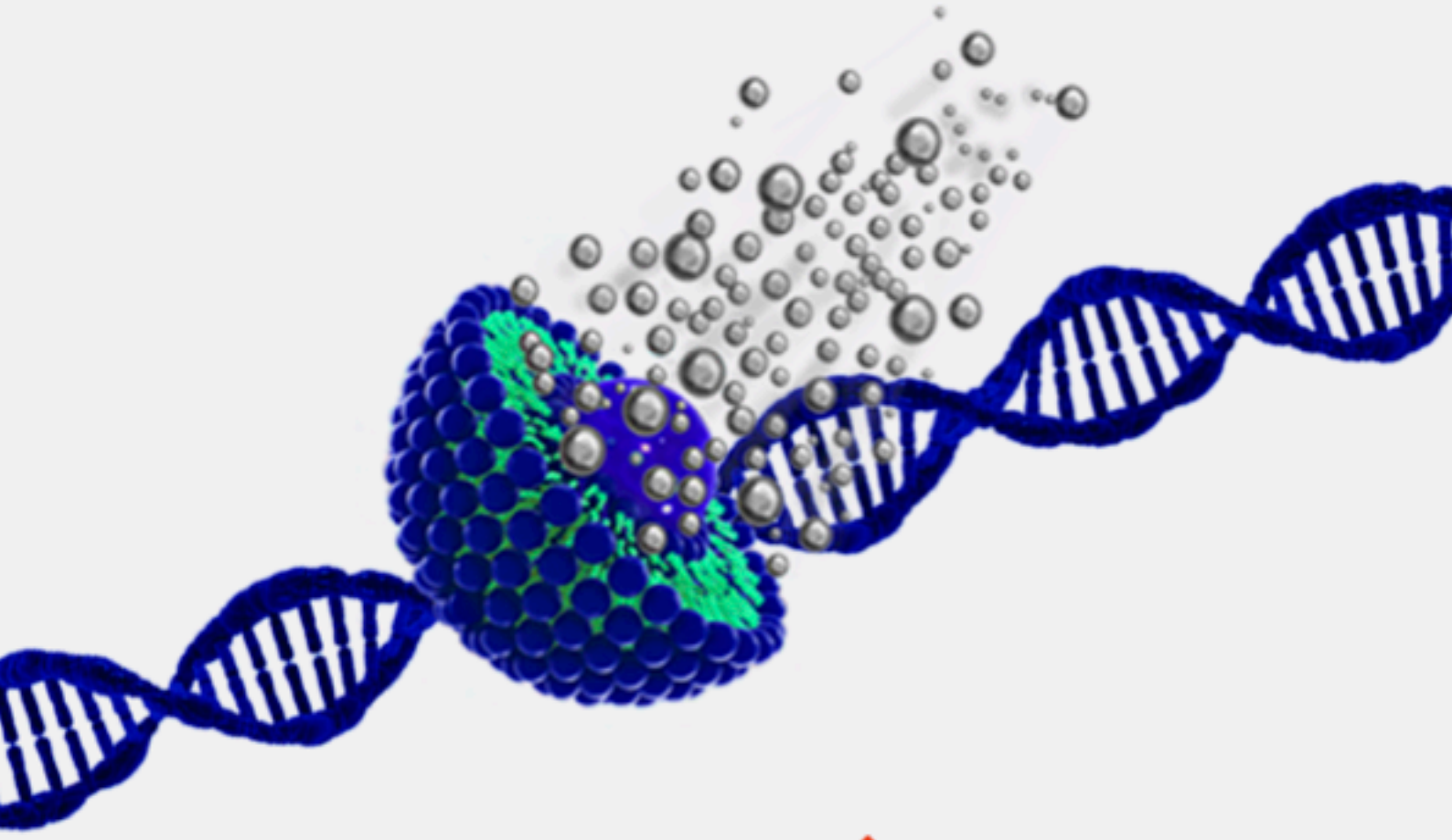
SUPRABIO 2024

International Symposium on SupraBiomolecular Systems

BOOK OF ABSTARCTS

Bordeaux, France

April 15- 17 2024



PremC

premc.org

Table of Contents

Exploring the structure and recognition of G-quadruplex in the B-MYB proto-oncogene promoter 1
Dr. Carla Cruz

Molecular architectures and druggable space of nucleic acid targets 2
Prof. Srivatsan Seergazhi Gopalan

Singlet oxygen as a tool for suprabiomolecular ligations 3
Prof. Annemieke Madder

Damage, repair and degradation of mitochondrial nucleic acids 4
Dr. Carlo Vascotto

Modified Self-amplifying RNA is the Next RNA Therapeutic 5
Prof. Mark Grinstaff

Supramolecular Mechanochemistry for the Activation of Drugs, Proteins and Genes 6
Prof. Andreas Herrmann

Permeation studies on new nanovesicles for drug delivery with multiphoton microscopy 7
Mrs. Ilaria Ferraboschi, Mrs. Marta Alcaina-Hernando, Dr. Nora Ventosa, Dr. Alba Cordoba, Prof. Silvia Pescina, Prof. Cristina Sissa

Targeting the IRES structure of mRNA for modulating gene translation 9
Mr. Albert Ferriol Monjo, Dr. Eugen Stulz, Dr. Tilman Sanchez-Elsner, Ms. Tina-Thien Ho, Dr. Gabriela Barbeta, Dr. Elena Vladimirovna

Targeting mucus hyperproduction in COPD using siRNA-loaded switchable lipid nanoparticles to silence SPDEF 11
Ms. Aurore GUEDIN-BEAUREPAIRE, Dr. Thais Pivetta, Mr. Kim-Nghi Hoang, Ms. Eloïse LATOUILLE, Dr. Isabelle Dupin, Mrs. Elise Maurat, Dr. Jeanne Leblond Chain

Porphyrin Stabilised G-Quadruplexes 12
Dr. Chloe Howells, Dr. Daniel Singleton, Dr. Teresa Lauria, Dr. Eugen Stulz

VITAMIN B12- A DRUG DELIVERY VEHICLE INTO THE CELLS 13
Ms. Monica Fresta, Prof. Dorota Gryko

Using liposomes as nanoreactors with DNA-mediated fusion 15
Dr. Nikolaj Risgaard, Prof. Stefan Vogel, Dr. Xinwei Tian, Dr. Philipp Löffler

From a synthetic methodology towards a bioconjugation tool: 5HP2Os as next-generation maleimide alternatives 16
Mr. Jan H. Meffert, Prof. Annemieke Madder

Amphiphilic Dynamic Covalent Polymer Vectors for siRNA Delivery 17
Mr. José García Coll, Dr. Nadir Bettache, Dr. Sébastien Ulrich

Hybrid Peptide-Agarose Hydrogels for 3D Bioassays 18
Dr. Greta Bergamaschi, Dr. Alessandro Gori, Mr. Roberto Frigerio, Dr. Andrea Pizzi, Mr. Angelo Musicò, Dr. Marina Cretich

Biomimetic (multi)compartmental structures and functions through self-assembly, clustering and reorganisation processes 19
Dr. Dietmar Appelhans

Dynamic Chiral Silver Nanoclusters Stabilized by DNA 20
Dr. Manuel Nuñez-Martinez, Dr. Krishnadas Kumaranchira Ramankutty, Dr. Ivan Rivilla, Dr. Amaia Larumbe, Dr. Javier Calvo, Prof. Luis Liz-Marzán, Prof. Aitziber L. Cortajarena

Lipid assemblies control of G4-topology 21
Ms. Brune Vialet, Dr. Nitin Dattatraya Bansode, Dr. Arnaud Gissot, Prof. Philippe Barthélémy

Shaping and Patterning Supramolecular Multi-Gelator Objects by Diffusion-Adhesion Assembly 22
Ms. Chayanan Tangsombun, Prof. David K. Smith

Protein-inspired ligand functionalities incorporated into DNA G-Quadruplexes 23
Dr. Jennifer Bremer, Prof. Guido Clever

Directed Molecular Evolution at Ultrahigh Throughput and via Machine Learning 24
Mr. Florian Hollfelder

Vitamin B12 as a drug delivery vehicle 25
Prof. Dorota Gryko

To Synthesize Theragnostic Probes and its Target Delivery with Enhanced Affinity 26
Prof. Anil Kunar Mishra

Self-assembly of nucleic acid nanotubes for selective targeting of different cancers 27
Prof. Efie Kokkoli

Lipid Stabilized G-Quadruplex Decoys for Anticancer Therapeutic Application 28
Ms. Faith Kivunga, Mr. Jules Garcia, Ms. Julie Lagouarde, Ms. Patricia Korczack, Ms. Aurore GUEDIN-BEAUREPAIRE, Dr. Virginie Baylot, Ms. Brune Vialet, Prof. Tina Kauss, Prof. Philippe Barthélémy

Supramolecular assembly of chemically modified Lipid Oligonucleotide for antibiotic resistance application 29
Mr. Henri Barry, Mr. philippe-andre cleret, Ms. Lisa Scillia, Prof. Philippe Barthélémy, Prof. Corinne Arpin, Prof. Tina Kauss

Thermoresponsive lipo-polypeptide towards controlled aggregation on liposome surface 30
Ms. Rosanna Le Scouarnec, Mr. Emmanuel Ibarboure Ibarboure, Prof. Sebastien Lecommandoux, Dr. Colin Bonduelle, Dr. Jeanne Leblond Chain

Selective tumour cell killing with novel modular antibody-conjugate using self-assembling DNA nanostructures 31
Ms. Tina-Thien Ho, Mrs. Anna H Turaj, Dr. Teresa Lauria, Mr. Albert Ferriol Monjo, Prof. Sean H Lim, Dr. Eugen Stulz

Synthesis and Evaluation of Non-benzenoid aromatic scaffold comprising Nucleic Acid and Peptides 32
Dr. Nagendra K Sharma

Chaperon-inspired peptide nanofibers interrupt Cu (I) homeostasis in cancer cells 33
Dr. Jeena Thekkeyil

Designing carbohydrate supramolecular hydrogels for 3D cell culture. From sacrificial to long lasting. From disordered to ordered. 34
Dr. Juliette Fitremann, Ms. Nadia Kasmi, Mr. Mickael Chabbert, Ms. Faniry Andriamiseza, Ms. Eve Pitot, Ms. Laetitia Pieruccioni, Ms. Isabelle Fourqueaux, Dr. Laure Gibot

Utilizing Deep Learning Algorithms for Automated Identification and Measurement of Target Molecules From Electrochemical Biosensors 36
Mrs. Fatemeh Esmaeili, Dr. Erica Cassie, Dr. Hong Phan T. Nguyen, Prof. Natalie O. V. Plank, Prof. Charles P. Unsworth, Prof. Alan Wang

Optical spectroscopy as a tool for supramolecular chemistry 38
Prof. Cristina Sissa, Dr. Andrea Delledonne, Mrs. Ilaria Ferraboschi

Investigating the Effectiveness of CDK 4/6 Inhibitor loaded 4-Carboxy Phenyl Boronic acid Conjugated pH Sensitive Chitosan Lecithin Na-noparticles in the management of Breast Cancer 39
Mr. naveen rajana

Nucleolipids as a matrix for 3D cell culture 40
Dr. Bruno Alies, Dr. Tarek Sayde, Dr. Omar El Hamoui, Dr. Gaëlle Bégaud, Dr. Sabrina Lacomme, Prof. Karen Gaudin, Prof. Gaëtane Lepes, Prof. Philippe Barthélémy, Prof. Serge Battu

Potato virus A particles, a novel biomaterial for self-assembled nanopatterns for templating silver nanoparticles on substrate surfaces 41
Dr. Swarnalok De, Mr. Hoang Nguyen, Dr. Fangxin Zou, Dr. Fevzihan Basarir, Ms. Maryam Mousavi, Dr. Ville Liljeström, Prof. Kristiina Mäkinen, Prof. Mauri Kostiainen, Prof. Jaana Vapaavuori

Light-triggered stapling of biologically relevant DNA tetraplexes results in increased topological, thermodynamic and metabolic stability. 43
Mr. Jack Barr, Dr. Enrico Cadoni, Ms. Sofie Schellinck, Prof. Emiliano Laudadio, Prof. Jose Martins, Prof. Annemieke Madder

Supramolecular dendrimer-mediated delivery of RNA therapeutics 44
Ms. Jing Wu, Ms. Wenjun Lan, Dr. Juan Iovanna, Dr. Ling Peng

Artificial PNAbased RNases 45
Prof. Roger Stromberg

Modular and adaptive self-assembling supramolecular dendrimers for biomedical applications 46
Dr. Ling Peng

Peptide Nucleic Acids (PNA 2.00): 'JANUS' PNAs with non-identical faces for programmable biomimetic supramolecular assemblies 47
Mr. Krishna Ganesh

Solving RNA delivery by leveraging the body's evolved RNA transport pathways 48
Dr. Alice Ghidini

Exploring the stability of RNA-based delivery scaffolds in diverse biological matrices: methodology and implications for platform development **49**
Ms. Laura Reyes Fraile, Dr. Alice Ghidini, Dr. Piotr Klimkowski, Dr. Aurélie Lacroix, Dr. James Rushworth, Dr. Elena Sanna, Dr. Eugen Stulz, Dr. George Foot, Dr. Anna Perdrix

Study of Nanovectors for lysosome-based therapeutic strategies against neurodegenerative diseases **51**
Mr. Rémi Kinet, Ms. Sarah Nieto, Dr. Angela Mutschler, Ms. Lea Mulot, Dr. Alexandra Gaubert, Prof. Philippe Barthélémy, Prof. Sebastien Lecommandoux, Dr. Benjamin Dehay

Targeting Genomic DNA with Oligonucleotides **52**
Prof. Edvard Smith

Exploring the structure and recognition of G-quadruplex in the B-MYB proto-oncogene promoter

Monday, 15th April - 09:00: Plenary Session 1 - Oral - Abstract ID: 79

Dr. Carla Cruz [1]

1. *University of Beira Interior and CICS-UBI, Portugal*

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. Targeting this oncogene is a promising approach for anti-cancer drug design. B-MYB has been deemed undruggable in previous reports, requiring the search for novel therapeutic options. 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. This talk is divided into two parts:
1) 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 not only effectively stabilized the G4 structure but also exhibited moderate affinity. They could alter the G4 topology and exhibit enhanced fluorescence emission in the presence of this G4. Additionally, these ligands studies. Cellular studies confirmed the co-localization between the target sequence and the developed probe, and these ligands increased the number of G4s observed in cells;
2) we found that the B-MYB gene promoter contains several G/C-rich motifs compatible with G4 formation. We investigated and validated the existence of G4 structures in the promoter region of B-MYB, first in vitro using a combination of bioinformatics, biophysical, and biochemical methods, then in cells with the recently developed G4access method.

Molecular architectures and druggable space of nucleic acid targets

Monday, 15th April - 09:40: Plenary Session 1 - Oral - Abstract ID: 74

Prof. Srivatsan Seergazhi Gopalan [1]

1. *Indian Institute of Science Education and Research (IISER), Pune, Dr. Homi Bhabha Road, Pune 411008, India.*

Numerous biophysical tools have provided efficient systems to study nucleic acids. However, our current understanding on how nucleic acid structure complements its function, particularly in cellular environment, is limited. This general limitation is largely due to the lack of probes that can be used in both cell-free and cellular assays, and in more than one biophysical technique. In this context, moving away from the tradition approach of "one label one technique" we adopted an innovative approach to investigate the nucleic acid structure and function in cell-free and cellular environments by using conformation-sensitive multifunctional nucleoside analog probes. Based on this strategy, we develop nucleoside analogs equipped with two or more labels (eg., fluorophore, ^{19}F NMR isotope label and X-ray crystallography phasing atom), which serve as common probes for analyzing nucleic acid motifs simultaneously by using a combination of fluorescence, NMR and X-ray crystallography techniques.[1-6] In parallel, we also develop chemo-enzymatic labeling technologies to functionalize and image nucleic acids in vitro and live cells. In this presentation, I will discuss the utility of our nucleoside probes in elucidating the structure, ligand recognition and population equilibrium of G-quadruplexes formed by oncogenic EGFR promoter region and HIV-1 long terminal repeat (LTR). Structural analysis and ligand binding properties in *in vitro* and in cell models by using fluorescence, ^{19}F NMR and MD simulation techniques will be presented.

