comparison of the two MycG4-PEQ complex structures (MycG4 with mutant or wt 3′-flanking) clearly shows the effect of the 3′-mutation on the flanking residue-ligand interface. Overall, our results establish a general concept of MycG4 recognition by small molecules for future rational design of drugs targeting MycG4.

P2.STR.67 – Structural Investigation of Intramolecular Left-Handed G-Quadruplexes and their Interactions with G4 Ligands

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Left-handed G-Quadruplexes (G4) are a non-canonical DNA structure discovered in 2015, distinguished from their right-handed counterparts in their helical twist. Formation of left-handed (LH) G4s has been found to be highly sequence dependent, being limited to variations of two minimal sequences, Motif1 ($G(TGG)_3TG$) and Motif2 ($((GGT)_3GTG$). All reported LHG4s form a distinct four-layered, two-subunit structure and display thymine capping, where T-loops fold over the outer G-tetrads. A prior study suggests that the presence of thymine caps prevents G4 ligands from directly binding LHG4s, but research on the subject is still sparse. We used biophysical methods (UV-vis spectroscopy, circular dichroism) and X-ray crystallography to characterize the interaction of four sequences designed from the LH minimal motifs (M1M1, M1M2, M2M2, and M2M1) with the G4-binding ligand N-methylmesoporphyrin IX (NMM), known to select for parallel G4s. The sequences display differing interactions with NMM, with biophysical data indicating that M2M2 refolds into an RH conformation while the other sequences refold into LH/RH hybrid. M1M2 displays a 2 DNA: 1 NMM binding stoichiometry, while all other sequences display a 1:1 stoichiometry. All sequences display weak binding constants of 0.3-1.2 μM-1 with NMM. Two crystal structures of M1M2 suggest the existence of LH and LH/RH hybrid conformations, confirming the possibility of conversion through conformational selection. The structure of M2M1 is fully LH.

P2.STR.68 – Decoding the KRAS G-Quadruplex: Insights into Structural Dynamics and Ligand Identification

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Cancer remains a leading cause of mortality, with mutations in the KRAS signaling protein driving many aggressive malignancies. Due to its high affinity for GTP/GDP, the lack of well-defined druggable pockets, and frequent mutations, targeting the KRAS protein is challenging. An alternative approach is targeting G-Quadruplexes (GQs) in the KRAS promoter, which regulate gene expression. Two distinct GQ conformations have been resolved from 32- and 22-nucleotide (nt) sequences in the KRAS promoter, with the 22-nt structure forming only in the presence of specific ligands. Thus, stabilizing the KRAS promoter GQs with small molecules may decrease KRAS expression and would not depend on mutations in the coding sequence. To characterize the KRAS GQ structural ensemble, we conducted Drude polarizable molecular dynamics (MD) simulations on wild-type and experimentally mutated 22-nt GQs. Our results revealed dynamic loop sampling and transient base stacking that was not observed in previous simulations. Clustering analysis identified dominant loop subpopulations as potential druggable motifs. We further performed Site Identification by Ligand Competitive Saturation (SILCS) simulations, generating solute occupancy maps to identify features of small molecules that may preferentially bind the KRAS GQ. Our findings provide atomistic insights into KRAS GQ dynamics and establish a framework for rational ligand design for KRAS-driven cancers.

P2.TAR.69 – Two Original Strategies Based on Cyclic RGD to Deliver G4 Ligands for Selective Targeting Tumoral Cells

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G-Quadruplexes are compelling anticancer targets due to their involvement in regulating key biological processes. To enhance the effectiveness of G4-targeted therapies, we developed two new approaches to deliver G4-ligands to cancer cells selectively exploiting the overexpression of αvβ3/αvβ5 integrins. Both strategies exploit the cyclic peptide Arg-Gly-Asp (cRGD) for targeted cell adherence to integrins, enhancing selective ligand internalization. As G4-ligands, we used naphthalene diimides (NDIs), known for high G4-stabilizing ability and inherent toxicity. cRGD has been conjugated via click chemistry to: i) silk fibroin nanoparticles encapsulating a tetrasubstituted NDI (cRGD-SFNs-NDI) and ii) a nearly identical tetrasubstituted NDI (cRGD-NDI). In vitro studies show encouraging results: while NDI alone displayed broad high toxicity, the new conjugates showed targeted activity based on αvβ3/αvβ5 overexpression. NDI-encapsulation within cRGD-SFNs significantly reduced off-target cytotoxicity, directing the therapeutic payload to U373 and U251 glioblastoma cell lines, overexpressing αvβ3/αvβ5 integrins. Similarly, cRGD-NDI treatment markedly decreased cell viability in these cell lines, while exhibiting minimal toxicity towards D384 cells, which lack αvβ3/αvβ5 integrins, even after 72 h. These findings confirm the potential of cRGD-mediated, site-specific drug delivery for enhancing selectivity in anticancer therapies.

