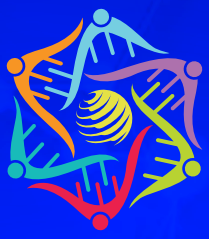

IRT International Roundtable

2021 VIRTUAL SYMPOSIUM

AUGUST 26–27

Phosphates Run the World: Chemical Biology and Applications of Nucleosides, Nucleotides and Nucleic Acids

WELCOME YOU TO THE IS3NA-IRT VIRTUAL SYMPOSIUM OF 2021

August 26, 2021

Dear friends and colleagues,

We welcome you to the IS3NA virtual symposium of 2021. We are gathering nearly 3 years after the wonderful meeting organized by Professor Yitzhak Tor and colleagues in San Diego in 2018. The 24th IRT planned for Stockholm has had to be postponed two years in a row, and planning for this virtual meeting began only four months ago. We initially thought to host a one-day virtual event for students, but due to considerable interest, here we are today with nearly 40 speakers, 90 posters, and 300 registrants around the globe. We realize this may not be the most convenient time for many of you. Your participation demonstrates the success of the field and your interest in the science behind nucleosides, nucleotides, and nucleic acids (3Ns).

Nucleosides, nucleotides, and oligonucleotides are no longer back seat passengers but real drivers. Over the past several decades, nucleoside and nucleotide derivatives have proven to be life-saving antiviral and anti-cancer drugs, and the more recent success of oligonucleotide-based therapeutics is equally impressive. Thanks to mRNA vaccines, "nucleic acid-based medicine" has become a common term. We expect that additional 3N-based therapies will be developed in the coming years and that many of you who have assembled for this virtual conference will contribute to future successful development of 3N-based drugs. The theme of this meeting is "Phosphates run the world: chemistry and biology of nucleic acids". During the next two days, presenters will discuss the biology of how phosphate-based molecules control various life processes and how chemistry makes that happen.

The first International Round Table (IRT) of 3N was organized by professors Jean-Louis Imbach and Leroy Townsend back in 1974. This symposium is dedicated to these two pioneers of 3N-based therapeutics. Since that first IRT, 23 conferences have been held around the world. These meetings have been well-attended by researchers from academia and industry. The interactions on the conference floor and outside the conference rooms have resulted in numerous productive collaborations, job offers, unforgettable heated discussions, and educational opportunities.

Special thanks go to our colleagues Piet Herdewyn, Kathie Seley-Radtke, Jean-Jacques Vasseur, and Suzanne Peyrottes for helping us put together a very exciting scientific program.

We thank all speakers, discussion leaders, short talk presenters, poster presenters for their enthusiasm, contributions and being part of our 3N community. Over the next two days, we will have the keynote address by Professor Kelvin K. Ogilvie, Dr. Victor E. Marquez will discuss the success story of nucleosides and nucleotides, and Professor David R. Corey will speak about recent triumphs of oligonucleotide therapeutics. We believe we have provided a well-balanced program where, 'veteran' and rising superstars will present 20 plenary talks. There will be 14 short talks given by our students, and nearly 90 posters summarized as short videos.

We have invited several important leaders of the field to Chair the talks and Discussion sessions in which all of us can participate and interact. In addition, there are various mechanisms for interactions made possible by our online platform.

We welcome and honor the many pioneers in our fields who have agreed to attend as our guests to participate at this first virtual symposium.

We would like to thank the sponsors who made this event possible for all of us. These sponsors include ChemGenes, Atea, Alnylam, Ionis, Aligos, Olix, Biospring, Hongene, Intellia, GenEdit, Oxeltis, Stoke, Generation Bio, ARTHEx Biotech, and AM Chemicals.

This event was very efficiently organized by Event Innovations, Inc. Special thanks go to Geri Beaty, Alexis Secka, Cindy Komlenic and their IT and AV teams. We also extend special thanks to Kim Altenbach of Alnylam for her assistance.

Finally, take a moment to visit the newly revamped IS3NA website, and make sure to follow updates regarding our future IRT meeting being organized by Dr. Roger Stromberg and colleagues in Sweden (August 28-31, 2022).