References:

1. Nuthanakanti, A.; Boerneke, M. A.; Hermann, T.; Srivatsan S. G. *Angew. Chem. Int. Ed.* **2017**, *56*, 2640–2644.
2. Manna, S.; Sarkar, D.; Srivatsan, S. G. *J. Am. Chem. Soc.* **2018**, *140*, 12622–12633.
3. Ashok, N.; Ishtiyaq, A.; Saddam, Y. K.; Kayarat, S.; Srivatsan, S. G. *Nucl. Acid. Res.* **2019**, *47*, 6059–6072.
4. Ghosh, P.; Kropp, H. M.; Betz, K.; Ludmann, S.; Diederichs, K.; Marx, A.; Srivatsan, S. G. *J. Am. Chem. Soc.* **2022**, *144*, 10556–10569.
5. Khatik, S. Y.; Sudhakar, S.; Mishra, S.; Kalia, J.; Pradeepkumar, P.I.; Srivatsan, S. G. *Chem. Sci.* **2023**, *14*, 5627–5637.
6. Khatik, S. Y.; Roy, S.; Srivatsan, S. G. *ACS Chem. Biol.* **2024**, doi.org/10.1021/acschembio.3c00676

Singlet oxygen as a tool for suprabiomolecular ligations

Monday, 15th April - 10:45: Plenary Session 2 - Oral - Abstract ID: 77

Prof. Annemieke Madder [1]

1. *Ghent University*

Within OBCR, we have developed a highly selective and efficient singlet oxygen mediated crosslink technology which is applicable to peptide-protein, peptide-nucleic acid and nucleic acid interstrand crosslink scenarios.[1] A furan 'warhead' is introduced and subsequently activated by oxidation trigger with singlet oxygen generating a nucleophile-sensitive keto-enal moiety.[2]

We developed furan-modified oligonucleotide probes which can be used for efficient and selective crosslinking to natural nucleic acid targets[3] as well as protein targets.[4] Furthermore, in the context of peptide ligand-receptor interactions, we have described, in live cells under normal growth conditions, selective crosslinking of furan-modified peptide ligands to their membrane receptor with zero toxicity, high efficiency and spatio-specificity.[5] Specific singlet oxygen based chemistries were further developed for versatile and site-selective modification of proteins.[6]

Financil support from FWO-Vlaanderen, BOF-UGent, European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 721613 (MMBio ITN), No 956070 (OligoMed ITN), No. 665501 (Pegasus[2] fellowship) and SynAb4Toxin - 101111255.

References:

[1] a) Carrette, L.L.G, Morii, T.; Madder, A. *Bioconj. Chem.* **2013**, *24(12),* 2008-2014; b) L. L. G. Carrette, E. Gyssels, N. De Laet, A. Madder. *Chem Comm*. **2016**, 52, 1539.

[2] a) Op de Beeck, M., Madder, A. *JACS* **2012**, *134*, 10737–10740; b) De Laet, N., Llamas, E.M., Madder, A. *ChemPhotoChem* **2018** *2*, 575–579;

[3] a) Stevens, K., Madder, A. *Nucleic Acids Research* **2009**, 1555; b) De Beeck, M.O., Madder, A. *JACS **2011**. 133*, 796–807; c) Manicardi, A., Gyssels, E., Corradini, R., Madder, A. *Chem. Comm*. **2016**, *52*, 6930–3; d) Manicardi, A. Cadoni, E., Madder, A. *Chem. Sci.*, **2020**, 11, 11729–11739; e) E. Cadoni, A. Manicardi, M. Fosséprè, K. Heirwegh, M. Surin, A. Madder, *Chem. Commun.*, **2021**, 57, 1010–1013.

[4] a) Miret-Casals, L.; Vannecke, W.; Hoogewijs, Madder, A. *et al. Chem. Comm.*, **2021**, ***57***, 6054 – 6057; b) Miret Casals, L.; Van De Putte, S. ; Madder, A. *et al.* Frontiers in Chemistry, **2022.** 9:799706.

[5] a) Vannecke, Van Troys, Ampe & Madder, ACS Chemical Biology **2017**, 2191; b) EP10196898.0.; c) EP 15176415.6.

[6] De Geyter, E.; Antonatou, E.; Kalaitzakis, D.; Madder, A. *Chemical Science*, **2021**, **12**, 5246 – 5252.

Damage, repair and degradation of mitochondrial nucleic acids

Monday, 15th April - 11:25: Plenary Session 2 - Oral - Abstract ID: 80

Dr. Carlo Vascotto [1]

1. *Polish Academy of Sciences, Poland*

Mitochondria play a crucial role in energy production, metabolism and signaling pathways. Central to their function is the integrity of mitochondrial DNA (mtDNA) and RNA, which encode essential components of the oxidative phosphorylation system. However, both mtDNA and mtRNA are susceptible to damage from endogenous and exogenous sources, leading to various cellular dysfunctions and contributing to aging and disease progression. Apurinic/apyrimidinic endonuclease 1 (APE1) is a multifunctional protein that plays a crucial role in DNA repair, redox regulation and transcriptional regulation. It is mainly localized in the nuclear compartment, but is also present within the mitochondrial matrix, where it functions as a key component of the mitochondrial base excision repair (BER) pathway, the primary pathway responsible for the recognition and repair of non-helix distorting base modifications such as oxidation, alkylation and abasic sites. In addition, recent evidence points to a novel role of APE1 in the degradation processes of oxidatively damaged mitochondrial mRNAs. This presentation emphasized the importance of understanding the dynamic interplay between damage, repair and degradation in the maintenance of mitochondrial nucleic acid homeostasis. Insights into these mechanisms not only deepen our understanding of basic cell biology, but also hold promising opportunities for therapeutic intervention in mitochondrial disorders and age-related diseases.

Modified Self-amplifying RNA is the Next RNA Therapeutic

Monday, 15th April - 14:30: Nanomedicine - Oral - Abstract ID: 22

Prof. Mark Grinstaff [1]

1. *Boston University*

Karikó and Weissman discovery of the role of modified nucleoside triphosphates (modNTPs) in RNA catalyzed the advancement of messenger ribonucleic acid (mRNA) to the forefront of modern medicine. Unfortunately, the inherent short half-life of mRNA necessitates a large dose to be effective, which increases the risk of adverse side effects, limits global accessibility, and restricts applications. More recently developed RNA technologies, such as self-amplifying RNA (saRNA) offers the potential of potent vaccines and *in situ* therapeutics by enabling protein expression for longer duration at lower doses. However, a major barrier to saRNA efficacy is the potent early interferon response triggered upon cellular entry, resulting in saRNA degradation and translational inhibition. Substitution of mRNA with modified nucleotides, such as N1-methylpseudouridine (N1mΨU), reduces the interferon response and enhances protein expression. Multiple attempts to use modNTPs in saRNA have been unsuccessful, leading to the decades' long dogma that modNTPs are incompatible with saRNA. We unexpectedly discovered several modNTPs (e.g., 5-methylcytidine triphosphate, 5mC) that, when incorporated into saRNA at 100% substitution, confer immune evasion and enhance protein expression potency and duration. Transfection of 5mC saRNA, encoding for mCherry protein, significantly enhances protein expression in mouse muscle myoblast C2C12 cells as well as human immortalized HEK293-T and Jurkat cells, and primary foreskin fibroblasts (HFF) and CD3+ T cells. A single intra-muscular injection of 5mC saRNA, encoding for luciferase, results in 30+ day protein expression and significantly greater performance than N1meΨU mRNA. Further, the type I interferon response is less in transfected human PBMCs *in vitro* and after intra-muscular administration *in vivo* compared to wild-type saRNA. As a first case study, we created a 5mC saRNA COVID vaccine, and observed significant *in vitro* expression of viral antigen and *in vivo* protection against a lethal challenge with a mouse-adapted SARS-CoV-2 strain. At a 10 ng dose, the 5mC saRNA vaccine confers statistically improved survival with increased antibody titers compared to unmodified saRNA or N1meΨU mRNA. This discovery considerably broadens the potential scope of saRNA to vaccines with increased potency and to cell therapy and protein replacement therapies.

Supramolecular Mechanochemistry for the Activation of Drugs, Proteins and Genes

Monday, 15th April - 14:47: Nanomedicine - Oral - Abstract ID: 10

Prof. Andreas Herrmann [1]

1. *DWI - Leibniz-Institute for Interactive Materials*

The field of optogenetics has enabled the fundamental understanding of neural circuits and disorders.[1,2] However, current optogenetic techniques require invasive surgical procedures to deliver light to target cells due to the low penetration depth of light into tissue. Therefore, ultrasound (US) was used as alternative trigger since US can deeply penetrate tissue with high spatiotemporal control. Our group develops general molecular technologies based on nucleic acid aptamers and mechanochemistry to control the activity of proteins and drugs (Fig. 1) by US.[3,4,5] Therefore, we produce high molecular weight polynucleic acids by rolling circle amplification or transcription that encode multiple aptamer binding sites for proteins or drugs. Once these loaded nucleic acid carriers are subjected to ultrasonication, covalent and non-covalent bond cleavage occurs by collapse of US-induced cavitation bubbles leading to activation of protein or drug cargoes. Similarly, we liberate small bioactive trigger molecules by US that initiate gene expression involving riboswitches relying on modified tRNA scaffolds.[6] A particular emphasis is paid to reducing US energies to make these sonogenetic and sonopharmacological systems compatible with living matter.[3]

References

[1] W. Haubensak, P. S. Kunwar, S. Ciocchi, J. Biag, H.-W. Dong, K. Deisseroth, E. M. Callaway, M. S. Fanselow, A. Lüthi, A. J. Anderson, Nature **468**, 270 (2010).

[2] A. V. Kravitz, B. S. Freeze, P. R. Parker, K. Kay, M. T. Thwin, K. Deisseroth, A. C. Kreitzer, Nature **466**, 622 (2010).

[3] P. Zhao, S. Huo, J. Fan, J. Chen, F. Kiessling, A. J. Boersma, R. Göstl, A. Herrmann, Angew. Chem. Int. Ed. **60**, 14707 (2021).

[4] S. Huo, P. Zhao, Z. Shi, M. Zou, X. Yang, E. M. Warszawik, M. Loznik, R. Göstl, A. Herrmann, Nat. Chem. **13**, 131 (2021).

[5] D. Yildiz, R. Göstl, A. Herrmann, Chem. Sci. **13**, 13708 (2022).

[6] A. Paul, E. M. Warszawik, M. Loznik, A. J. Boersma, A. Herrmann, Angew. Chem. Int. Ed. **59**, 20328 (2020).

Permeation studies on new nanovesicles for drug delivery with multiphoton microscopy

Monday, 15th April - 15:04: Nanomedicine - Oral - Abstract ID: 14

Mrs. Ilaria Ferraboschi [1], ***Mrs. Marta Alcaina-Hernando*** [2], ***Dr. Nora Ventosa*** [3], ***Dr. Alba Cordoba*** [4], ***Prof. Silvia Pescina*** [5], ***Prof. Cristina Sissa*** [6]

1. *Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Area Parco delle Scienze 17/A, 43124 Parma, Italy.,* ***2.*** *Nanomol Technologies S.L. and Institut de Ciència de Materials de Barcelona, ICMAB-CSIC/CIBER-BBN, Campus UAB, Bellaterra, 08193 Spain.,* ***3.*** *Institut de Ciència de Materials de Barcelona, ICMAB-CSIC/CIBER-BBN, Campus UAB, Bellaterra, 08193 Spain.,* ***4.*** *Nanomol Technologies S.L., Campus UAB, Bellaterra, 08193 Spain.,* ***5.*** *ADDResLab, Department of Food and Drug, University of Parma, Area Parco delle Scienze 27/a, 43124 Parma, Italy.,* ***6.*** *Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Area Parco delle Scienze 17/A, 43124 Parma, Italy*

Introduction

The delivery of non-water-soluble pharmaceutical compounds commonly exploits (nano)carriers, which improve the availability of the drug at the target location in the patient's body. Therefore, the interaction of the carrier with the biological tissues is of paramount importance to gain the therapeutic efficiency. For this reason, the optimization of the formulations intended for skin administration commonly comprises *ex vivo* permeation studies, using porcine tissues as model.

Methods

Multiphoton microscopy (MPM) exploits nonlinear interactions between light and matter (e.g., two photon excited fluorescence and second harmonic generation) to obtain 2D and 3D images of the sample. The involved phenomena are achieved focusing red or near-infrared light inside the sample, producing low scattering and low photo-bleaching, and allowing for deep penetration in the tissue up to 1-2 mm. Thus, MPM is a promising tool to follow the permeation of carriers inside *ex vivo* models, and, combined with other techniques, to validate their efficacy[1].

Results

In this contribution, MPM is applied to follow the permeation of new plant based nanovesicles (NVs) in the delivery of a small, non-water-soluble molecule in skin tissue. To perform the permeation study, several differently treated skin specimens were analyzed with the MPM. Skin treatment was performed using a Franz-type diffusion cell and, later, the specimens were placed under the microscope objective for imaging analysis (Fig.1). Nile Red (NR), a small non-water-soluble fluorescent molecule, was loaded inside the NVs to make the carrier fluorescent, and hence detectable by MPM. Moreover, NR emission is strongly affected by medium polarity, providing interesting hints about the molecule surrounding.

Discussion

Thanks to the advantages of MPM, it was possible to visualize the first hundreds of micrometers of full thickness skin samples (Fig.2), unveiling important features of the carrier behavior inside the samples.

Information about the permeation and the release of the cargo within the tissue were obtained from the tissues imaging and the acquisition of emission spectra directly on the treated specimens.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 101007804-Micro4Nano

[1] Int. J. Pharm., 2023, 638, 122911.

Figure1-analysis-protocol.png

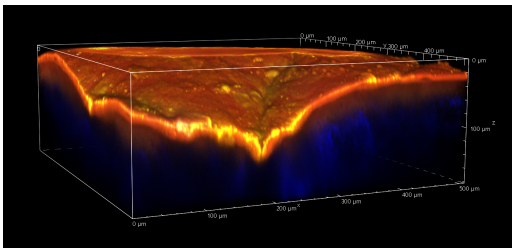


Figure2-skin-treated-nr-loaded-nvs-3h-3d.png

Targeting the IRES structure of mRNA for modulating gene translation

Monday, 15th April - 15:21: Nanomedicine - Oral - Abstract ID: 23

Mr. Albert Ferriol Monjo [1], ***Dr. Eugen Stulz*** [1], ***Dr. Tilman Sanchez-Elsner*** [1], ***Ms. Tina-Thien Ho*** [1], ***Dr. Gabriela Barbeta*** [1], ***Dr. Elena Vladimirovna*** [1]

1. *University of Southampton*

Cellular proteins are commonly synthesized by the cap-dependent mechanism. Some studies suggest that under stress conditions where this process is compromised, the ribosomes are recruited by a complex structural element called Internal Ribosome Entry Segment (IRES)[1]. The IRES is a sequence of the mRNA with complex secondary structures, located within the mRNA 5'UTR that allows the ribosome to be recruited near the initiation codon[2].

The proto-oncogene *c-MYC*, which encodes a transcription factor c-MYC, is one example where the IRES promoted expression provides an important alternative pathway. Its expression is strictly controlled in normal cells but becomes disregulated and overexpressed in over 70 % human cancers. The activity of the *c-MYC* IRES is increased in malignant cells compared to the healthy cells, providing a potential window for cancer selective *c-MYC* inhibition.

We are using specific short oligonucleotides (ODNs) for highly selective targeting of the *c-MYC* IRES structure. The designed ODNs contain modifiers for increased nuclease resistance and increased RNA-DNA duplex formation (Locked Nucleic Acid (LNA), phosphorothioate). The binding of the ODNs to different regions of the IRES could potentially be used to either downregulate or upregulate gene expression through either stabilising or destabilising the IRES structure.

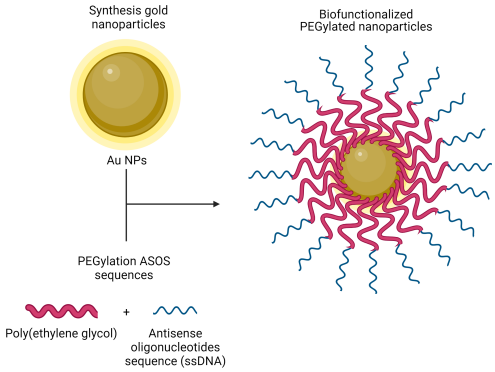
The synthesized oligonucleotides are bioconjugated to gold nanoparticles (AuNPs), previously functionalized with PEG, in order to increase the efficiency of the delivery of the sequences into the cell. These delivery systems were characterized by UV-Visible spectrophotometry, dynamic light scattering (DLS), and transmission electron microscopy (TEM) in order to determine the physicochemical properties of the colloids and the optimal biofunctionalization of the sequences onto the surface.

Cell tests were performed in order to analyse the impact of the sequences in terms of protein expression and gene regulation. Different techniques were used to study the effect of the different oligonucleotides' modifications on c-MYC IRES activity in vitro.

