Quadruplexes Made in France

29 novembre 2024, Amphi Becquerel, Ecole Polytechnique

Program

8h30 *Registration* Amphi Becquerel

8h45 Opening remarks

J. L. Mergny (LOB, Ecole Polytechnique / E4H ; Palaiseau) *Quadruplexes are everywhere in France...*

9h00 Session 1: *3 talks (15 min + 5min questions)*

- J. Dejeu (FEMTO-ST, Besançon) & J. Boissieras (Institut Curie, Orsay), *Non selectivity of I-mab for I-motif demonstrated by Bio-Layer Interferometry and Circular dichroism.*
- M. Ranz (ARNA, Université de Bordeaux), *Binding modes and structural dynamics of G4/ligands complexes probed by Hydrogen-Deuterium eXchange coupled to native Mass Spectrometry (HDX/MS).*
- A. Froux (CRAN, Université de Lorraine, Nancy), *New Schiff Base Metal Complexes for G-quadruplex binding: from synthesis to anticancer applications.*

10h05 Photo & Coffee break: poster session

11h00 Session 2: *4 talks (15 min + 5min questions)*

- L. Mouawad (Institut Curie, Orsay), *ASC-G4: a website to calculate the Advanced Structural Characteristics of G4.*
- A. Rainot (ITODYS, Paris Cité & STEBICEF, Université de Palerme), *In Silico study: Detection of Guanine Quadruplexes via Surface Gated Graphene Transistors.*
- V. Balanikas (LOB, Ecole Polytechnique, Palaiseau), *Spectroscopic study of low-energy DNA photoionization using nanosecond transient absorption.*
- R. Veitia (Oncologie moléculaire et pathologies ovariennes, Institut Jacques Monot, Paris Cité), *The forkhead DNA-binding domain binds specific G2-rich RNA sequences.*

12h20 Lunch Break: Salon d'Honneur & Coffee break: Poster session

13h40 Session 3: *4 talks (15 min + 5min questions)*

- A. Cammas (Cancer Research Center, Université de Toulouse), *RG4s couple translation to therapy-induced autophagy.*
- A. Peynet (GGB, Faculté de Médecine et des Sciences pour la Santé, Brest), *Role of non-canonical secondary structures of RNA in the alternative splicing of apoptosis regulatory genes and use as intervention points for cancers resistant to chemotherapies.*
- J. C. Andrau (IGMM, Montpellier), *G-quadruplexes are promoter elements involved in nucleosome exclusion and transcriptional pause release*
- L. Ferret (Centre de recherche des Cordeliers, Paris), *Involvement of lysosomes in cancer resistance to transcription inhibitors.*

15h00 Coffee break: Poster session

15h30 Session 4*: 4 talks (15 min + 5min questions)*

- G. Giraud (CRCL, Lyon), *G-quadruplexes control hepatitis B virus replication by promoting cccDNA transcription and phase separation in hepatocytes.*
- K. Mosca (LLB, CEA Saclay), *A Novel Workflow for Classifying Nucleic Acid Circular Dichroism Spectra.*
- F. Krikava (Biophysics of nucleic acids, Institute of Biophysics of the Czech Academy of Sciences, Brno), *Imperfect G-quadruplexes: Structural and thermodynamic studies on dGMP alterations in sequence and environment.*
- N. Da Rocha (Institut Pasteur, Paris), *Biochemical and Functional Characterization of SARS-CoV-2 Unique Domain (SUD) in NSP3 / RNA G4 / Protein Complexes and Therapeutic Properties of G4-Ligands Inhibiting their Formation.*

16h50 Closing remarks

P. Changenet (LOB, Ecole Polytechnique, Palaiseau)

Abstracts
Oral presentations

Binding modes and structural dynamics of G4/ligands complexes probed by Hydrogen-Deuterium eXchange coupled to native Mass Spectrometry (HDX/MS)

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Abstract:

The binding of small molecules onto G-quadruplexes (G4) structures mostly consists in π-stacking interactions between an external G-tetrad and the aromatic core of the ligand (1). However some ligands can interact differently (e.g. by intercalating between two tetrads (2)) and can shift conformational ensemble equilibria, including towards unstructured forms.