We hope that this virtual symposium will be both informative and fun!

Muthiah (Mano) Manoharan and Masad J. Damha

Muthiah (Mano) Manoharan, PhD
President, IS3NA & Alnylam Pharmaceuticals

Masad J. Damha, FCIC, PhD
Immediate Past President, IS3NA, & McGill Univ.

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IS3NA IRT

21 VIRTUAL SYMPOSIUM

AUGUST 26–27

Phosphates Run the World: Chemical Biology and Applications of Nucleosides, Nucleotides and Nucleic Acids

Contents

MISSION

Established in 2000, the IS3NA was the brainchild of Prof. J.-L. Imbach and Prof. L. B. Townsend, the two "fathers" of the International Roundtable meetings. They wanted the IRTs to continue but wanted control of the IRTs to become the responsibility of a new Society formed for that purpose. This new Society would not only take over the operation of the IRTs but would also bring together people interested in each aspect of research related to nucleotides, oligonucleotides, and nucleic acids.

The aim of the IS3NA is to capitalize on the knowledge of practicing members across several disciplines to understand the impact of nucleic acids in a plethora of cutting-edge scientific questions ranging from the origins of life to the development of novel therapeutics.

AIMS OF IS3NA:

- To coordinate and sponsor meetings and to promote publications.
- To provide means for the dissemination of knowledge dealing with current research.
- To act as a mediator for communication, cooperation, and understanding between scientists of all nationalities.
- To encourage national and international collaborations on research and application among academic, industrial, governmental and private institutional organizations.

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21 VIRTUAL SYMPOSIUM

AUGUST 26–27

Phosphates Run the World: Chemical Biology and Applications of Nucleosides, Nucleotides and Nucleic Acids

ABSTRACTS

Invited Speakers

KL01

Invention of Ganciclovir and First Total Chemical Synthesis of a tRNA Sequence

Kelvin Ogilvie PhD

Acadia University, Nova Scotia, Canada

Abstract

The author left graduate school in 1968 with the goal to be the first to chemically synthesize large RNA molecules setting a transfer RNA sequence as an objective that would clearly demonstrate the success of the method. His first innovative approach to RNA synthesis was unsuccessful but provided insights that led to the invention of ganciclovir. His pursuit of the silyl protecting group and the development of an automated coupling process led to the first total chemical synthesis of a transfer RNA sequence.

PL01

Design and therapeutic potential of purinergic receptor ligands

Kenneth A. Jacobson PhD

NIDDK, National Institutes of Health, Bethesda, Maryland, USA

Abstract

The purinergic signaling system responds to extracellular nucleosides and mono- and dinucleotides. Over several decades, it became apparent that it represents a complex signaling system present in every organ that is involved in the response to tissue stress. Purinergic receptors include four adenosine receptors and eight P2Y G protein-coupled receptors, as well as seven P2X ligand-gated ion channels. The P2X receptors are activated by ATP and the P2Y receptors by various nucleosides (ATP, UTP, ADP, UDP and UDP-glucose). Thus, ATP and other nucleotide derivatives that are essential intracellularly also have a fundamental role in extracellular signaling. Every cell in the body has some combination of purinergic P2X, P2Y or adenosine receptors, which are relevant to many diseases. For example, elevated adenosine in the tumor microenvironment suppresses the immune response – thus, adenosine A_{2A} antagonists or inhibitors of CD73 that produces adenosine are efficacious in cancer immunotherapy. Our lab has introduced many chemical modulators of the signaling purinome, both for the receptors and for the associated enzymes and transporters. With X-ray or cryo-EM structures now available for a few receptors, we used computational modeling methods to guide our ligand design. The A_1 and A_{2A} adenosine receptor structures serve as templates for the modeling by homology of the A_3 receptor. A structure-based approach has been taken to enhance the selectivity of A_3 agonists to >3000-fold. The A_1 and A_{2A} receptor agonists, but not A_3 agonists, have cardiovascular side effects that limit their clinical utility. We introduced the first A_3 adenosine agonists, which are better tolerated systemically, and two of which are now in advanced clinical trials for psoriasis, liver cancer, no alcoholic steatohepatitis, with no serious adverse effects in >2000 human subjects. Hopefully, there will soon be clinical trials of adenosine agonists for chronic neuropathic pain and brain ischemia. P2Y and P2X receptors boost the immune response, and therefore antagonists are being designed as treatment for inflammatory, allergic and ischemic conditions. P2X4 agonists protect cardiac tissue in heart failure. Thus, we are modulating purinergic signaling with small molecules, both nucleoside and non-nucleoside derived, for potential therapeutic advantage.