Bibliography

1 J. P. C. Le Quesne, M. Stoneley, G. A. Fraser and A. E. Willis, *J. Mol. Biol.*, 2001, **310**, 111–126.

2 A. L. Gutiérrez-Escolano, *Rev Educ Bioquim.*, 2006, 25, 12–19.



Delivery system scheme.png

Targeting mucus hyperproduction in COPD using siRNA-loaded switchable lipid nanoparticles to silence SPDEF

Monday, 15th April - 15:38: Nanomedicine - Oral - Abstract ID: 59

Ms. Aurore GUEDIN-BEAUREPAIRE [1], ***Dr. Thais Pivetta*** [2], ***Mr. Kim-Nghi Hoang*** [3], ***Ms. Eloïse LATOUILLE*** [3], ***Dr. Isabelle Dupin*** [2], ***Mrs. Elise Maurat*** [2], ***Dr. Jeanne Leblond Chain*** [4]

1. *ARNA U1212 INSERM,* ***2.*** *U1045,* ***3.*** *Bordeaux University,* ***4.*** *ARNA*

RNA therapies have been pioneered for lung diseases,since clinical trials involving RNA inhalation have been conducted for cystic fibrosis or asthma. However, several biological barriers such as mucus penetration and bronchial epithelial delivery still hamper the efficacy of RNA therapies for lung diseases. This issue is even more important in chronic obstructive pulmonary disease (COPD), as mucus hyperproduction is participating to airflow limitation and associated with an increased all-cause mortality. Small interfering RNA (siRNA) against SAM-pointed domain-containing ETS transcription factor (SPDEF), an intracellular transcription factor required for goblet cell differentiation, is a therapeutic option to reduce mucus hyperproduction and restore mucociliary clearance. Here, using an air-liquid interface (ALI) model, we prepared and tested switchable lipid nanoparticles (LNP) to deliver siRNA against SPDEF. Primary bronchial epithelial cells derived from pulmonary samples were collected after thoracic surgery of COPD and non-COPD patients. Cells were cultivated at the ALI culture to obtain a fully differentiated epithelium. Lipid nanoparticles were prepared by rapid mixing of lipids in ethanol and siRNA in buffer. Interestingly, PEG shielding was compared to another shielding polymer, Poly(ethyloxazoline). After dialysis, their size, charge and encapsulation efficiency were characterized. After the exposure of epithelial cells to siRNA-LNP during 4h, siRNA uptake was evaluated by flow cytometry and confocal imaging. Silencing efficiency was assessed by RTqPCR and western blot. The siRNA-LNP were able to efficiently penetrate into differentiated cells in ALI culture. Confocal imaging confirmed that siRNA have crossed the mucus layer and penetrated within the cytoplasm of epithelial cells. SPDEF silencing was achieved at 72 and 120 hours after siRNA-LNPs exposure at the RNA and protein level. These results highlight the potential of this switchable lipid nanoparticle formulation to carry out siRNA drugs for COPD treatment.

Porphyrin Stabilised G-Quadruplexes

Monday, 15th April - 14:30: Bioorganic chemistry - Oral - Abstract ID: 40

Dr. Chloe Howells [1], ***Dr. Daniel Singleton*** [1], ***Dr. Teresa Lauria*** [1], ***Dr. Eugen Stulz*** [1]

1. *University of Southampton*

DNA-porphyrin Interactions have been extensively investigated over the last few decades, especially in the case of the stabilisation of G-quadruplex (G4) formation.[1] It is known that DNA sequences rich in guanine (GGGX)4 can form both inter- and intramolecular structures composed of stacking G-quartets surrounding monovalent cations such as potassium.[2] G4 structures play a crucial role in the maintenance of genomic integrity by protecting the terminal DNA of chromosomes (telomeric regions) from repair enzymes, especially telomerase.[3]

Telomerase is a ribonucleoprotein enzyme which can add short telomeric DNA strands onto the 3' terminal overhang of chromosomes, which is normally shortened during mitosis. The reduction in overhang length is essential in determining how many times a cell can divide, eventually causing cell death.[4] Telomerase counteracts strand shortening and therefore stops cells from dying. Its expression in supressed in most somatic cells, but telomerase presence is detected in 85% of cancers.[5]

Here, the synthesis of a novel G4-forming DNA sequence based on the human telomeric repeat (GGGTTA) where porphyrins are covalently attached to thymidine at the 7 and 13 positions is presented. It is shown by thermal denaturing experiments that the addition of porphyrins to the sequence greatly stabilises the G4 formation. We will present further results regarding stability with different cations, structure analysis via molecular dynamic simulations, and biological activity using telomerase assays.

References

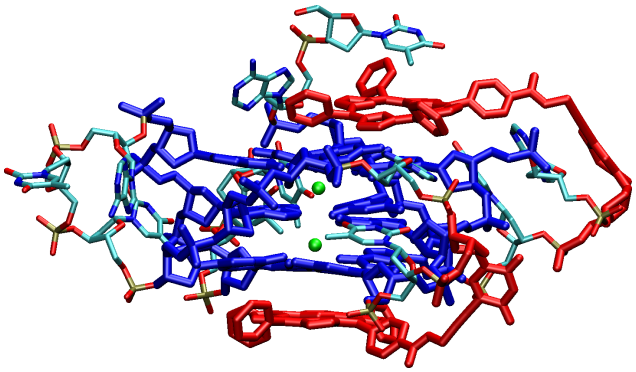
1 U. Chilakamarthi, D. Koteshwar, S. Jinka, N. Vamsi Krishna, K. Sridharan, N. Nagesh and L. Giribabu, *Biochemistry*, 2018, **57**, 6514–6527.

2 B. R. Vummidi, J. Alzeer and N. W. Luedtke, *ChemBioChem*, 2013, **14**, 540–558.

3 C. G. Wu and M. Spies, *Nucleic Acids Res*, 2016, **44**, 8742–8753.

4 J. T. Chang, Y. Chen, H. Yang, C. Chen and A. Cheng, *Eur J Biochem*, 2002, **269**, 3442–3450.

5 N. A. Kovalenko, D. D. Zhdanov and T. F. Kovalenko, *Mol Biol*, 2013, **47**, 476–487.



Gq-5546-crop.png

VITAMIN B12- A DRUG DELIVERY VEHICLE INTO THE CELLS

Monday, 15th April - 14:47: Bioorganic chemistry - Oral - Abstract ID: 41

Ms. Monica Fresta [1], ***Prof. Dorota Gryko*** [1]

1. *Polish Academy of Science*

The delivery of an active compound (cargo) to a target biological site is often a very challenging task and combining a natural compound possessing the ability to pass through the cell membrane with a therapeutic agent in a hybrid/conjugate seems to be a promising approach. Despite the all clear advantages in use the Oligonucleotide therapeutic, like the fact they are able to target previously “undruggable” targets providing new approaches for complex disease, the manly problem looks like to be the delivery of them into the cells. Endocytosis is the main pathway for oligonucleotides to enter cells and various endocytic pathways are involved in the internalization of oligonucleotides. So in order to overcoming such endosomal barriers it is necessary to install some chemical modifications or create some conjugates or nanocarriers. For instance, different type of small molecules and anionic polymer conjugates are used to alter membrane stability of endosomes to release oligonucleotides. Our strategy is to utilize the Vit-B_{12} , which has a unique uptake pathway, as a vehicle to transport oligonucleotides into cells. Vit-B_{12} acts like a delivery vehicle due to its unique dietary uptake pathway, the constant demand for it in dividing cells and also well-established transport through Glycoproteins. In order for Vit-B_{12} to act as a carrier, its structure must be modified to allows selective coupling of biological active compound and at the same time high affinity to transport proteins. To utilize this system means to find an appropriate functionalization of Vit-B_{12} that both allows father conjugation of therapeutics and does not interrupt its recognition by transport proteins.

Screenshot 2024-02-06 alle 11.35.34.png

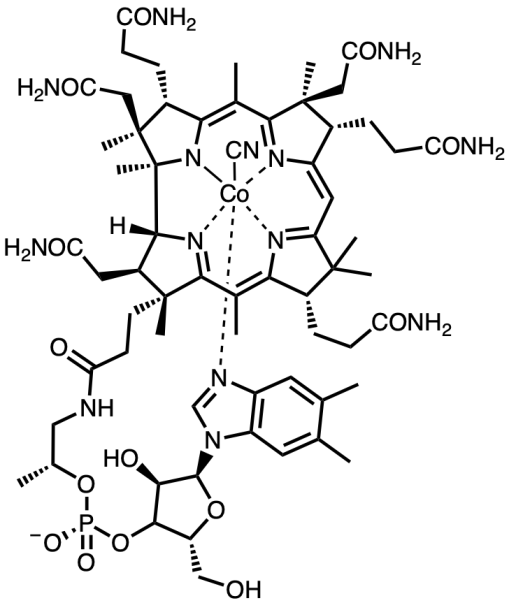


Image-2.png

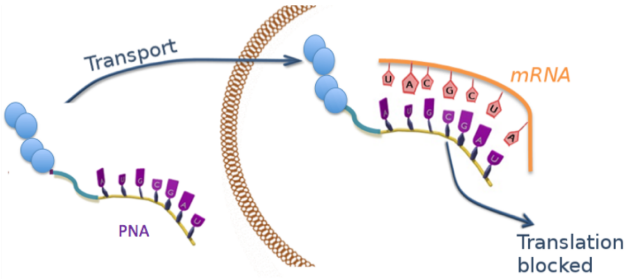


Image.png

Using liposomes as nanoreactors with DNA-mediated fusion

Monday, 15th April - 15:04: Bioorganic chemistry - Oral - Abstract ID: 43

Dr. Nikolaj Risgaard [1], ***Prof. Stefan Vogel*** [1], ***Dr. Xinwei Tian*** [1], ***Dr. Philipp Löffler*** [1]

1. *University of Southern Denmark*

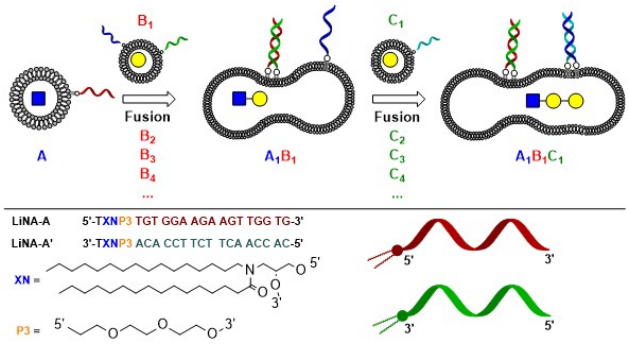
A major obstacle to mimic cellular compartmentalization has been the lack of technology for leakage-free fusion of multiple lipid nanoreactors in sequential or parallel fusion. We have previously demonstrated that lipidated DNA (LiNA) can be used to promote fusion with low leakage and high content mixing.[1] The LiNA fusion system have shown up to 7 sequential fusion rounds with minimal cross-talk between different LiNA pairs in a combinatorial single-particle fusion setup with >93% fusion efficiency.[2] Using the LiNAs, we have developed DNA-programmed lipid nanoreactors (PLNs) to synthesize a set of carbohydrate mimetics with mono- to hexasaccharide azide building block connected by click-chemistry, including parallel and two-step reaction schemes[3].

Figure. *Illustration of a two-step fusion cascade with encapsulated substrates in liposomes for multi-step synthesis using lipidated DNA (LiNAs) for fusion and content mixing. An example of sequence and structure of one of the LiNA pairs are shown.*

The LiNA liposome fusion technology has been established as a suitable platform for combinatorial chemistry with the initial examples from the synthesis of carbohydrate mimetics. However, with currently employed reaction types the full potential demonstrated in the single-particle studies has not been explored. Future work includes optimizing the LiNA design and performing enzymatic reactions in PLNs, some of which will be presented with the current PLN system.

This work was supported by grants from The VILLUM Foundation, grant no. VKR022710.

References: [1] P. M. G. Löffler, O. Ries, A. Rabe, A. H. Okholm, R. P. Thomsen, J. Kjems, S. Vogel, *Angewandte Chemie (International ed.)* **2017**, *56*, 13228-13231. [2] M. G. Malle, P. M. G. Löffler, S. S. R. Bohr, M. B. Sletfjerding, N. A. Risgaard, S. B. Jensen, M. Zhang, P. Hedegard, S. Vogel, N. S. Hatzakis, *Nature chemistry* **2022**, *14*, 558-565. [3] X. Tian, N. A. Risgaard, P. M. G. Löffler, S. Vogel, *Journal of the American Chemical Society* **2023**, *145*, 19633-19641.



Using liposomes as nanoreactors with dna-mediated fusion.jpg

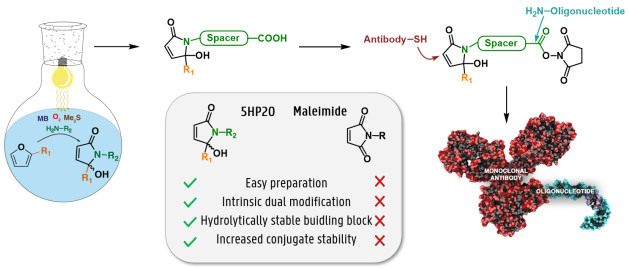
From a synthetic methodology towards a bioconjugation tool: 5HP2Os as next-generation maleimide alternatives

Monday, 15th April - 15:21: Bioorganic chemistry - Oral - Abstract ID: 20

Mr. Jan H. Meffert [1], ***Prof. Annemieke Madder*** [1]

1. *Ghent University*

5-Hydroxy-1H-**p**yrrol-**2**(5H)-**o**nes (5HP2Os) can be obtained in an efficient manner starting from modified furan building blocks employing methylene blue as photosensitizer and redox agent, a chemistry previously used as a starting point for the synthesis of various natural products by the group of Prof. Vassilikogiannakis[1].
The presence of a Michael acceptor and the similarity to classical maleimides, led us to investigate the possibility to employ 5HP2Os for bioconjugation purposes[2]. Bioconjugation of small molecule drugs or oligonucleotides to proteins (mainly antibodies) is one of the main technologies in obtaining today's highly specific therapeutics. Furthermore, such technology is also the key to allow for various precise imaging technologies.[3]
After determination of the conjugation capabilities of 5HP2O model building blocks on small molecule level and on protein level, several 5HP2O-based multifunctional linkers were synthesized for the purpose of oligonucleotide to protein conjugation. In comparison with classical maleimides, which are often used for bioconjugation, 5HP2O derivatives display increased stability and can hold two functional handles (e.g. NHS ester and fluorescent probe, NHS ester and click handle), allowing for some exciting applications.



Abstract suprabio.png

Amphiphilic Dynamic Covalent Polymer Vectors for siRNA Delivery

Monday, 15th April - 15:38: Bioorganic chemistry - Oral - Abstract ID: 5

Mr. José García Coll [1], ***Dr. Nadir Bettache*** [1], ***Dr. Sébastien Ulrich*** [1]

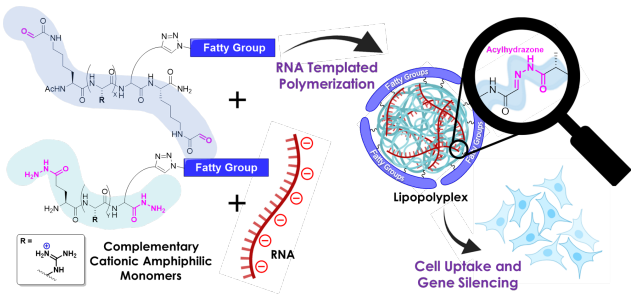
1. *IBMM, Institut des Biomolécules Max Mousseron, Université de Montpellier, CNRS, ENSCM, 34095 - Montpellier (France)*

Dynamic Covalent Polymers (DCPs) are emerging as promising vectors for gene delivery.[1-4] In previous works,[5,6] we showed that a dynamic library of complementary cationic peptides can undergo a siRNA-templated polymerization through acylhydrazone and oxime ligations, leading to the formation of a glycosylated DCP which in turn triggers cell-selective uptake and siRNA delivery.

In this communication, we will present the design and synthesis of a new family of modified peptides that give access to amphiphilic DCPs. The physicochemical properties of these original vectors were characterized by DLS and ζ Potential and their potency to complex and deliver siRNA-Luc was assessed by gel electrophoresis and cell assays on a MCF-7-Luc cancer cell line. The results reveal that these amphiphilic DCPs have a better ability to complex siRNA, which also translates into an improved efficacy for siRNA transfection on cells.[7]

We will show our *in situ* screening approach that deciphered the structure-activity relationships within this new series of multi-component dynamic covalent co-polymers. Overall, these results highlight the potential of hybrid systems that combine the best of lipid nanoparticles and cationic polymers in order to design smart vectors of nucleic acids.