Native mass spectrometry allows probing G4, cation and ligand interactions as it gives direct information on the binding affinity and stoichiometry (3–5). Going further, cation stoichiometry can inform on conformational change (6).

Recently, our lab developed Hydrogen-deuterium exchange coupled to native mass spectrometry (HDX/MS) technique to the study of structured oligonucleotides, including G4s (7). The H/D exchange rates of G4s inform on their structures and isothermal stability, and further analysis provide additional information on their dynamics, notably their unfolding rates (8, 9).

We found that different binding modes lead to different exchange patterns: external stacking further protects the external guanines, which exchange normally within the first few minutes, while intercalation via unfolding/refolding of the oligo leads to a significant protection of the whole structure over a large exchange time range.

Subsequently, we used this knowledge to probe different combinations of G4/ligands to gain insights on the ligands binding modes and dynamics, and found potential candidates to intercalation binding. Additionally, some results do not fit in any of the two categories mentioned before, suggesting potential non-canonical binding modes or different refolding pathways than the ones already observed.

HDX/MS offers a promising complement to structural elucidation techniques like NMR, giving access to different species and stoichiometries simultaneously, with samples at low DNA and ligands concentrations and in a high-throughput way. Future developments of the technique could allow to determine the binding sites of ligands onto G4 structures or even higher order complexes as G4/peptides or even G4/proteins interactions.

References:

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NEW SCHIFF BASE METAL COMPLEXES FOR G-QUADRUPLEX BINDING: FROM SYNTHESIS TO ANTICANCER APPLICATIONS

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Abstract:

G-rich sequences within nucleic acids can adopt non-conventional secondary structures, known as G-quadruplexes (G4). These motifs are notably enriched at critical genomic regions, such as gene promoters, highlighting their essential role in regulating many cellular processes. The identification of G4 in the promoters of numerous oncogenes positions them as promising therapeutic targets in cancer treatment. In this context, we have employed a transdisciplinary approach to selectively bind and stabilize G4 in cancer cells, with the goal of identifying novel stabilizers to control cancer progression.

In this multidisciplinary study, we synthesized 12 novel transition metal complexes, based on the Salphen scaffold, incorporating Zn2+, Ni2+, Cu2+, Pd2+ and Pt2+ coordination. The DNA-binding properties of these complexes were assessed in solution using various spectroscopy techniques, complemented by molecular dynamic simulations. Their anticancer activity was investigated in pancreatic and breast cancer cell lines (T3M4, MDA-MB-231 and T47D), by evaluating their effects on cell proliferation, viability, and gene expression modulation.

In-solution studies of the 12 newly synthesized complexes demonstrated their ability to bind and stabilize G4 structures, with a notable preference for G4 structures over double-stranded DNA. Molecular dynamic simulations confirmed stable interactions between the complexes and the *kRAS* G4, revealing an unconventional binding mode, where some molecules interact with the G4 loop. In cancer cells, immunofluorescence assays confirmed the ligands' ability to increase G4 formation, suggesting stabilization of these structures in a cellular environment. Additionally, our compounds were shown to early downregulate the expression of several G4-driven oncogenes, likely due to G4 stabilization. Based on these promising findings, we evaluated the effects of the compounds on cell proliferation and viability in cancer cell lines, and interestingly showed those molecules can significantly impair such cellular parameters.

In this study, we introduced a large library of novel molecules capable of specifically stabilizing G4s, demonstrating promising anticancer properties. Future research will aim to deepen our understanding of the roles of G4s in cancerous and tumoral environments, potentially advancing therapeutic strategies in cancerology.