PL02

Discovery of Remdesivir and Beyond

Joy Y. Feng

Gilead Sciences, Foster City, CA, USA

Abstract

Remdesivir (RDV; GS-5734; Veklury®) is the first FDA-approved antiviral to treat COVID-19 and currently remains the only approved direct-acting antiviral for hospitalized COVID-19 patients. RDV is a single diastereomer monophosphoramidate prodrug of an adenosine analogue that is metabolized intracellularly in multiple steps to form the active nucleoside triphosphate analog, which acts as a potent and selective inhibitor of multiple viral RNA polymerases. This presentation will share the history of RDV discovery, its in vitro and in vivo broad-spectrum antiviral activity against multiple RNA viruses, and the characterization of its potential off-target effects. In addition, the overview will include a summary of RDV clinical development, its efficacy against new COVID-19 variants, and ongoing efforts to develop other forms of RDV.

PL03

Rapidly Advancing Transformational Therapeutic Solutions for Patients with Severe Viral Diseases

Jean-Pierre Sommadossi PhD

Atea Pharmaceuticals, Boston, MA, USA

Abstract

AT-527, an oral direct-acting antiviral, was specifically designed as a purine nucleotide prodrug to inhibit the viral RNA polymerase of single stranded RNA viruses. AT-527 is a potent antiviral agent against HCV and has demonstrated robust viral reduction in HCV infected individuals, regardless of genotype and stage of disease, including cirrhosis.

Early on in the COVID-19 pandemic, AT-527 was tested on SARS-CoV-2 and demonstrated *in vitro* to have very potent antiviral activity against SARS-CoV-2.

Given the evolving dynamics of COVID-19, medical interventions and oral therapeutics will be essential in stemming the tide of this pandemic, which is becoming endemic.

Consistent with *in vitro* data, results from a Phase 2 study in hospitalized patients with COVID-19, demonstrated rapid and sustained antiviral activity of AT-527 as well as viral clearance of SARS-CoV-2 RNA. Through a collaboration with Roche, AT-527 is currently in a global Phase 3 trial (MORNINGSKY).

A direct-acting antiviral against COVID-19 aims to prevent disease progression by minimizing or eliminating viral replication and thereby reducing the severity of the disease, preventing or shortening hospitalization, and also potentially preventing transmission of the virus to others. This makes AT-527 well suited for use in treatment and prophylactic settings and complementary to vaccines for COVID-19.

PL04

The Second Dimension of the Genetic Code

Thomas Carell PhD

Ludwig-Maximilians-University, Munich, Germany

Abstract

DNA stores genetic information in the form of the sequence of the four canonical bases dA, dC, dG and dT. DNA contains in addition epigenetic information, which is established by the four modified cytidine bases 5-methylcytidine (mdC), 5-hydroxymethylcytidine (hmdC), 5-formylcytidine (fdC) and 5-carboxycytidine (cadC) (Fig. 1, left).[1]Additionally, 5-hydroxymethyluridine may play a role as well. These bases are generated by Tet enzymes. The position and the kind of modified dC-base at a specific position in the genome establishes an unknown 2nd code in our genetic system (Fig. 1, left). Setting and erasing of these epigenetic bases controls the complete development process starting from an omnipotent stem cells and ending with an adult specialized cell (Fig. 1, right). I am going to discuss results about the function and the distribution of the new epigenetic bases hmdC, fdC and cadC in the genome.[2] I am showing how metabolic states influence the chemistry by setting and erasing these modified bases. Synthetic routes to these new bases will be discussed that enable the preparation of oligonucleotides containing these bases embedded. Particularly, isotope dilution and isotope tracing mass spectrometry was used us to understand the chemistry that occurs on these bases in the genome.[3] Interesting is the fact that base excision repair seems to play a central role during erasure of the bases and again mass spectrometry helped to quantify the repair processes involved epigenetics.[4] Finally, I am discussing potential präbiotic origins of modified bases.[5]