1. García Coll, J.; Ulrich, S. *ChemBioChem* **2023**, 24 (19), e202300333.
2. Ulrich, S. *Acc. Chem. Res.* **2019**, 52 (2), 510–519.
3. Zhang, Y.; Qi, Y.; Ulrich, S.; Barboiu, M.; Ramström, O. *Mater. Chem. Front.* **2020**, 4 (2), 489–506.
4. Su, D.; Coste, M.; Diaconu, A.; Barboiu, M.; Ulrich, S. Cationic. *J. Mater. Chem. B* **2020**, 8 (41), 9385–9403.
5. Laroui, N.; Coste, M.; Su, D.; Ali, L. M. A.; Bessin, Y.; Barboiu, M.; Gary-Bobo, M.; Bettache, N.; Ulrich, S. *Angew. Chem. Int. Ed.* **2021**, 60 (11), 5783–5787.
6. Su, D.-D.; Ali, L. M. A.; Coste, M.; Laroui, N.; Bessin, Y.; Barboiu, M.; Bettache, N.; Ulrich, S. *Chem. Eur. J.* **2023**, 29 (7), e202202921.
7. García Coll, J.; Bettache, N.; Ulrich, S. Manuscript in preparation.



Rna-templated polymerization of amphiphilic sirna delivery vectors.png

Hybrid Peptide-Agarose Hydrogels for 3D Bioassays

Monday, 15th April - 16:30: Biomaterials, Soft matter - I - Oral - Abstract ID: 2

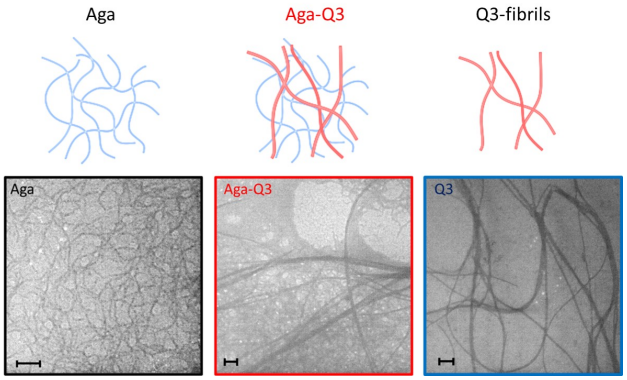
Dr. Greta Bergamaschi [1], ***Dr. Alessandro Gori*** [1], ***Mr. Roberto Frigerio*** [1], ***Dr. Andrea Pizzi*** [2], ***Mr. Angelo Musicò*** [1], ***Dr. Marina Cretich*** [1]

1. *National Research Council, Istituto di Scienze e Tecnologie Chimiche "Giulio Natta" (SCITEC-CNR), Milan, Italy,* ***2.*** *Politecnico di Milano*

Hydrogels represent ideal environments to locally confine biomolecular probes onto analytical surfaces under solution-mimetic conditions. Yet, severe limitations still exist in matching unimpaired analytes diffusion with stable probe entrapment. Herein, we report on a hybrid hydrogel obtained by combining a self-assembling peptide with low-temperature gelling agarose that proved of simple and robust application for the fabrication of microdroplet arrays.

The two distinct hydrogel components favorably combine to provide a novel material entailing unique features towards 3D assays, as here demonstrated in microdroplet arrays fabrication. The hydrogel microstructural and functional properties are easily tunable, resulting in a unique combination of favorable features which include: 1) spontaneous and rapid formation; 2) direct use in microarrays fabrication due to favorable viscoelastic properties; 3) self-adhesiveness on hydrophobic surfaces.

Most importantly, the 3D assay format showed greatly superior performances with respect to conventional planar 2D assays, as here demonstrated both in model antibody-epitope recognition and in real case scenario IgG immunoreactivity. Overall, the developed material overcomes many of the limitations that currently plague hydrogel for immunoassays limiting their widespread applications (limited mass transport, complex fabrication, poor versatility of use), while being user-friendly, robust and cost-effective.



Abs 1.jpg

Biomimetic (multi)compartmental structures and functions through self-assembly, clustering and reorganisation processes

Monday, 15th April - 16:47: Biomaterials, Soft matter - I - Oral - Abstract ID: 12

Dr. Dietmar Appelhans [1]

1. *Leibniz-Institut für Polymerforschung Dresden e.V.*

Cellular compartments and functions attract many scientist to understand their functions, but also to mimic their structures and functions. From the point of polymer science it is of high interest to substitute liposomal structures by polymeric materials, equipped with higher membrane stability and (multi)responsive membrane characteristics to mimic the mass transport of metabolites and biomolecules for studying enzymatic cascade, autonomous, out-of-equilibrium, feedback and (self-)oscillating reactions. Especially, the use of pH-, salt-, light-, and/or redox-responsive polymeric vesicles (Psomes) in our working group offers the possibility to study various biomimetic (eukaryotic) cell aspects. This results in the establishment of therapeutic organelles and in the mimicking of artificial organelles and their protocells/synthetic cells for potential applications in synthetic biology, enzyme replacement therapy, and systems biology.[1-9]

Here, in this contribution we report on (i) the cyclic clustering and declustering of Psomes in larger µm-sized biomimetic cell structures and functions[10] and (ii) the nanoparticle- and Psomes-induced transformation of membrane-less coacervates into Psomes-membranized coacervates and final (multicompartmental) giant vesicles.[11] The competition of different non-covalent interactions (ionic, H-bonds, and hydrophobic interactions etc.) allows us to fabricate stabilized, metastable and dynamic supramolecular structures with (multi)compartmental and (multi)phasic confinements with the potential to integrate enzymes in defined locations.

References

1. H. Gumz et al., *Adv. Sci.* **2019**, 6, 1801299.
2. S. Moreno et al., *Small* **2020**, *16*, 2002135.
3. E. Geervliet et al., *J. Controlled Release* **2021**, *332*, 594-607.
4. P. Wen et al., *Small* **2021**, *17*, 2005749.
5. F. Rajabasadi et al., *Adv. Mater.* **2022**, *34*, 2204257.
6. S. Moreno et al., *Biomacromolecules* **2022**, *23*, 4655-4667.
7. K. Zhang et al., *Biomacromolecules* **2023**, *24*, 2489-2500.
8. (a) X. Xu et al., *Adv. Sci.* **2023**, *10*, 2207214; (b) X. Xu et al., *Small Methods* **2023**, 2300257.
9. D. Wang et al., *Adv. Funct. Mater.* **2023**, *33*, 2306904.
10. (a) P. Wang et al., *Biomacromolecules* **2022**, *23*, 3648-3662; (b) K. Zhang et al., *J. Colloid Interface Sci.* **2024**, *654*, Part B, 1469-1482.
11. Y. Zhang et al., manuscript to being submitted.

Dynamic Chiral Silver Nanoclusters Stabilized by DNA

Monday, 15th April - 17:04: Biomaterials, Soft matter - I - Oral - Abstract ID: 66

Dr. Manuel Nuñez-Martinez [1], ***Dr. Krishnadas Kumaranchira Ramankutty*** [1], ***Dr. Ivan Rivilla*** [2], ***Dr. Amaia Larumbe*** [3], ***Dr. Javier Calvo*** [4], ***Prof. Luis Liz-Marzán*** [4], ***Prof. Aitziber L. Cortajarena*** [1]

1. *CIC biomaGUNE, Basque Researchand Technology Alliance (BRTA), Donostia-SanSebastián 20014, Spain; Ikerbasque, Basque Foundation for Science, Bilbao 43009, Spain,* ***2.*** *Departamento de Química Orgánica I, University of the Basque Country, Faculty of Chemistry, P° Manuel Lardizabal 3, E-20018 Donostia, Spain. b. IKERBASQUE-Basque Foundation for Science, Plaza Euskadi 5, E-48009 Bilbao, Spain,* ***3.*** *Spain3 Departamento de Química Orgánica I, Centro de Innovación en Química Avanzada (ORFEO−CINQA), Universityof the Basque Country (UPV/EHU), Faculty of Chemistry, P° Manuel Lardizabal 3, E-20018 Donostia, Spain.,* ***4.*** *CIC biomaGUNE, Basque Research and Technology Alliance (BRTA), Donostia-SanSebastián 20014, Spain; Ikerbasque, Basque Foundation for Science, Bilbao 43009, Spain*

Photoluminescent nanomaterials based on DNA-stabilized silver nanoclusters (AgNCs@DNA) have attracted increasing interest, due to their potential applications as biosensors and biomarkers.[1-3] The optical properties of these biomaterials are strongly related to the interactions between the ssDNA and the AgNCs and, therefore, the design of specific DNA sequences is of prime relevance. Moreover, DNA templates can also act as chiral inducers, potentially leading to chiral photoluminescent AgNCs@DNA, where the chiral information is transmitted from the DNA structure to the luminescent AgNCs.

We report dynamic AgNCs@DNA with circularly polarized luminescence (CPL), in which the CPL signal can be tuned through the design of ssDNA strands. The dynamic behavior of these chiral AgNCs@DNA was evaluated under different external stimuli (hydration state and temperature) obtaining opposite chiroptical properties (CD and CPL signals) . This work represents an effort to understand the relationship between structure and chirality in this type of biomaterials.

1. *ChemPhotoChem 2021, 5, 369–375*
2. *Anal. Chem. 2015, 87, 5302−5309*
3. *J. Phys. Chem. C 2018, 122, 28382−28392*

Lipid assemblies control of G4-topology

Monday, 15th April - 16:30: Supramolecular chemistry - I - Oral - Abstract ID: 48

Ms. Brune Vialet [1], ***Dr. Nitin Dattatraya Bansode*** [1], ***Dr. Arnaud Gissot*** [1], ***Prof. Philippe Barthélémy*** [2]

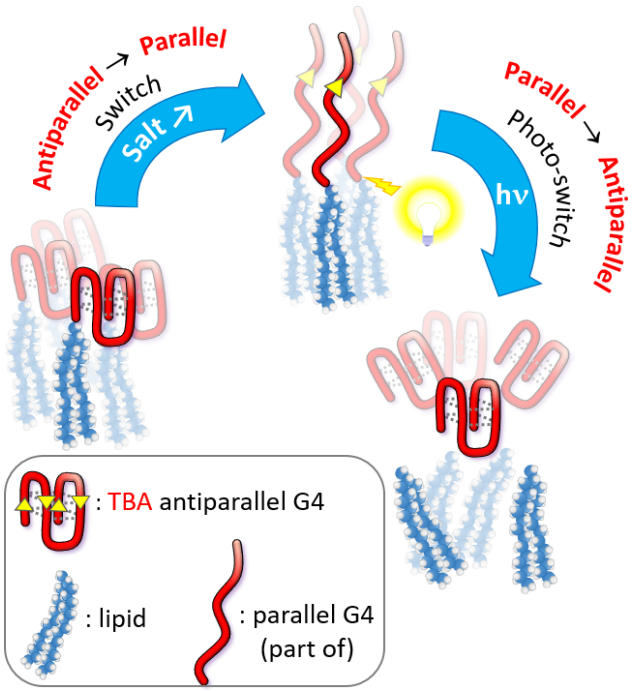
1. *ARNA U1212 INSERM,* ***2.*** *University of Bordeaux*

Nucleic acids carrying strands of guanines are able to self-assemble into G-quartets, the staking of which leads to G-quadruplexes (G4s). These structures display an important degree of polymorphic diversity with distinct orientations (parallel, anti-parallel, and hybrid), strand stoichiometry (1-4 strands) and various numbers of G-quartets. Several factors such as strand concentrations, G4-ligands, temperature and more can impact this conformational diversity. Controlling G4 polymorphism expresses a challenge for potential applications of G4s in biotechnology.

This communication presents structural behaviors of lipid G4 supramolecular assemblies[1] originating with the Thrombin Binding Aptamer (TBA). This aptamer is a well-described G4-quadruplex (G4) exhibiting an anti-parallel conformation with relevant biological activity. The modification by a double chain lipid shows a conformational switch to an inactive parallel topology. To restore its biological activity, an original photo-cleavable link was designed and incorporated between the sequence and the lipid. The parallel conformer is chemo-selectively switched back to the native anti-parallel conformation upon light irradiation. Our lipid assembly constitutes an original prodrug of the TBA aptamer.

Reference:

[1]: B. Vialet, N. Bansode, A. Gissot, P. Barthélémy, ***Bioconjugate Chem.***, 2023, **34**, 7, 1198-1204



Graphical abstract.png

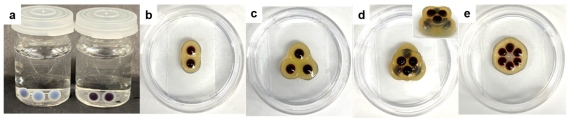
Shaping and Patterning Supramolecular Multi-Gelator Objects by Diffusion-Adhesion Assembly

Monday, 15th April - 16:47: Supramolecular chemistry - I - Oral - Abstract ID: 8

Ms. Chayanan Tangsombun [1], ***Prof. David K. Smith*** [1]

1. *Department of Chemistry, University of York, Heslington, York, YO10 5DD*

Supramolecular hydrogels are soft and reversible materials which derive from the self-assembly of low-molecular-weight gelators (LMWGs) to form a fibrillar network in aqueous solvents underpinned by non-covalent interactions. Shaped hydrogels with spatiotemporally-controlled properties are appealing platforms for biological and pharmaceutical applications. Here, we demonstrate that the diffusion of an acid from a gel bead core assembled from the LMWG 1,3:2,4-di(4-acylhydrazide)-benzylidenesorbitol (DBS-$CONHNH_2$) reinforced with agarose leads to the self-assembly of a second gelator, DBS-COOH, as a shell. The kinetics of DBS-COOH assembly is dependent on the amount of acid in the system and the shell of the core-shell beads can be further reinforced by co-assembly with gellan gum. By arranging the acid-loaded gel bead cores in predefined positions in a petri dish, the dynamic self-assembly of the DBS-COOH gel led to adhesion between the different gel beads allowing different patterned gels to be fabricated. Gel beads loaded with DBS-carboxylate could be used to restrict DBS-COOH shell assembly, allowing them to be removed at the end of diffusion adhesion assembly, acting as imprints. More complex objects could be developed using a layer-by-layer approach. The DBS-$CONHNH_2$ within the gel bead cores retains its unique feature of inducing *in situ* formation of silver or gold nanoparticles, allowing the fabrication of objects which have metal nanoparticles incorporated in specific domains within them.



The formation of (a) core-shell gel beads, (b) oval, (c) triangle, (d) double-triangle and (e) donut-shaped hydrogel objects based on acetic-acid-loaded gel bead incorporating gold nanoparticles (dark-purple).

Photographs of shaped and patterned hydrogel.png

Protein-inspired ligand functionalities incorporated into DNA G-Quadruplexes

Monday, 15th April - 17:04: Supramolecular chemistry - I - Oral - Abstract ID: 31

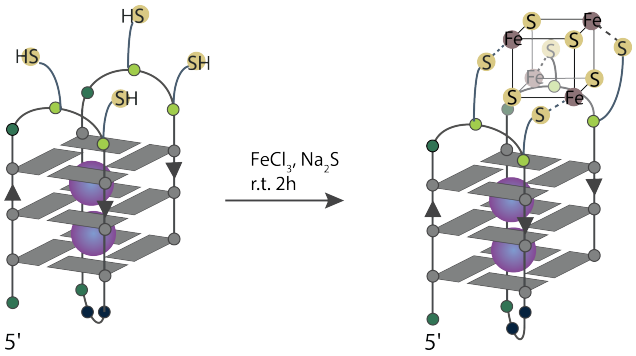
Dr. Jennifer Bremer [1], ***Prof. Guido Clever*** [1]

1. *TU Dortmund*

DNA can fold into a variety of structures besides the widely known double helix structure motif.[1] An exemplary non-standard DNA structure is the G-Quadruplex (G4), which is formed by Guanine rich sequences.[1] In this four stranded secondary structure, four Guanine nucleobases construct a planar G tetrad with eight Hoogsten hydrogen bonds and normally a sodium or potassium ion in the middle.[1] These tetrads can stack via π- π stacking onto each other.[1,2] Sequences which are able to form G4's are observed in the promoter region of oncogene and human telomeric DNA.[1-4] This in addition to the involvement of G4's in diseases makes this special secondary structure motif an interesting target.[1-4] Furthermore, modified nucleobases can be incorporated into G4 sequences via solid phase synthesis leading to different functions like acting as MetalloDNAymes for catalytic C-C bond formations[5] or metal induced topology changes.[6] In this context, the incorporation of amino acid-like ligands into the backbone of G4 forming sequences to act like DNAzymes is shown. The aim is to mimic the coordination environment of the enzyme hydrogenase, which have cysteine residues and an iron-sulfur cluster in its catalytic center.[7] For this matter, ligands with a threoninol backbone and thiol functions were synthesized and incorporated into G4-DNA. The idea is, that these cysteine functionalities can maybe form iron sulfur clusters.