ASC-G4: a website to calculate the Advanced Structural Characteristics of G4

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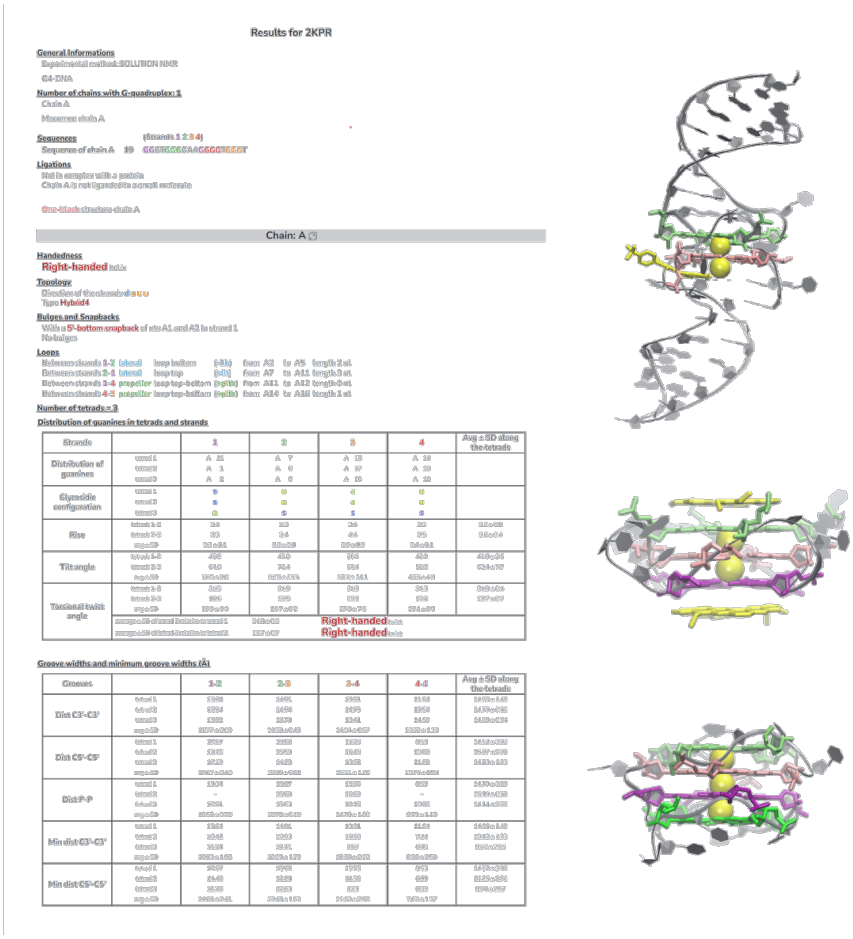
Abstract:

Intramolecular G-quadruplex folds are far more complex than presented for the canonical structures and there are some hesitations to qualify them in the literature. Hence the need for clear and objective rules to describe all G4s. This is what we have done when we created ASC-G4 (http://tiny.cc/ASC-G4), a website where one uploads the G4 structure and obtains all its characteristics: dimerization, ligation, handedness, strand directions, topology, bulges, snapbacks, loops, nucleotide identification in each tetrad and strand, glycosidic configuration, rise between tetrads, tilt and twist angles, groove widths based on C3', C5' and P atoms, minimum groove widths based on C3' and C5' atoms, all the main chain and sugar torsion angles, and the sugar pseudo rotation angles. Here, I will present the website and clarify the most important concepts. Indeed, there are eight distinguishable topologies, not five, there are four types of snapbacks, not only one, the experimental ranges to define the *syn* and *anti* glycosidic configurations are different from those of the IUPAC-IUB…



In Silico study: Detection of Guanine Quadruplexes via Surface Gated Graphene Transistors

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Abstract:

The ability to quantitatively detect guanine quadruplexes (G4s) is important, as they are present in viral genomes (1) and in the promoters of oncogenes (2). Quickly and accurately identifying G4s would be beneficial during a viral epidemic or in the context of cancer pre-diagnosis. Thus, having dependable and quantitative sensors is vital for various applications. In this proof of concept, we present an *in silico* model for a potential universal electrochemical sensor designed aimed at detecting G4s.