P2.TAR.70 – Interactions of Simple BODIPY Dyes with Various DNA G-Quadruplexes

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A class of compound with a skeleton origin of 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) is a vital and diverse group of dyes, which hence to their attractive optical properties have found the application in sensing, bioimaging, bio-labeling, photodynamic therapy or in drug delivery systems. Several reports have been made on BODIPY derivatives ability to recognize and bind with DNA G-Quadruplexes (G4). Recently we have synthesized a series of simple derivatives, bearing modified or unmodified phenyl ring substituted at the meso position of BODIPY core, aiming to elucidate the potential of their utilization as GQuadruplex ligands. However the emissive properties of studied compounds were found out to be highly-dependent on the experimental condition (mainly due to their tendency for aggregates formation), we managed to select two derivatives showing clear preference towards DNA GQuadruplexes of different topologies, over single or double stranded DNA. Results of spectroscopic investigations (UV-vis absorbance, fluorescence and circular dichroism spectroscopy) will be presented to elucidate the mode of observed interactions, together with the thermodynamic analysis of melting profiles and the results of G4/hemin peroxidase inhibition assay. The evaluation of tested compounds cytotoxicity has also been made and the results obtained from the MTT assays will be presented.

P2.TAR.71 – Accessibility of Telomeric Overhangs to Stabilizing Small Molecules

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Human chromosomes terminate in telomeres, which contain a 50–300 nt single-stranded overhangs composed of GGGTTA repeating sequences. These sequences can fold into G-Quadruplexes (GQs), protective structures that regulate telomerase activity. While GQs stabilize telomeres, intervening unfolded regions remain accessible to various agents, influencing telomere maintenance and stability. Extensive efforts have been made to develop GQ-stabilizing small molecules to inhibit telomerase activity. However, it remains unclear how the length of telomeric overhangs, particularly in the physiologically relevant range, impacts the accessibility to such molecules. For example, binding of one or a few molecules might compact longer overhangs, reducing further access to additional molecules. Alternatively, the individual GQs might remain independent, regardless of overhang length, allowing continuous small molecule binding until saturation. This study investigated the binding of a fluorescently labeled small molecule, Cy5-labeled oxazole telomestatin derivative (Cy5-7OTD), to telomeric sequences of varying lengths using single-molecule fluorescence microscopy. Photobleaching step analysis was used to quantify the distribution of the number of bound small molecules to telomeric constructs containing 1–7 GQs (up to ~160 nt long overhang). These findings enhance our understanding of how telomere length affects small molecule binding, offering new insights into telomere stability and regulation.

P2.TAR.72 – Discovery Of Small Molecules to Target DDX5 Unfolding of MYC Promoter G-Quadruplex For MYC Inhibition

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MYC is a highly deregulated oncogene in human cancers. Direct targeting of MYC protein has proven to be challenging due to its disordered structure. Importantly, the G-Quadruplex formed in the proximal promoter of the MYC gene (MycG4) acts as a transcriptional silencer. Our lab has previously discovered the DDX5 helicase actively unfolds MycG4 and transactivates MYC in human cancer cells. Therefore, the DDX5-MycG4 interaction and unfolding presents a promising target for effective MYC downregulation. However, no inhibitors are available that directly bind DDX5 and specifically inhibit its G4 unfolding activity. Here, we report our progress to identify specific inhibitors of the DDX5-MycG4 interaction for MYC downregulation. We adapted a FRET-based G4 unfolding assay for high-throughput screening of ~20,000 diverse drug-like compounds. Compounds that reduced DDX5 unfolding by more than 40% were defined as a hit, resulting in 31 initial hits. Notably, we identified five different classes of hit compound chemical structures. Several hits are substantially structurally deviating from classic G4 interacting compounds, suggesting a different mechanism of action for inhibition of the DDX5 protein-DNA interaction. We conducted hit validation and tested the identified compounds for DDX5 G4 and RNA unfolding. In the future, we want to further characterize our identified lead compounds and optimize them for improved MYC downregulation as future anti-cancer therapeutics.