References[1] Lejault, P., Mitteaux, J., Sperti, F. R. & Monchaud, D. Cell Chem. Biol., 2021, 28, 436–455.[2] Choi, J. & Majima, T. Chem. Soc. Rev.,2011, 40, 5893–5909.[3] Brooks, T. A. & Hurley, L. H. Nat Rev Cancer, 2009, 9, 849–861.[4] Brooks, T. A., Kendrick, S. & Hurley, L. Febs J, 2010, 277, 3459–3469.[5] Punt, P. M., Langenberg, M. D., Altan, O. & Clever, G. H., J. Am. Chem. Soc., 2021, 43, 9, 3555–3561.[6] Engelhard, D. M., Nowack, J. & Clever, G. H., Angewandte Chemie Int Ed., 2017, 56, 11640–11644.[7] Lubitz, W., Ogata, H., Rüdiger, O. & Reijerse, E. Chem. Rev., 2014, 114, 8, 4081–4148.

8 Stratmann, L. M. Metal-Mediated DNA G-Quadruplexes, 2021.



G4konf.png

Directed Molecular Evolution at Ultrahigh Throughput and via Machine Learning

Tuesday, 16th April - 09:00: Plenary Session 3 - Oral - Abstract ID: 81

Mr. Florian Hollfelder [1]

1. *University of Cambridge, UK*

TBC

Vitamin B12 as a drug delivery vehicle

Tuesday, 16th April - 09:40: Plenary Session 3 - Oral - Abstract ID: 76

Prof. Dorota Gryko [1]

1. Polish Academy of Science

Delivery of an active compound to its site of action is one of the crucial issues in drug development. A promising strategy is to use naturally occurring compounds, such as vitamin B_{12} due to its unique ability to penetrate cells via a system of transport proteins.[1]

In order for B_{12} to act as a carrier, its structure must be modified to allow selective coupling of biologically active compounds and at the same time high affinity to transport proteins must be retained. Selective and high-yielding functionalizations of B_{12} are desirable; however, the complexity of cobalamin's structure makes this extremely challenging. Our group has introduced methods allowing to achieve this goal (**Figure 1: A**). B_{12} can be selectively attached to alkynes (via CuAAC),[2] acids (via amide bond formation)[3] or thiols (via disulfide bond formation).[4] Also reduction-free, direct alkynylation of vitamin B_{12} at cobalt center that leads to previously unknown heat and light stable acetylide cobalamins has been developed.[5] The selective orthogonal conjugation at both the Co center and 5'-OH group can also be achieved.[6] Recently, we have developed modifications at previously unexplored *meso* position.[7]

The idea of using cobalamin as a delivery vehicle is documented in mammals and was applied to increase bioavailability of different therapeutics. However, such approach has not been applied to bacteria. Thus, in our work we focus on creating a connection of B_{12} and PNA that will be targeted at bacterial DNA or RNA (**Figure 1: B**).[8] The use of short, modified oligonucleotides as inhibitors of bacterial translation seems a promising alternative for antibiotics, which are currently overused leading to bacterial resistance. We found that vitamin B_{12} transports antisense PNA into *E. coli* cells more efficiently than cell-penetrating peptide (KFF)3K. Moreover, we have analyzed the structure and conformational dynamics of conjugates of Cbl with a PNA monomer and oligomer and B_{12} was found to increase the flexibility of PNA in a way that could be beneficial for its hybridization with natural nucleic acid oligomers.[9] The results of our study provide the foundation for considering vitamin B_{12} as a delivery tool for PNA oligonucleotides into bacterial cells.

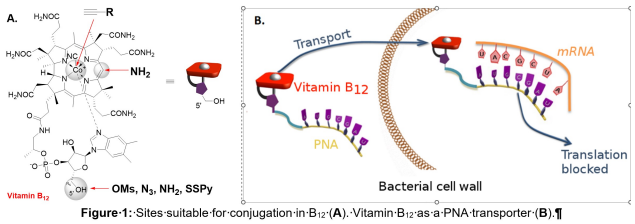


Figure 1: Sites suitable for conjugation in B_{12} (**A**). Vitamin B_{12} as a PNA transporter (**B**).

Figure 1.jpg

To Synthesize Theragnostic Probes and its Target Delivery with Enhanced Affinity

Tuesday, 16th April - 10:45: Plenary Session 4 - Oral - Abstract ID: 71

Prof. Anil Kunar Mishra [1]

1. *Indian Institute of Technology, Roorkee*

To develop smart and specific tracers to investigate *in vivo* topography, and connections and titration of biochemical's non-invasively using imaging techniques are essence of time in diagnosis and therapy or to use it in the combination as theragnostic. Our ongoing efforts are to innovate and translate multimodal molecular pharmaceuticals of broad interest in health care through state of art construction of chemical chaperons.
These probes and agents are being developed to visualize specific molecular targets and pathways in live cells, tissues and organism (from plants, mouse to human).
The specific aim is to 1) design and synthesize new imaging probes/agents, 2) develop and use novel amplification schemes for the development of 'next generation' imaging probes, 3) optimize pharmacokinetics and 'imagability', 4) efficiently synthesize and produce complex and diverse small molecules, and test their ability as imaging agents.
To design and synthesis the next generation of imaging probes/agents for MRI, PET and SPECT, and optical imaging is the core mandate of our research lab. The development of drug delivery using nanocarriers/supramolecular approaches are emerging as a promising therapeutic tool to transport theragnostic molecules to tumors. The construction of NPs using a "layer-by-layer" approach allows surface functionalization with neutralizing and targeting moieties.

Self-assembly of nucleic acid nanotubes for selective targeting of different cancers

Tuesday, 16th April - 11:25: Plenary Session 4 - Oral - Abstract ID: 78

Prof. Efie Kokkoli [1]

1. *Johns Hopkins University*

Self-assembly of amphiphilic molecules is an attractive method to engineer supramolecular materials for biomedical and other applications. In my group, we focus on the design of DNA- and RNA-amphiphiles and evaluate how different building blocks of the amphiphiles affect their tendency to self-assemble spontaneously into different nanostructures, as well as their potential to be used for different applications. In this presentation, I will discuss aspects of the molecular design of nucleic acid amphiphiles that control the formation of functional nanotubes, along with their use as targeted delivery vehicles for glioblastoma and triple negative breast cancer.

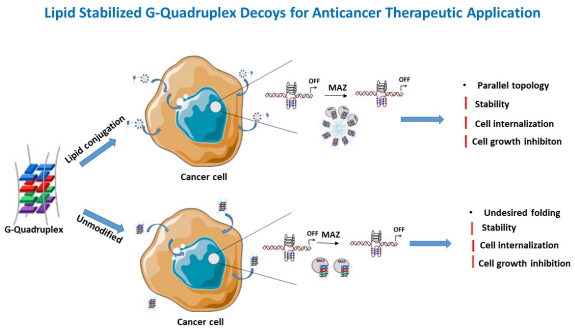
Lipid Stabilized G-Quadruplex Decoys for Anticancer Therapeutic Application

Tuesday, 16th April - 14:30: Bioconjugates self-assembly - Oral - Abstract ID: 46

Ms. Faith Kivunga [1], ***Mr. Jules Garcia*** [1], ***Ms. Julie Lagouarde*** [1], ***Ms. Patricia Korczack*** [2], ***Ms. Aurore GUEDIN-BEAUREPAIRE*** [2], ***Dr. Virginie Baylot*** [2], ***Ms. Brune Vialet*** [2], ***Prof. Tina Kauss*** [2], ***Prof. Philippe Barthélémy*** [3]

1. *Bordeaux University,* ***2.*** *ARNA U1212 INSERM,* ***3.*** *University of Bordeaux*

G-quadruplexes are noncanonical DNA structures present in the promoter region of most oncogenes and play a crucial role in transcription activation. DNA binding proteins that recognize and unwind these structures to activate oncogenes' expression are potential therapeutic targets to treat oncogene-driven cancers. G quadruplex forming oligonucleotides successfully down-regulate the expression of the targeted oncogenes by acting as decoys for the DNA binding proteins. Our project focuses on developing novel oligonucleotides that mimic the G quadruplexes and their topology, to inhibit the oncogenes' transcription activation in aggressive cancers. To optimize our oligonucleotide-based treatment delivery method and increase their stability, bioavailability, and uptake in tumor cells, we grafted lipid moieties to the oligonucleotides' DNA sequence. These oligonucleotides were synthesized using phosphoramidite chemistry, purified, and characterized using high-performance liquid chromatography (HPLC) and mass spectrometry. G quadruplex structure formation and stability were studied using Circular Dichroism at 4°C, 37°C, and 90°C in the presence and absence of salts. The formation of micelles was confirmed by dynamic light scattering (DLS). To assess the antiproliferative and protein knockdown effect, pancreatic cancer cell lines were treated with oligonucleotides. We demonstrated that lipid modification conferred stability of the G4 quadruplex mimics in the desired topology, similar to that of the native sequence at the promoter region. In addition, lipid conjugation increased the internalization by cancer cells and the pharmacological effectiveness of oligonucleotides in comparison to their unmodified counterparts. Our preliminary results show that these lipid-stabilized G4 quadruplexes can inhibit the growth of cancer cells. Our results demonstrate, for the first time, that our innovative G4 quadruplex oligonucleotide-based potential therapy displays high antitumor activity. They may represent a new class of anticancer drugs that can be developed for the treatment of the world's deadliest cancers.



Graphical abstract symposium.jpg

Supramolecular assembly of chemically modified Lipid Oligonucleotide for antibiotic resistance application

Tuesday, 16th April - 14:47: Bioconjugates self-assembly - Oral - Abstract ID: 34

Mr. Henri Barry [1], ***Mr. philippe-andre cleret*** [2], ***Ms. Lisa Scillia*** [2], ***Prof. Philippe Barthélémy*** [3], ***Prof. Corinne Arpin*** [4], ***Prof. Tina Kauss*** [2]

1. *ARNA,* ***2.*** *ARNA U1212 INSERM,* ***3.*** *University of Bordeaux,* ***4.*** *MFP*

Introduction

Oligonucleotides (ONs) have been shown to be effective in the treatment of a range of diseases, and with 16 ONs approved by the FDA, they represent an opportunity for the treatment of rare and/or developmental diseases. Currently approved indications concern direct regulation of human gene expression. Bacterial applications and more precisely targeting of resistance genes using ON have been considered, but one of major hurdles is the efficient penetration of ON in bacteria. Formulation and chemical conjugation have been explored to address this challenge. In particular, conjugation with lipids and a nucleolipid has been described [1] effective for intrabacterial penetration.

Methods

All ONs are synthesized in-house using ChemBioPharm's Opto-oligo ON synthesis and characterization platform. They are purified and analyzed using techniques such as HPLC, dialysis and mass spectrometry. In the case of LONs, a nucleolipid conjugate is attached at the 5' position at the end of the synthesis. Supramolecular assemblies are monitored using dynamic light scattering.

Results

With the nucleolipid in the 5' position LONs acquire the property to auto assemble in micelles. The size of these objects has been measured at 11 nm by DLS. Various modifications have been made to the structure of the ONs, such as ribose or backbone modifications. These modifications affect the ability of the ON to form micelles and to reduce antibiotic resistance.

Discussion

The auto-assembly of LON along with various chemical structures can modulate their biological activity. Micelles may act as a delivery agent. Further studies will explore the impact of the micelle formation on the exact mechanism of the entry of LONs, which is still not fully understood. Some other types of formulation can also take advantage of Auto assembly.

[1] T. Kauss *et al.*, « Lipid oligonucleotides as a new strategy for tackling the antibiotic resistance », *Sci. Rep.*, vol. 10, n° 1, Art. n° 1, déc. 2020, doi: 10.1038/s41598-020-58047-x.

Acknowledgments

The authors wish to thank the Opto-oligo synthesis platform run by Brune Vialet and Patricia Korczak for the synthesis of all LON and Magali Mondin from the Bordeaux imaging center imaging. This project has been funded by ANR-22-CE35-0015.

Thermoresponsive lipo-polypeptide towards controlled aggregation on liposome surface

Tuesday, 16th April - 15:04: Bioconjugates self-assembly - Oral - Abstract ID: 54

Ms. Rosanna Le Scouarnec [1], ***Mr. Emmanuel Ibarboure Ibarboure*** [2], ***Prof. Sebastien Lecommandoux*** [2], ***Dr. Colin Bonduelle*** [2], ***Dr. Jeanne Leblond Chain*** [1]

1. *ARNA U1212 INSERM,* ***2.*** *Laboratoire de Chimie des polymères organiques (LCPO), UMR5629*

Pore-forming proteins like α-haemolysine are dynamic systems made of inactive monomers able to form complex multimeric transmembrane assemblies, generating then transport across the cell membrane. In order to mimic such biological assemblies, stimuli-responsive polymers have been studied to modulate membrane permeability in the presence of ions, light, temperature or redox conditions. This work develops polymer-based biomimetic scaffold to enhance and control the membrane destabilization effect. This polymeric backbone is synthesized via the ring-opening polymerization of N-carboxyanhydrides and initiated by a lipid moiety. We fully report here on the synthesis of a thermoresponsive lipo-polypeptide based on proline repeat units, characterized by NMR and MALDI and its thermoresponsive behavior. Such lipo-polypeptides were formulated into giant unilamelar vesicles through electroformation. Upon an increase in temperature, a phase separation at the surface of vesicles was observed by confocal microscopy, which lead to local domains enriched in polymer. The permeability of such vesicles was monitored by fluorescence. Interestingly, such polymers could be used to build synthetic cells with controlled passive transport across their membranes.

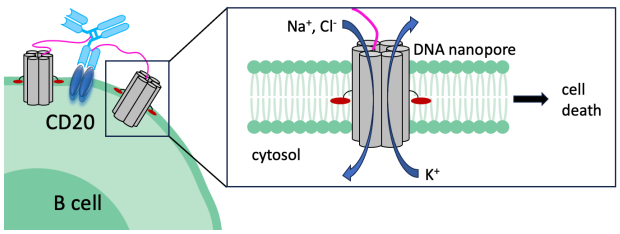
Selective tumour cell killing with novel modular antibody-conjugate using self-assembling DNA nanostructures

Tuesday, 16th April - 15:21: Bioconjugates self-assembly - Oral - Abstract ID: 55

Ms. Tina-Thien Ho [1], ***Mrs. Anna H Turaj*** [2], ***Dr. Teresa Lauria*** [3], ***Mr. Albert Ferriol Monjo*** [3], ***Prof. Sean H Lim*** [2], ***Dr. Eugen Stulz*** [3]

1. *University of Southampton & Centre for Cancer Immunology,* ***2.*** *Centre for Cancer Immunology,* ***3.*** *University of Southampton*

The development of potent but specific cytotoxics is critical for safe but effective cancer therapy. Self-assembling DNA nanostructures are based on the simple principle of Watson-Crick base pairing which enable the creation of an indefinite range of structures of different sizes and shapes; potential applications include cell drug delivery, genetic analysis, biological imaging, bionics and as biophysical tools on account of their good biocompatibility and biosafety (Liu, Duan et al. 2021). We previously developed membrane-spanning DNA nanopores (NP) which perforate cell membranes and trigger cell death (Burns, Al-Juffali et al. 2014). Here, we aim to develop a modular system comprising cytotoxic NP combined with a targeting moiety (e.g. rituximab, a CD20 monoclonal antibody) for killing of malignant B cells (Fig. 1) to produce a tumour-targeted and highly cytotoxic cancer therapeutic.
We designed 6-helix bundle structured NP of 14 nm length and a diameter of 6 nm with two external hydrophobic moieties - comprising 10 interconnected oligonucleotides ranging from 42 to 92 bases. NP were produced in a one-step assembly process and the formation was verified by electromobility shift assay (EMSA). Subsequently, NP were conjugated site-specifically with dibromopyridazinedione-linker which rebridge the disulphides in rituximab to produce a 4:1 nanopore:antibody ratio.
Successful antibody-oligonucleotide conjugation was shown by SDS-PAGE. Using Flow cytometry, we confirmed preservation of rituximab's binding specificity to a CD20+ lymphoma cell line and stability of the construct through detection of fluorophore- labelled oligonucleotides. Serum stability of NP has been demonstrated for 3 weeks at 37°C through EMSA up to 3 weeks. On co-culture with Ramos cells, increased cell death was observed with the cholesterol-modified NP at 48h compared to the no-treatment control.
Ongoing studies are aimed at validating the cytotoxicity of the modular system in different cancer types *in vitro*, and subsequently, *in vivo*.