Our approach involves the development of a device based on a Solution-Gated Graphene Transistor (SGGT). By applying an electrical potential to the graphene electrode, we can monitor changes in current that result from interactions with the analyte (3, 4).

The sensitivity of the graphene surface can be enhanced through modification to target specific molecules, such as G4s. Using molecular dynamics simulation, we performed free energy profile analyses of G4 desorption and several other relevant molecules to determine the best pairing for effective anchoring and selectivity. Next, we simulated how these sensitizers interact with G4s. The promising results led us to calculate ionic density profiles to determine the detectability of the surface modifications and G4 capture.

References:

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Spectroscopic study of low-energy DNA photoionization using nanosecond transient absorption

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Abstract:

DNA photoionization typically occurs when exposed to UV radiation, particularly at wavelengths shorter than 200 nm. However, in the past two decades, it has been discovered that this phenomenon extends into the UVB range (>200 nm), although with smaller quantum yields (ϕ). Using time-resolved spectroscopy, we investigated the effects of UV-induced photoionization in both synthetic and genomic DNA in aqueous solution, with a focus on guanine-rich G-quadruplex structures. These exhibit significantly higher quantum yields (3.5×10^{-3} to 15×10^{-3}) compared to double-stranded DNA (2×10^{-3}), with the photoionization efficiency being highly sensitive to structural characteristics, including the type of metal cations and the arrangement of peripheral guanine bases.

Our findings show that guanine radical cations (G^{+}•) generated within G-quadruplexes undergo distinct deprotonation processes. In duplex DNA, more than 95% of G^{+}• radicals rapidly deprotonate to form the oxidative marker 8-oxo-7,8-dihydroguanine. By contrast, in G-quadruplexes, deprotonation occurs in two phases: a fast initial step followed by a slower process. The resulting radical species, (G-H2)•, differ from the (G-H1)• radicals typically observed in duplex DNA. This distinction highlights a unique pathway for oxidative damage in G-quadruplexes, which may have broader implications for understanding the mechanisms of DNA damage and repair, particularly in guanine-rich regions of the genome.

While focused on DNA damage, these findings could also be useful in biosensor technologies by utilizing the unique charge separation dynamics of G-quadruplexes. Time-resolved spectroscopy helps to characterize these dynamics and may contribute to future developments in photoconductivity and sensing devices. Further research into the final lesions formed by these radicals could improve our understanding of their biological significance, particularly in relation to genomic stability and cellular responses to oxidative stress.

References:

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The forkhead DNA-binding domain binds specific G2-rich RNA sequences

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Abstract:

Transcription factors contain a DNA-binding domain ensuring specific recognition of DNA target sequences. The family of forkhead (FOX) transcription factors is composed of dozens of paralogs in mammals. The forkhead domain (FHD) is a segment of about 100 amino acids that binds an A-rich DNA sequence. We have recently reported a previously unnoticed feature of FOXL2, a member of the FOX family. Using RNA SELEX (Systematic Evolution of Ligands by Exponential Enrichment), we were able to show that FOXL2 exhibits a strong affinity for G2-rich RNA sequences, predicted to fold into G-quadruplex (rG4) structures, while showing minimal or no binding affinity to similar G-rich motifs when they occur in DNA. This selective binding to RNA rather than DNA G4 sequences introduces a new layer to FOXL2's functionality, indicating that it may have specific roles in RNA regulation in addition to its classical role as a transcription factor.

By analyzing FOXL2-regulated genes, we observed that a statistically significant subset of these genes included G2-rich motifs in their untranslated regions (UTRs). This enrichment suggests that FOXL2 could play a role in post-transcriptional regulation. Such genes are involved in crucial signaling pathways and cellular processes, such as apoptosis regulation and the MAPK signaling cascade, indicating that FOXL2's interaction with G-rich RNA structures might have regulatory consequences for these pathways. We also observed that other FHD-containing transcription factors, such as FOXA1 and FOXO3a, as well as chimeric FOXL2 proteins containing these forkhead domains, could also bind some of FOXL2's preferred G-rich RNA sequences. This shared RNA-binding feature across different FOX family members points to an unexpected functional versatility of the FHD, suggesting that RNA binding may be a broader characteristic of the this domain.