Dna-np mechanism-of-action scheme ho.png

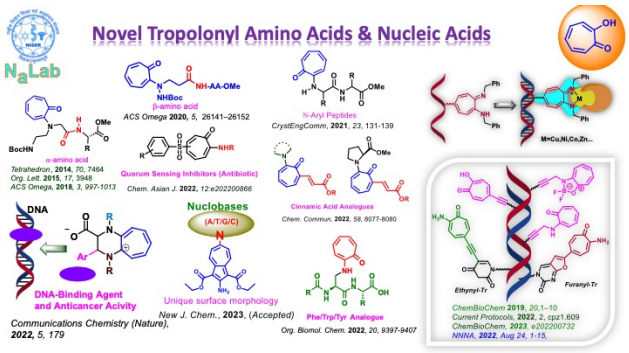
Synthesis and Evaluation of Non-benzenoid aromatic scaffold comprising Nucleic Acid and Peptides

Tuesday, 16th April - 15:38: Bioconjugates self-assembly - Oral - Abstract ID: 18

Dr. Nagendra K Sharma [1]

1. *National Institute of Science Education and Research (NISER),*

Aromatic scaffold of nucleic acid (DNA/RNA) and peptides are derived from benzenoid aromatic moieties. However non-benzenoid aromatic scaffold such as Tropolone and Azulene are constituents of various natural products, possessing wide range of bioactivities such as antifungal, antibiotics, and anticancer. Thus, exploring these non-benzenoid aromatic scaffolds in the context of nucleic acids and peptides could open up new avenues in drug discovery and biotechnology. It may lead to the development of molecules with improved therapeutic properties or unique mechanisms of action, which could be valuable in the treatment of various diseases, including cancer, infections, and other medical conditions. However, extensive research and experimentation would be required to fully understand the potential and limitations of such modifications.



Slide1.jpeg

Chaperon-inspired peptide nanofibers interrupt Cu (I) homeostasis in cancer cells

Tuesday, 16th April - 14:30: Biomaterials, Soft matter) - II - Oral - Abstract ID: 69

Dr. Jeena Thekkeyil [1]

1. *Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany*

Supramolecular assembly of peptides within the cancer cell has emerged as a promising strategy for inducing cancer cell death. In this innovative approach, soluble amphiphilic peptide self-assembles into well-defined nanostructures inside the cancer cell in response to specific stimuli, under the condition of molecular crowding or due to the localization inside the confined space such as mitochondria. Initially non-functional in their soluble state, these peptides become activated within the cell upon forming supramolecular structures, typically nanofibers. Previous studies have illustrated the ability of intramitochondrial formed nanofibers to interact with the mitochondrial membrane, ultimately initiating apoptosis. In here, we demonstrate the capability of *in cellulo* formed peptide fibers to disturb the copper (I) (Cu^+) homeostasis of cancer cells to induce cell death, which could not be achieved by the small molecules. Cu^+ homeostasis is upregulated in many cancers and contributes to tumorigenesis. However, interrupting Cu^+ homeostasis inside the cell by small molecule is challenging because of their poor binding affinity compared to the intracellular ligands that bind Cu^+ with extremely high binding affinity. This challenge was successfully addressed by designing a Cu^+ chaperon derived peptide sequence Nap-FFMTCGGCR that assembles into nanofibers inside the cell under the condition of molecular crowding. Nap-FFMTCGGCR fibers demonstrated high affinity towards the Cu^+ which was comparable to the intracellular Cu^+ binding chaperons or ligands. Nap-FFMTCGGCR fibers induce over production of ROS, reduced the activity of Super oxide dismutase 1 (SOD 1), and ultimately induced the cellular apoptosis of triple negative breast cancer (MDA-MB-231) cells. In contrast, Nap-FFMTCGGCR has minimal impact on normal HEK 293T cells. Control peptides show that the self-assembly and Cu^+ binding properties must work in synergy to successfully disrupt Cu^+ homeostasis. We show the assembly-enhanced affinity for metal ions opens new therapeutic strategies to address disease-relevant metal ion homeostasis.

Designing carbohydrate supramolecular hydrogels for 3D cell culture. From sacrificial to long lasting. From disordered to ordered.

Tuesday, 16th April - 14:47: Biomaterials, Soft matter) - II - Oral - Abstract ID: 6

Dr. Juliette Fitremann [1]***, Ms. Nadia Kasmi*** [1]***, Mr. Mickael Chabbert*** [1]***, Ms. Faniry Andriamiseza*** [1]***, Ms. Eve Pitot*** [2]***, Ms. Laetitia Pieruccioni*** [3]***, Ms. Isabelle Fourqueaux*** [4]***, Dr. Laure Gibot*** [1]

1. *Softmat (ex-IMRCP), UMR5623 CNRS-Université Toulouse 3,* ***2.*** *IPBS-CNRS-Toulouse,* ***3.*** *RESTORE-INSERM-Toulouse,* ***4.*** *CMEAB-Université Toulouse 3*

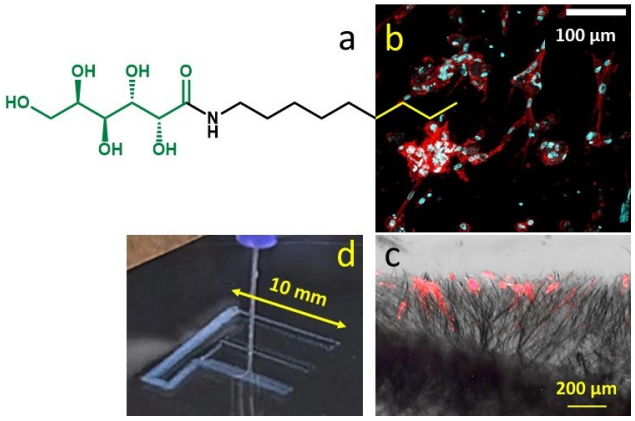
Introduction: Some low molecular weight supramolecular hydrogel based on carbohydrate fatty amides give fibrillar hydrogels compatible for cell culture. These new materials are interesting because cells can go in depth in these very porous hydrogels. Also the fibers tend to help the growth of cell extensions. Our previous results, related to the culture of human neural stem cells on N-heptyl-D-galactonamide hydrogels, have shown that cell clusters connected by bundles of neurites have grown in the hydrogel [1]. However, this gel tends to be washed out after 10 days in vitro which is not enough for culture maturity. To improve the scope of these hydrogels, we synthetized a galactonamide with a longer nonyl chain, in order to decrease the solubility of the molecule in the culture medium (Fig a). The culture of primary human dermal fibroblasts on N-nonyl-D-galactonamide hydrogels is presented here.

Methods: N-nonyl-D-galactonamide hydrogels are prepared in culture plates, seeded with primary human dermal fibroblasts that are grown up to 28 days. The organization of the cells and the gels has been characterized with the following methods: viability (MTT assays), confocal, biphoton, electron microscopy and observation of sections of gels.

Results and discussion: On N-nonyl-D-galactonamide hydrogels, it was possible to carry out the cell culture during at least 28 days. After this time, the gels are still well-shaped, showing that long lasting cell culture is possible on these non-covalent supramolecular hydrogels. Human dermal fibroblasts have grown in clusters preferentially where the density of fibers is higher (Fig b). The cell bodies, the cell extensions and the clusters tend to grow along the bundles of supramolecular fibers. Observation of hydrogel sections showed that cells colonized the gel up to approx. 300 µm (Fig c). We also observed that in some conditions, the preparation of the hydrogel led to well aligned fibers. This property could be used to align the cells. 3D printing is also possible and gives well defined patterns (Fig d)[2].

(1) Chalard, A. et al, *ACS Appl. Mater. Interfaces* **2018**, 17004

(2) Andriamiseza, F. et al. *J. Colloid Interface Sci.* **2022**, 156



Fitremann supramolecular gel 3d cell culture suprabio2024.jpg

Utilizing Deep Learning Algorithms for Automated Identification and Measurement of Target Molecules From Electrochemical Biosensors

Tuesday, 16th April - 15:04: Biomaterials, Soft matter) - II - Oral - Abstract ID: 38

Mrs. Fatemeh Esmaeili [1], ***Dr. Erica Cassie*** [2], ***Dr. Hong Phan T. Nguyen*** [2], ***Prof. Natalie O. V. Plank*** [2], ***Prof. Charles P. Unsworth*** [1], ***Prof. Alan Wang*** [3]

1. *Department of Engineering Science, University of Auckland, Auckland 1010, and The MacDiarmid Institute for Advanced Materials and Nanotechnology, Victoria University of Wellington, Wellington 6021, New Zealand,* ***2.*** *School of Chemical and Physical Sciences, Victoria University of Wellington, and The MacDiarmid Institute for Advanced Materials and Nanotechnology, Victoria University of Wellington, Wellington 6021, New Zealand,* ***3.*** *Auckland Bioengineering Institute, Center for Medical Imaging, Faculty of Medical and Health Sciences, and Center for Brain Research, University of Auckland, Auckland 1010, New Zealand*

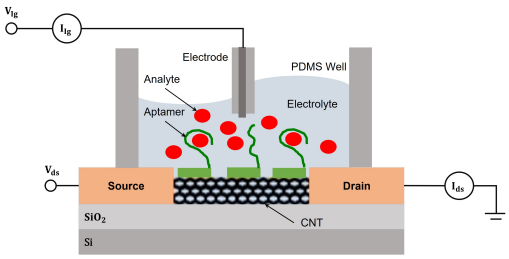
This research investigates the application of advanced deep learning (DL) algorithms to process signals collected from nanomaterial-based aptasensors, with a focus on automating the detection and measurement of target analytes. By employing DL techniques resembling a classification task, the study aims to enhance biosensor performance through refined identification and measurement of analytes.

Initially, the research categorizes analyte concentrations into six classes ranging from zero concentration to 10 μM and distinguishes abnormal data segments from normal ones, crucial for identifying the presence or absence of analytes in samples and determining the quantity of target molecules upon detection. However, the study faces a significant challenge due to data scarcity, a common issue in real-world scenarios. To address this challenge, two data augmentation techniques are proposed: scaling data augmentation and conditional variational autoencoder-based techniques.

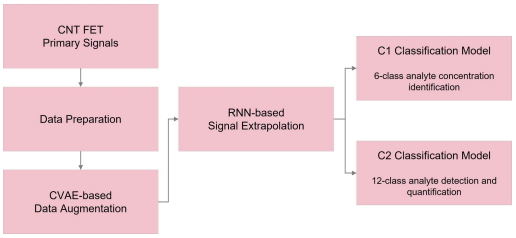
For the initial classification task, a DL model utilizing long short-term memory (LSTM) networks is constructed, highlighting the substantial impact of data labelling and segment length on model performance. Building on these insights, an automatic anomaly detection approach is employed, based on a semi-supervised learning strategy using autoencoder networks and kernel density estimation. While the proposed models show promise in automatically identifying abnormal data, uncertainties persist regarding the influence of segment lengths on their performance.

To ensure uniform signal lengths across datasets, recurrent-based networks are proposed. Subsequently, seven distinct DL classification models including GRU, ULSTM, BLSTM, Conv-GRU, Conv-ULSTM, Conv-BLSTM, and CNN are developed for both initial and secondary classification tasks. The results underscore the effectiveness of appropriate data pre-processing techniques in enhancing neural network performance, thereby facilitating the automated recognition and measurement of analytes from biosensor signals.

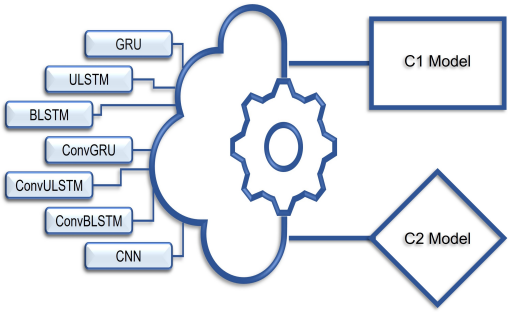
In conclusion, this research significantly contributes to the automated identification and measurement of target analytes using aptamer-based sensors, while also offering practical methods to enhance biosensor performance and biomedical signal processing. The suggested data augmentation techniques effectively address challenges associated with limited data availability, and the introduced autoencoder models serve a dual function, facilitating signal reconstruction and offering insights into unfamiliar datasets. Additionally, the developed classification models serve as inspiration for the creation of innovative classification approaches.



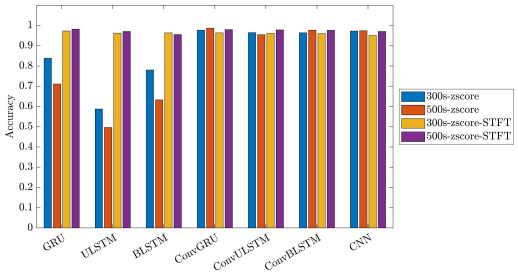
Biosensor.jpg



Workflow.jpg



Deeplearningmodels.jpg



Accuracybarchart.jpg

Optical spectroscopy as a tool for supramolecular chemistry

Tuesday, 16th April - 15:21: Biomaterials, Soft matter) - II - Oral - Abstract ID: 15

Prof. Cristina Sissa [1], ***Dr. Andrea Delledonne*** [1], ***Mrs. Ilaria Ferraboschi*** [1]

1. *Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Area Parco delle Scienze 17/A, 43124 Parma, Italy*

Introduction

The study of nanometric-sized supramolecular objects (micelles, nanovesicles, etc.) is a complex task as their formation and stability are strongly influenced by the environment. The removal of the solvent (often water) usually results in the loss of weak intermolecular interactions crucial for nanoparticles stability. In this context, advanced optical spectroscopic techniques can provide valuable information about the small molecules embedded in the nanoparticle, the integrity of the carrier, and its diffusion, without altering the environment of the supramolecular assembly.

Methods

Absorption and emission spectra are collected to investigate nanoparticles loaded with small fluorescent organic molecules. Depending on the size of the nanoparticle under examination, scattering could significantly impact on the results, and appropriate measures (e.g., the use of filters) should be taken to avoid interference between scattering phenomena and absorption/emission. Steady-state and time-resolved fluorescence anisotropy are powerful tools for studying the mobility of small fluorophores interacting with the nanoparticle. Absorption and fluorescence spectroscopies are also applied to investigate Förster resonance energy transfer phenomena.

Results

Nile red and Coumarin 153 are used as solvatochromic probes to assess the local polarity of the environment, not only in suspension but also in biological settings (*ex vivo* tissues) [1]. Fluorescence anisotropy has successfully demonstrated the loading of small fluorescent molecules in unimer micelles, and the anisotropy decay is exploited to study the mobility of the small fluorescent probe embedded in the nanoparticle [2]. Förster resonance energy transfer is adopted to evaluate the integrity of the carrier in different environments [3].

Discussion

Advanced optical spectroscopic techniques are valuable tools for the investigation of nanoparticles without altering their environment, allowing for information retrieval not only about the optical probe but also about the nanoparticle itself. A detailed spectroscopic characterization of dye-loaded nanoparticles is always necessary when the system is adopted for investigations in complex environments, such as biological tissues.

[1] Journal of Controlled Release 2022, 349, 744-755

[2] ACS Applied Nano Materials 2023, 6, 17, 15551-15562

[3] J. Mater. Chem. C 2021, 9, 10952

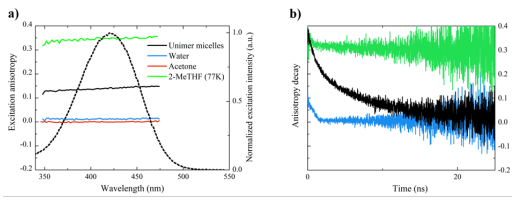


Figure 1: Steady state (a) and time-resolved (b) fluorescence anisotropy of C153 in different environments.

Fluorescence-anisotropy.png

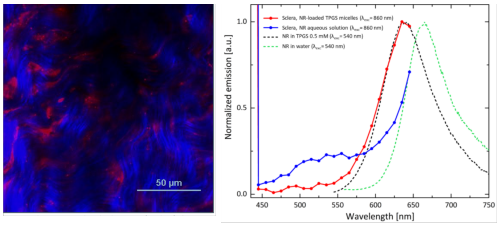


Figure 2: Multiphoton microscopy images (left) of porcine sclera stained with Nile Red (NR) loaded micelles. Emission spectra (right) of NR collected in solution, in suspension and in the scleral tissue.

Multiphon-emission.png

Investigating the Effectiveness of CDK 4/6 Inhibitor loaded 4-Carboxy Phenyl Boronic acid Conjugated pH Sensitive Chitosan Lecithin Na-noparticles in the management of Breast Cancer

Tuesday, 16th April - 15:38: Biomaterials, Soft matter) - II - Oral - Abstract ID: 1

Mr. naveen rajana [1]

1. *Pharmaceutical Nanotechnology Research Laboratory, Department of Pharmaceutics, National Institute of Pharmaceutical Edu-cation and Research (NIPER), Hyderabad, Telangana INDIA*

Over the past few decades, anti-cancer drugs have been suffering from off-target toxicity and non-specific effects in preclinical, and clinical setups; targeting the specific cancer tissue is the main approach to triumph this burdensome(1). Recently, many receptors are known to be overexpressed in cancer and are explored as docking sites for targeting tumor tissues. In the current research, we aim to design and develop a novel 4-carboxy phenyl boronic acid (4- CPBA) conjugated Palbociclib (PALB) loaded pH-sensitive chitosan lipid nanoparticles (PPCL). The objectives of the study are to synthesis of 4-CPBA conjugated chitosan, overcome the drawbacks and to facilitate the targeted delivery of PALB, enhance the anti-cancer efficacy of the PALB in in-vitro cell line studies by loading into 4-CPBA conjugated chitosan lipid nanoparticles. 4-CPBA was conjugated to chitosan by carbodiimide chemistry (2) and formation of conjugate was confirmed by 1HNMR, ATR-FTIR spectroscopic techniques. Ionic-gelation method was used for the fabrication of PPCL and particles size, PDI, zeta potential was found to be 226.5 ± 4.3 nm, 0.271± 0.014 and 5.03 ± 0.42 mV. Presence of pH-sensitive biological macromolecule, i.e., chitosan in the carrier system provides pH-senstivity to PPCL and sustainedly released the drug upto 144 h. The PPCL exhibited approximately 7.2, 6.6, and 5- fold reduction in IC50 values than PALB in MCF-7, MDA-MB-231 and 4T1 cells. Receptor blocking assay concluded that the fabricated nanoparticles were internalized into MCF-7 cells might be through sialic acid-mediated endocytosis. PPCL caused extensive mitochondrial depolarization, enhanced ROS generation, apoptosis (DAPI nuclear staining, acridine orange/ ethidium bromide dual staining), and reduced % cell migration than pure PALB. Thus, it reported that delivering PALB by 4-carboxy phenyl boronic acid conjugated chitosan lipid nanoparticles provides an optimistic approach to treatment of breast cancer.

Nucleolipids as a matrix for 3D cell culture

Tuesday, 16th April - 15:55: Biomaterials, Soft matter) - II - Oral - Abstract ID: 65

Dr. Bruno Alies [1], ***Dr. Tarek Sayde*** [2], ***Dr. Omar El Hamoui*** [1], ***Dr. Gaëlle Bégaud*** [2], ***Dr. Sabrina Lacomme*** [3], ***Prof. Karen Gaudin*** [1], ***Prof. Gaëtane Lepes*** [4], ***Prof. Philippe Barthélémy*** [5], ***Prof. Serge Battu*** [2]

1. *Univ. Bordeaux, CNRS, INSERM, ARNA, UMR 5320, U1212, F-33000 Bordeaux, France,* ***2.*** *Univ. Limoges, UMR INSERM 1308 CAPTuR, Faculté de Médecine, 87025 Limoges, France,* ***3.*** *Bordeaux Imaging Center, UMS 3420 CNRS-INSERM, Université de Bordeaux, Bordeaux 33076, France,* ***4.*** *UPPA, CNRS, IPREM, UMR 5254, 64053 Pau, France,* ***5.*** *University of Bordeaux*

Thanks to cell cultures, research has made drastic progresses. Yet limitations persist in 2D as they differ from those in vivo because of the flattened way cells grow on plate. In vivo studies are strictly regulated, costly, time-consuming, and ethically challenging. To address this, 3D cell culture in a supramolecular gel matrix emerges as a solution, bridging the gap between in vitro and in vivo. In this work, we present a comprehensive exploration of a specific class hydrogels of single molecules, unraveling their intricate self-assembly dynamics, applications in 3D cell culture, and innovative solutions for enhancing reproducibility in cancer models. We investigated the impacts of concentration, temperature, and solvent. Based on this knowledge, we evaluated the hydrogel features in order to fulfill the requirements of the cell culture microenvironment, in terms of the mechanical properties, architecture, molecular diffusion, porosity, and experimental practicality. Finally, we addressed the critical challenge of reproducibility in 3D cancer models. This well-defined supramolecular hydrogel emerges as a constant and reproducible support for cancer stem cell culture. Leveraging sedimentation field-flow fractionation cell sorting, we demonstrates the hydrogel's ability to yield spheroids with uniform size and reproducible growth kinetics.

References :

Saydé T, El Hamoui O, Alies B, Gaudin K, Lespes G, Battu S. *Nanomaterials* 2021 11(2), 481

El Hamoui O, Gaudin K, Battu S, Barthélémy P, Lespes G, Alies B. *Langmuir* 2021, 37, 1, 297

El Hamoui O, Saydé T, Svahn I, Gudin A, Gontier E, Le Coustumer P, Verget J, Barthélémy P, Gaudin K, Battu S, Lespes G, Alies B. *ACS Biomater. Sci. Eng.* 2022, 8 (8), 3387

Potato virus A particles, a novel biomaterial for self-assembled nanopatterns for templating silver nanoparticles on substrate surfaces

Tuesday, 16th April - 16:40: Supramolecular chemistry - II - Oral - Abstract ID: 11

Dr. Swarnalok De [1], ***Mr. Hoang Nguyen*** [1], ***Dr. Fangxin Zou*** [1], ***Dr. Fevzihan Basarir*** [1], ***Ms. Maryam Mousavi*** [1], ***Dr. Ville Liljeström*** [2], ***Prof. Kristiina Mäkinen*** [3], ***Prof. Mauri Kostiainen*** [4], ***Prof. Jaana Vapaavuori*** [1]

1. *Department of Chemistry and Materials Science, Aalto University,* ***2.*** *Nanomicroscopy Center, OtaNano, Aalto University,* ***3.*** *Department of Agricultural Sciences, University of Helsinki,* ***4.*** *Department of Bioproducts and Biosystems, Aalto University*

Introduction

The ability to control the assembly of building blocks in an organized manner is a powerful tool for the fabrication of nano-to-large scale functional structures. However, arranging such building blocks into functional structures has been a challenge for many areas of science and engineering. In this work, we explored the unique self-assembly behavior and potential applications of plant virus (potato virus A) nanoparticles (PVANPs). From the point of the view of nanomaterials, PVANPs are flexible high aspect ratio rod-shaped viruses. They are safe and uniform as compared to most commercially available rod-like colloids or polymers.

Methods

We analyzed the liquid crystal (LC) behavior of PVANPs under different solution conditions using polarized optical microscopy (Figure 1A-C). Followed by this, we developed different methods for LC-directed self-assembly of PVANPs onto glass/Si surfaces. PVANP patterns were visualized using scanning electron microscopy.

Results and Discussion

In this work we created random, directionally aligned and interconnected network like patterns of PVANPs onto substrate surfaces (Figure 1D-F). Next, we exploited the surface charge of the PVANP coat proteins to specifically capture plasmonic silver nanoparticles AgNPs, via electrostatic interaction. Taking advantage of the self-assembly behavior of PVANPs we templated glass surfaces with continuous network of AgNPs interspersed with vacant spaces (Figure 2A,B). Such dense network-like arrays of plasmonic hotspots acted as a photothermal coating that achieved a temperature increase of 21 ^{o}C above ambient temperature under 1-sun radiation, while retaining 78% transmittance of visible light measured at 550 nm wavelength (Figure 2C,D). The PVA-AgNP hybrid showed excellent potential as antifogging coating, exhibiting 2 – 3 times faster defogging rates compared to uncoated samples (Figure 2E).

In another line of study, PVANPs templated with silver via electroless deposition were shown to function as piezoresistive metamaterial. This hybrid material is hypothesized to conduct electricity by tunneling the electrons between the closely arranged arrays of AgNPs. The conductivity is sensitive to the distance between the AgNPs. Therefore, miniscule variation in the distance between the AgNPs lead to large variation in the resistance (Figure 3A-C). Currently we are integrating this piezoresistive element into wearables for detection of different physiological cues.

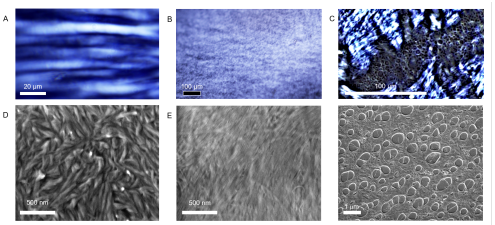


Figure 1. (A-C) LC-profiles of PVANP solutions in water, 0.1 M ammonium acetate buffer at pH8 and in 1% Triton X-100 respectively (D-F) PVANP patterns from the corresponding solutions respectively deposited on Si-surface. (D,E) Drop-cast patterns. (F) Spin-coated patterns.

De abstract figure 1.png

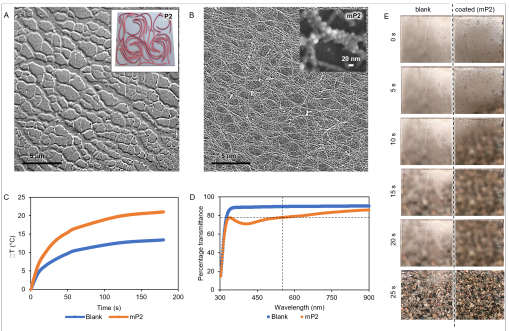


Figure 2. (A) PVANP pattern (P2) with interspersed vacant spaces. (B) Metallized PVANP patterns (mP2) templated with dense arrays of AgNPs specifically bound to the particles. (C) Temperature increase profiles of the coated and uncoated samples under 1-sun radiation. (D) Visible light transmittance profiles of coated and blank samples. (E) time resolved defogging profiles of coated and uncoated samples outdoor in real-life conditions.

De abstract figure 2.png

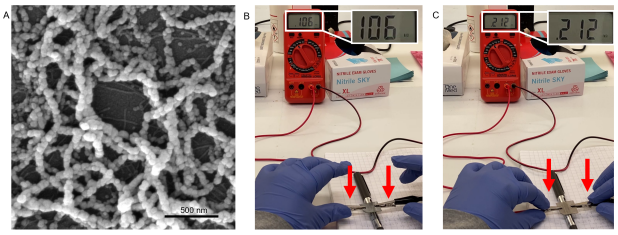


Figure 3. (A) Highly specific binding of AgNPs to PVANPs (scale 500 nm). (B,C) This photographs show demonstration for piezoresistive nature of PVA-AgNP films. The resistance changed (in B and C insets) upon application of mild pressure on the PVA-AgNP films (showed with red arrow).

De abstract figure 3.png

Light-triggered stapling of biologically relevant DNA tetraplexes results in increased topological, thermodynamic and metabolic stability.

Tuesday, 16th April - 16:57: Supramolecular chemistry - II - Oral - Abstract ID: 25

Mr. Jack Barr [1], ***Dr. Enrico Cadoni*** [1], ***Ms. Sofie Schellinck*** [1], ***Prof. Emiliano Laudadio*** [2], ***Prof. Jose Martins*** [1], ***Prof. Annemieke Madder*** [1]

1. *Ghent University,* ***2.*** *Polytechnic University of Marche*

Guanine (G) and cytosine(C)-rich DNA strands have a propensity, under certain conditions, to form four-stranded (tetraplexed), non-canonical secondary structures. These structures are believed to be involved in the regulation of multiple cellular processes, including gene expression and telomere maintenance[1]. G-quadruplexes (G4s), formed in G-rich sequences, have a substantial body of evidence for their biological roles and have found wide use in the fields of nanotechnology and medicine[2]. I-motifs (IM), formed from C-rich strands, while less well studied are also growing in relevance in these fields. An intrinsic property of these structures is the polymorphism of their topologies which are influenced by many factors such as sequence, salts, and the presence of binding ligands. This has biological consequences as it has been demonstrated that G4-binding proteins often only interact with one specific topology[3].

In the context of aptamer therapy, controlling this polymorphism has been identified as one of the key issues damaging their performance both in-vivo and in-vitro, along with low biological stability and high clearance from the body. To remediate this issue, we have developed a robust secondary structure stapling methodology by benchmarking a variety of pre-existing DNA crosslinking moieties. The benefits of the developed stapling approach include an increased melting temperature and exonuclease resistance. Crucially, in this way a steric lock is created that allows the secondary structure to resist external influences on its topology.

1 D. Varshney, J. Spiegel, K. Zyner, D. Tannahill and S. Balasubramanian, *Nat Rev Mol Cell Biol*, 2020, **21**, 459–474.

2 J. Carvalho, J. L. Mergny, G. F. Salgado, J. A. Queiroz and C. Cruz, *Trends Mol Med*, 2020, **26**, 848–861.

3 A. L. Moye, K. C. Porter, S. B. Cohen, T. Phan, K. G. Zyner, N. Sasaki, G. O. Lovrecz, J. L. Beck and T. M. Bryan, *Nat Commun*, 2015, **6**, 7643.

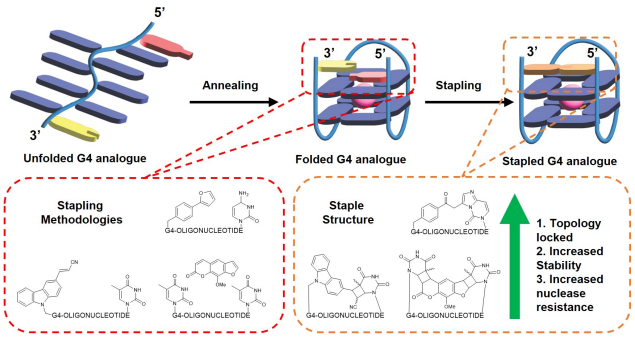


Figure 1. schematic demonstrating a general stapling methodology and chemistries used.jpg

Supramolecular dendrimer-mediated delivery of RNA therapeutics

Tuesday, 16th April - 17:14: Supramolecular chemistry - II - Oral - Abstract ID: 27

Ms. Jing Wu [1], ***Ms. Wenjun Lan*** [2], ***Dr. Juan Iovanna*** [2], ***Dr. Ling Peng*** [3]

1. *Centre Interdisciplinaire de Nanoscience de Marseille (CINaM) UMR 7325, CNRS, Aix Marseille University, Marseille,,* ***2.*** *Centre de Recherche en Cancerologie de Marseille, CRCM, Inserm, CNRS, Institut Paoli-Calmettes, Aix-Marseille University, Marseill,* ***3.*** *Centre Interdisciplinaire de Nanoscience de Marseille (CINaM) UMR 7325, CNRS, Aix Marseille University, Marseille*

Nucleic acid therapeutics are fast becoming an important drug modality for cancer therapy, because they can address undruggable targets and evolving pathogens, and provide precision medicine. [1] However, nucleic acid therapeutics are unstable and have poor bioavailability. Therefore, they require delivery vectors to protect them and safely deliver them to the site of action to achieve the desired therapeutic effect.[1] We have developed innovative supramolecular dendrimer vectors, which are composed of lipid/dendrimer conjugates and able to harness the delivery advantages of lipid and polymeric vectors, the two most advanced nonviral vectors for nucleic acid delivery.[2] In particular, these dendrimer vectors are very effective for the delivery of both small interfering RNA (siRNA) [3-4] and small activation RNA (saRNA).[5] I will present our recent results on the supramolecular dendrimers for the co-delivery of siRNA and saRNA in treating pancreatic cancer.

Keywords: dendrimer, nucleic acid delivery, siRNA, saRNA, cancer therapy

Acknowledgments:

This work was supported by the European project H2020 Marie Sklodowska-Curie Innovative Training Network “OLIGOMED” (No. 956070).

References:

[1] Kulkarni, J.A *et al.*, The current landscape of nucleic acid therapeutics. *Nat. Nanotechnol.* **16**, 630–643 (2021).

[2] J. Chen *et al.*, Amphiphilic dendrimer vectors for RNA delivery: State-of-the-art and future perspective. *Acc. Mater. Res.* **3**, 484–497 (2022).

[3] J. Chen et al., Synthesis and use of an amphiphilic dendrimer for siRNA delivery into primary immune cells. *Nat. Protoc.* **16**, 327–351 (2021).

[4] J. Chen et al., Cargo-selective and adaptive delivery of nucleic acid therapeutics by bola-amphiphilic dendrimers. *Proc. Natl. Acad. Sci. U.S.A* **120**, e2220787120(2023).

[5] Xiong Y *et al.*, Small Activating RNA Modulation of the G Protein□Coupled Receptor for Cancer Treatment. *Adv. Sci.* **9**, 2200562 (2022).

Artificial PNAbased RNases

Wednesday, 17th April - 09:00: Plenary Session 5 - Oral - Abstract ID: 82

Prof. Roger Stromberg [1]

1. *Karolinska Institutet*

TBC

Modular and adaptive self-assembling supramolecular dendrimers for biomedical applications

Wednesday, 17th April - 09:40: Plenary Session 5 - Oral - Abstract ID: 73

Dr. Ling Peng [1]

1. *Centre Interdisciplinaire de Nanoscience de Marseille (CINaM) UMR 7325, CNRS, Aix Marseille University, Marseille*

Dendrimers are ideal precision materials for elaborating nanomedicine by virtue of their well-defined structure, multivalent cooperativity and nanosize per se. We have pioneered modular and adaptive self-assembling supramolecular dendrimers(1) for the delivery of anticancer drugs(2) and nucleic acid therapeutics(3) as well as imaging agents(4) for cancer detection and treatment. Remarkably, these supramolecular dendrimer nanosystems are able to exploit the in situ tumor-secreted extracellular vesicles for effective delivery and deep penetration in tumor tissue, while overcoming tumor heterogeneity and dynamic evolution(2). Also, we have recently developed self-assembling dendrimer nanosystems against infectious diseases caused by antimicrobial resistant pathogens(5). Our findings offer a fresh perspective for exploiting the advantageous features of supramolecular dendrimers to reach the ultimate goal of nanomedicine

References:

[1] Lyu et al, Acc. Chem. Res. 2020, 53, 2936-2946.