We hypothesize that FOXL2 and other FHD-containing proteins may transition between binding DNA and RNA, depending on the molecular environment or cellular context. This implies that FOX factors might function not only as a conventional transcription factors but also as mediators in RNA metabolism. This functional plasticity could position FOX factors as active participants in processes such as RNA maturation or stability.

Our results opens up new directions for understanding the multifunctionality of FOX proteins. The implications for both normal physiology and disease are substantial, as pathogenic variants in FOX genes are associated with many different phenotypes including developmental diseases and cancer. Further investigation into the broader RNA-binding capabilities of the FHD, including structural studies to determine how FOX factors interacts with G4 RNA in vivo and the potential consequences for gene expression regulation is still required.

RG4s couple translation to therapy-induced autophagy

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Abstract:

Therapy-induced reprogramming of protein synthesis shapes cell stress processes involved in cancer development and treatment, yet the underlying molecular mechanisms remain to be fully characterized. Here, we addressed the underinvestigated link between mRNA translation and autophagy by focusing on RNA G-quadruplexes (RG4s), non-canonical RNA structures driving translational plasticity. Our results identified RG4s bound to the RNA helicase DDX3X, together with PRPF8 and EFTUD2, two novel translational regulators and RG4 binders, as critical regulators of autophagy mRNA translation, protein expression and flux. Chemotherapy-induced increased recruitement of DDX3X, PRPF8 and EFTUD2 on RG4, allow RG4 unwinding, thus facilitating protein synthesis of autophagy factors. This mechanism, which rescues autophagy proteins from therapy-induced global translational inhibition, leads to an increase in prosurvival autophagy that underscores chemotherapy resistance in glioblastoma (GBM). RG4 stabilization sensitizes GBM cells to chemotherapy, indicating that RG4-dependent mechanisms confer new vulnerabilities that can be harnessed for therapeutic purposes.

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

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Abstract:

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

Références:

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2- A. Tyson-Capper, H. Gautrey. *RNA Biology*, **2018**

G-quadruplexes control hepatitis B virus replication by promoting cccDNA transcription and phase separation in hepatocytes

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Abstract :

Phase separation regulates fundamental processes in gene expression and is mediated by the local concentration of proteins and nucleic acids, as well as nucleic acid secondary structures such as G-quadruplexes (G4s). These structures play fundamental roles in both host gene expression and in viral replication due to their peculiar localisation in regulatory sequences. Hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) is an episomal minichromosome whose persistence is at the basis of chronic infection. Identifying the mechanisms controlling its transcriptional activity is indispensable to develop new therapeutic strategies against chronic hepatitis B. The aim of this study was to determine whether G4s are formed in cccDNA and regulate viral replication. Combining biochemistry and functional studies, we demonstrate that cccDNA indeed contains ten G4s structures. Furthermore, mutations disrupting two G4s located in the enhancer I HBV regulatory region altered cccDNA transcription and viral replication. Finally, we showed for the first time that cccDNA undergoes phase separation in a G4-dependent manner to promote its transcription. Altogether, our data give new insight in the transcriptional regulation of the HBV minichromosome that might pave the way for the identification of novel targets to destabilize or silence cccDNA.

Imperfect G-quadruplexes: Structural and thermodynamic studies on dGMP alterations in sequence and environment

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Abstract:

G-quadruplexes (G4s) are non-canonical DNA structures formed by guanine-rich sequences. G4s have significant roles in regulation of genes expression or genome stability. While ideal sequences capable of G4 formation, such as those with stretches of three guanines, preferentially with short thymine loops in between, are often studied, the reality is that most G4 sequences found in living systems are imperfect. In this work, I focus on imperfect G-quadruplexes, specifically those with deletions of guanosine monophosphate (GMP), to understand how such alterations affect their stability and structure.