[2] a) Jiang et al, Proc. Natl. Acad. Sci. U.S.A. 2023, 120, e2215308120; b) Wei et al, Proc. Natl. Acad. Sci. USA, 2015, 112, 2978-2983.

[3] a) Chen et al, Proc. Natl. Acad. Sci. U.S.A. 2023, 120, e2220787120; b) Chen et al, Acc. Mater. Res. 2022, 3, 5, 484-497.

[4] a) Ding et al, Adv Mater. 2023, 2308262; b) Ding et al, Chem. Commun. 2020, 56, 301-304; c) Garrigue et al, Proc. Natl. Acad. Sci. USA, 2018, 115, 11454-11459.

[5] King et al, ACS Infectious Diseases, 2024, 10, 453-466; b) Dhumal et al, Nanoscale, 2022, 14, 9286-9296.

Peptide Nucleic Acids (PNA 2.00): 'JANUS' PNAs with non-identical faces for programmable biomimetic supramolecular assemblies

Wednesday, 17th April - 10:45: Plenary Session 6 - Oral - Abstract ID: 83

Mr. Krishna Ganesh [1]

1. *ISER Pune and IISER Tirupati*

DNA and RNA are naturally endowed with structural features for self-assembly through complementary base pairing, leading to a variety of structures ranging from hairpins, duplexes, triplexes to cruciform and tetraplexes etc. Several chemically modified analogues involving modification of sugar-phosphate backbone have been developed in the context of antisense and siRNA therapeutics. These analogues bind to complementary sequences via WC H-bonding from bases, but only from one side of the backbone.

Peptide Nucleic Acids (PNA) are DNA analogues with nucleobases linked to pseudopeptide backbone instead of sugar-phosphate backbone and show sequence specific binding to complementary DNA and RNA with high affinity. We have now deisgned "Janus PNAs" that carry two different nucleobase sequences, one on each face of PNA. It is shown that such a Janus PNA can concurrently bind two different DNA/RNA sequences that enables them to target two different genes. We demonstrate the potential of "Janus" PNAs to form programmable assemblies such as double duplex, triplex of duplex and tetraduplex of tetraplex, with appropriate complementary DNA and RNA. Having multiple duplexes on a single backbone also leads to synergistic effects on the thermal stability of each of the duplexes. Multistrand complexes from Janus PNAs can also be generated using Ag to bridge two C's on one face and WC H-bonding on other face leading to supramolecular complexes with different types of pairing mechanisms in same complex.

Solving RNA delivery by leveraging the body's evolved RNA transport pathways

Wednesday, 17th April - 11:25: Plenary Session 6 - Oral - Abstract ID: 72

Dr. Alice Ghidini [1]

1. *Sixfold Bioscience*

The interaction of RNA with biofluids and intracellular proteins has received increased interest in the past decades. Several structural, molecular and functional aspects of this interaction have been clarified and characterized. With the recent arising of RNA-based therapeutics, it has also been highlighted the important contribution that the interaction with protein components has on the pharmacological properties of this new modality. Clarifying and quantifying these binding mechanisms is unanimously considered as an essential factor that could help rationalize the design of any drug formulation containing RNA.

The interaction with proteins is a crucial binding event that determines RNA-therapeutics distribution properties, including the one of ligand/nanoparticles used for their delivery. Despite its significance on the overall efficacy of the therapeutic, a lack of methodologies has lagged its understanding. In recent years several academic groups and pharmaceutical companies have taken over the challenge and proposed inventive analytical methods. At Sixfold we apply innovative in vitro and in vivo experimental solutions for the study of RNA/protein interactions and ultimately design our RNA-based delivery system, Mergo.

Mergo is a structured and chemically modified RNA scaffold, which is designed for cell-specific distribution of therapeutics. Mergos have shown unique plasma protein binding profiles and signature tissue distributions. The team is currently working on profiling the constructs based on several properties, such as secondary structure, motifs and chemical modifications patterns and on exploring several routes of administrations.

Exploring the stability of RNA-based delivery scaffolds in diverse biological matrices: methodology and implications for platform development

Wednesday, 17th April - 13:30: Drug Delivery - Oral - Abstract ID: 64

Ms. Laura Reyes Fraile [1], ***Dr. Alice Ghidini*** [1], ***Dr. Piotr Klimkowski*** [1], ***Dr. Aurélie Lacroix*** [1], ***Dr. James Rushworth*** [1], ***Dr. Elena Sanna*** [1], ***Dr. Eugen Stulz*** [2], ***Dr. George Foot*** [1], ***Dr. Anna Perdrix*** [1]

1. *Sixfold Bioscience,* ***2.*** *University of Southampton*

Over the last few decades, significant efforts have focused on enhancing the stability of oligonucleotides. Chemical modifications have been shown to reduce ON susceptibility to nucleases and extend their half-life in serum, all while shedding light on their direct impact on therapeutic effectiveness. In addition, advances in oligonucleotide design have overcome the need to use lipid nanoparticle formulations to shield oligonucleotide therapeutics from serum degradation, paving the way for innovative delivery systems. However, it remains crucial to predict and analyse the endogenous degradation mechanisms in intra and extracellular environments to ensure the functional activity of both oligonucleotides and delivery systems.

The work presented here elucidates the methodology developed to investigate the stability of a novel RNA delivery across distinct biological matrices, emulating the diverse conditions these molecules encounter from the site of injection to the site of action. The main objectives of this research are: i) to establish a systematic workflow to assess stability within biologically relevant matrices; ii) to subsequently evaluate how different chemical modifications and linkers influence the stability of ON against nuclease activity; iii) to assess their ability to endure in environments resembling endolysosomal vesicles, iv) development of new mass spectrometry tool to identify degradation products in biological matrices.

Our research demonstrates that chemical modifications within the RNA delivery system predominantly impact degradation, both extracellularly and intracellularly, while the oligo linker plays a role in therapeutic release rates. We observed a significant enhancement in serum stability for RNA delivery scaffolds compared to single-strand counterparts, with chemical modifications primarily driving degradation within endolysosomal vesicles. Additionally, none of the investigated delivery systems impacted RISC loading or siRNA activity, emphasising their role solely in biodistribution and stability enhancement.

In summary, this work outlines robust methodologies for stability evaluation prior to in vivo experimentation, providing a crucial tool for further scaffold designs. The approaches presented here can also be translated broadly to other therapeutically relevant oligonucleotides, ultimately enhancing their design in an informed and impactful way.

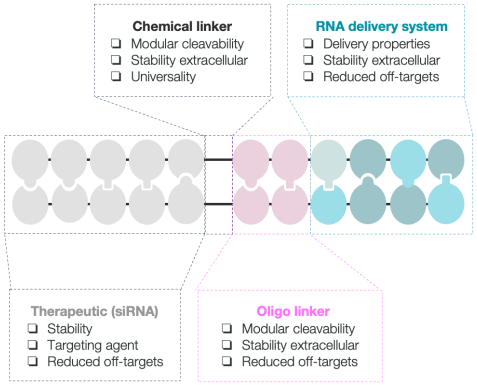


Figure 1. Graphical representation of construct components with the specific and ideal attributes of each one for proper functionality.

Figure1 suprabio.png

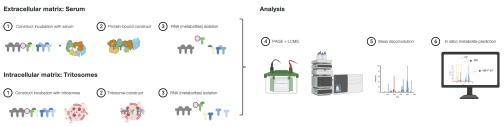


Figure2 suprabio.png

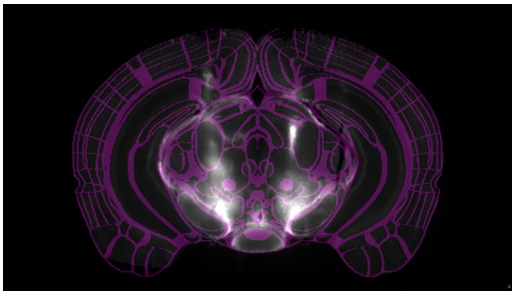
Study of Nanovectors for lysosome-based therapeutic strategies against neurodegenerative diseases

Wednesday, 17th April - 13:47: Drug Delivery - Oral - Abstract ID: 29

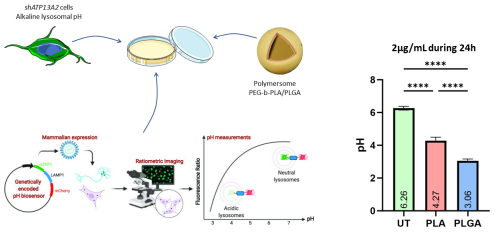
Mr. Rémi Kinet [1], ***Ms. Sarah Nieto*** [2], ***Dr. Angela Mutschler*** [2], ***Ms. Lea Mulot*** [3], ***Dr. Alexandra Gaubert*** [3], ***Prof. Philippe Barthélémy*** [4], ***Prof. Sebastien Lecommandoux*** [2], ***Dr. Benjamin Dehay*** [1]

1. *Institut des maladies neurodégénératives - UMR5293,* ***2.*** *Laboratoire de Chimie des polymères organiques (LCPO), UMR5629,* ***3.*** *ChemBioPharm - ARNA - Université de Bordeaux - Inserm U1212 - UMR CNRS 5320,* ***4.*** *University of Bordeaux*

Neurodegenerative disorders like Parkinson's disease (PD) have emerged as a critical health concern with these complex and age-related diseases, which are characterized by a selective neuronal vulnerability, including degeneration in specific brain regions hosting dopaminergic (DA) neurons and deposits of misfolded proteins. It has been hypothesized that autophagy-lysosomal pathway (ALP) failure partially accumulates pathogenic components in neurodegeneration. Lysosomes are responsible for the clearance of long-lived proteins, such as α-synuclein, which tends to aggregate due to poor folding. In the context of PD, this aggregation interferes with cell homeostasis and alters the system of cell degradation, leading to cell death. Overall, two possibilities can be envisioned to restore physiological conditions: an increase in the number of lysosomes or an increase in the lysosome's function. Regarding the first option, trehalose is a natural disaccharide that is a great candidate for neuroprotection against various neurodegenerative diseases. It bears a twofold nature: an mTOR-independent ALP biogenesis enhancer and a chemical chaperone. On another side, we demonstrated that using acidic nanoparticles (aNPs) made of PLGA could restore lysosomal pH and function in several models of lysosomal impairment. Thus, this project aims to develop nanosystem-based therapies for restoring ALP function and activity for treating lysosomal-related neurodegenerative diseases with the perspective of crossing the BBB and reaching the DA neurons. We will use trehalose-based derivatives to accommodate the different loading compartments available in polymersomes. To restore acidification defects, we will take advantage of the polymersome nature as a nanocarrier and an active compound. Indeed, the hydrolytic chain scission of PLGA provides lactic and glycolic acid units, which are expected to restore lysosomal pH and function.



Atlas.png



Ph.png

Targeting Genomic DNA with Oligonucleotides

Wednesday, 17th April - 14:05: Plenary Session 7 - Oral - Abstract ID: 84

Prof. Edvard Smith [1]

1. *Karolinska Institutet*

Efficient and sequence-specific targeting of double-stranded DNA (dsDNA) by synthetic ligands is a major objective for treatments aiming at the specific binding to selected chromosomal regions and also for certain aspects of biotechnology. Currently, most strategies targeting ds-DNA are dependent on engineered proteins, triplex forming oligonucleotides (TFOs) or minor groove binders. CRISPR-Cas9 can be directed to essentially any DNA sequence but relies on the ability of the very large, exogenous Cas9 protein to preopen the double helix. Double-helix invasion is a highly attractive mechanism for targeting dsDNA due to the theoretical simplicity of the design, which is based on Watson–Crick pairing rule. However, dsDNA remains difficult to access due to the stabilizing interactions in the double helix, i.e. base pairing and stacking. We have developed various forms of locked nucleic acid (LNA)—DNA mixmers to target dsDNA. The design has been in the form of TFOs, bisLNAs and clamps. The bisLNAs invade and hybridize to one of the DNA strands through both Watson-Crick and Hoogsteen base-pairing. The bisLNA forms a clamp structure with an extension, whereas clamps are LNA—DNA mixmers lacking the extension. These tools have been used both outside and inside cells and their properties and functional activity will be presented.

Authors Index

Alcaina-Hernando, M. 7
Alies, B. (Univ. Bordeaux, CNRS, INSERM, ARNA, UMR 5320, U1212, F-33000 Bordeaux, France) 40
Andriamiseza, F. 34
Appelhans, D. 19
Arpin, C. 29

Bansode, N. 21
Barbeta, G. 9
Barr, J. 43
Barry, H. 29
Barthélémy, P. 21, 28, 29, 40, 51
Basarir, F. 41
Battu, S. 40
Baylot, V. 28
Bergamaschi, G. 18
Bettache, N. 17
Bonduelle, C. 30
Bremer, J. 23
Bégaud, G. 40

Cadoni, E. 43
Calvo, J. 20
Cassie, E. 36
Chabbert, M. 34
cleret, p. 29
Clever, G. 23
Cordoba, A. 7
Cretich, M. 18
Cruz, C. 1

De, S. 41
Dehay, B. 51
Delledonne, A. 38
Dupin, I. 11

El Hamoui, O. 40
Esmaeili, F. 36

Ferraboschi, I. 7, 38
Ferriol Monjo, A. 9, 31
Fitremann, J. 34
Foot, G. 49
Fourqueaux, I. 34
Fresta, M. 13
Frigerio, R. 18

Ganesh, K. 47
Garcia, J. 28
García Coll, J. 17
Gaubert, A. 51
Gaudin, K. 40
Ghidini, A. 48, 49
Gibot, L. 34
Gissot, A. 21
Gori, A. 18
Grinstaff, M. 5
Gryko, D. 13, 25
GUEDIN-BEAUREPAIRE, A. 11, 28

Herrmann, A. 6
Ho, T. 9, 31
Hoang, K. 11
Hollfelder, F. 24
Howells, C. 12

Ibarboure, E. 30
Iovanna, J. 44

Kasmi, N. 34
Kauss, T. (ARNA U1212 INSERM) 28, 29
Kinet, R. 51
Kivunga, F. 28
Klimkowski, P. 49
Kokkoli, E. 27
Korczack, P. 28
Kostiainen, M. 41
Kumaranchira Ramankutty, K. 20

L. Cortajarena, A. 20
Lacomme, S. 40
Lacroix, A. 49
Lagouarde, J. 28
Lan, W. 44
Larumbe, A. 20
LATOUILLE, E. 11
Laudadio, E. 43

Lauria, T. 12, 31
Le Scouarnec, R. 30
Leblond Chain, J. (ARNA) 11, 30
Lecommandoux, S. 30, 51
Lepes, G. 40
Liljeström, V. 41
Lim, S. 31
Liz-Marzán, L. 20
Löffler, P. 15

Madder, A. 3, 16, 43
Martins, J. 43
Maurat, E. 11
Meffert, J. 16
Mishra, A. 26
Mousavi, M. 41
Mulot, L. 51
Musicò, A. 18
Mutschler, A. 51
Mäkinen, K. 41

Nguyen, H. (Department of Chemistry and Materials Science, Aalto University) 41
Nguyen, H. (School of Chemical and Physical Sciences, Victoria University of Wellington, and The MacDiarmid Institute for Advanced Materials and Nanotechnology, Victoria University of Wellington, Wellington 6021, New Zealand) 36
Nieto, S. 51
Nuñez-Martinez, M. 20

Peng, L. 44, 46
Perdrix, A. 49
Pescina, S. 7
Pieruccioni, L. 34
Pitot, E. 34
Pivetta, T. 11
Pizzi, A. 18
Plank, N. 36

rajana, n. 39
Reyes Fraile, L. 49
Risgaard, N. 15
Rivilla, I. 20
Rushworth, J. 49

Sanchez-Elsner, T. 9
Sanna, E. 49
Sayde, T. 40
Schellinck, S. 43
Scillia, L. 29
Seergazhi Gopalan, S. 2
Sharma, N. 32
Singleton, D. 12
Sissa, C. 7, 38
Smith, D. 22
Smith, E. 52
Stromberg, R. 45
Stulz, E. 9, 12, 31, 49

Tangsombun, C. 22
Thekkeyil, J. 33
Tian, X. 15
Turaj, A. 31

Ulrich, S. 17
Unsworth, C. 36

Vapaavuori, J. 41
Vascotto, C. 4
Ventosa, N. 7
Vialet, B. 21, 28
Vladimirovna, E. 9
Vogel, S. 15

Wang, A. 36
Wu, J. 44

Zou, F. 41

Conferences, Events & Workshops