Sequence AATGGGTGGGTTTGGGTGGGTAA was used as a model, to which several modifications involving GMP deletions at selected positions were introduced. Biophysical techniques, including Circular Dichroism (CD) and UV melting, were employed to characterize the structural and thermal properties of these imperfect G4s. These techniques allowed an in-depth analysis of the folding patterns and melting transitions, providing insights into the stability of these structures under various conditions. Even the deficiency of a single GMP significantly destabilizes the G4, which might not be very surprising, but also alters the G4 conformation with strong position dependency.

Additionally, preliminary kinetic experiments using stopped-flow coupled with CD to observe the folding dynamics of the modified sequences were performed. While these results are still under evaluation, they could provide further insight into the folding mechanisms of G4s with GMP deletions.

This work expands our understanding of how non-ideal sequences form G-quadruplexes, which are commonly found in biologically relevant contexts.

Posters

1. A C Ajello (CRCT, Toulouse), Mechanisms of DNA double-strand break induction by G4 ligands during transcription
2. M. Blondel (GGB, Faculté de Médecine, Brest), Role of non-canonical secondary structures of RNA in the alternative splicing of apoptosis regulatory genes and use as intervention points for cancers resistant to chemotherapies
3. Y. Chen (LOB, Ecole Polytechnique, Palaiseau), SubG4s: A Novel Class of Bulge-Rich G-Quadruplexes
4. A. Cucchiarini (LOB, Ecole Polytechnique, Palaiseau), Reevaluation of quadruplex propensity of Aptamer
5. A. Ganot (MFP, Université de Bordeaux), Role of G quadruplexes (G4s) in HIV-1 expression and impact on viral latency
6. J. Jiang (Laboratoire de Chimie Organique et Photochimie, Université libre de Bruxelles), Exploring the Interactions between Mononuclear Ru(II) Complexes with π-Extended Ligand Isomers and G-Quadruplexes
7. J. Lacoste (LBD, Paris Sorbonne Université), A first glimpse of G-quadruplexes in early Drosophila melanogaster development
8. S. Louvet (LBD, Paris Sorbonne Université), A first glimpse of G-quadruplexes in early Drosophila melanogaster development
9. J. Mitteaux (ICMUB, Université de Bourgogne, Dijon), Small molecule-based regulation of gene expression in human astrocytes switching on and off the G-quadruplex control systems
10. D. Ordanoska (MOBIDIC, Université de Rennes), G4 structures in human immunoglobulin gamma genes and their mirror images
11. Z. Othman (ARNA, Université de Bordeaux)
12. S. Panda, (LOB, Ecole Polytechnique, Palaiseau) Remodeling Ca2+ dynamics by targeting a promising E-box containing G-quadruplex at ORAI1 promoter in triple-negative breast cancer.
13. Anne Petitjean, (Queens University, Canada), A versatile Coordination Platform for G4 targeting
14. G. Psalmon (ICMUB, U. Bourgogne, Dijon), How nucleic acid secondary structures can be used to treat pancreatic cancer
15. S. Raeven (ICMUB, U. Bourgogne, Dijon), Synthesis and characterisation of multivalent ligands for the study of DNA and RNA G-quadruplexes
16. M. Ranz (ARNA, Université de Bordeaux), Hydrogen-Deuterium Exchange coupled to Native Mass Spectrometry (HDX/MS) to study telomeric G-quadruplex DNA structures
17. M. Trincas. (I2BM, Université Grenoble Alpes), Discovery of novel G4 binding peptidic derivatives identified via DNA encoded combinatorial libraries (DEL)
18. J. Wang (LOB, Ecole Polytechnique, Palaiseau)
19. D. Wikar (DV01, Institut Pasteur, Paris), Regulation of HIV-1 transcription by guanine quadruplexes (G4) folded in the viral promoter. Identification of new cellular proteins partners of these G4s and therapeutic potential of the identified G4/protein complexes