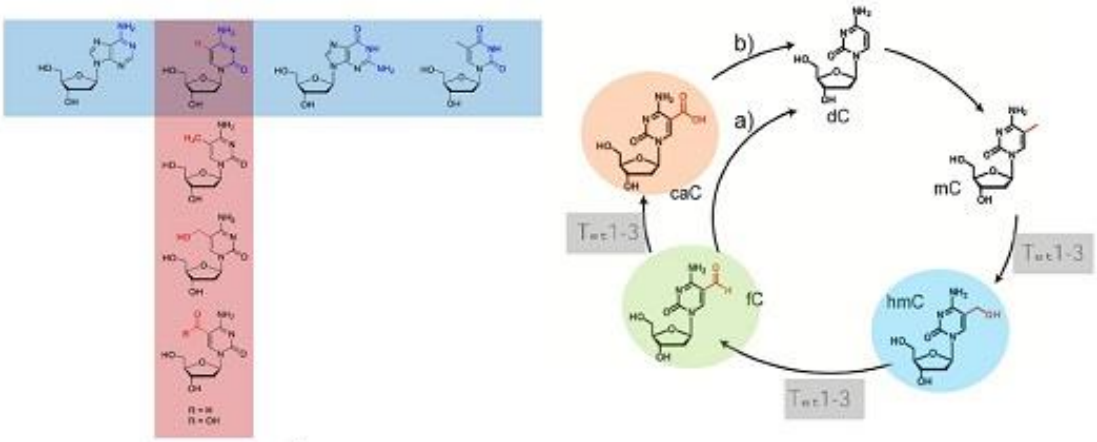

Figure 1. Illustrations of the 2nd orthogonal code that is present in DNA (left) and proposal of how the epigenetic bases are interconverted to establish dynamic changes of the epigenetic code during cellular development (right).

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ACKNOWLEDGEMENT

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PL05

Synthesis of 2’-dihalo ribonucleoside analogs

Franck Amblard PhD

Emory University, Atlanta, GA, USA

Abstract

We present herein an improved route to 2'-dihalo ribolactones which hinged on a completely diastereoselective fluorination reaction. These ribolactones were then utilized to prepare nucleosides in a newly optimized glycosylation reaction that took advantage of a crystallization induced dynamic resolution of the intermediate 1-lactols. The improved yields and diastereoselectivity in both phases of the synthesis allowed us to prepare 2'-bromo (or 2'-chloro)-2'-fluoro ribonucleosides more efficiently and with elimination of difficult diastereometric mixture separations.

PL06

A de novo Nucleoside Synthesis: New Opportunities for Medicinal and Process Chemistry

Robert Britton PhD

Simon Fraser University, British Columbia, Canada

Abstract

Nucleoside analogues are a major class of drugs used most commonly in the treatment of cancer and viral infections. While several decades of synthetic effort has provided reliable templates for nucleoside analogue synthesis, these processes are often protracted, not amenable to diversification and rely on a limited pool of chiral carbohydrate starting materials. These challenges are manifest for medicinal chemists tasked with lead diversification and can pose additional and significant complications to related process research efforts. Here, we report the collaborative efforts between researchers at Simon Fraser University and Merck that led to the development of a unique platform for rapidly constructing nucleosides and nucleoside analogues from simple achiral starting materials. Using only proline catalysis, we demonstrate that heteroaryl-substituted acetaldehydes can be fluorinated then directly engaged in enantioselective aldol reactions in a 'one-pot' reaction. A subsequent stereospecific intramolecular fluoride displacement reaction involving a stable N-F aminal provides a fully functionalized nucleoside analogue. The versatility and broad scope of this process is highlighted in the construction of D- and L-nucleosides and nucleoside analogues, locked nucleic acids, iminonucleosides, C4'-modified nucleosides and C2'-modified nucleosides. This process should become a valuable tool that supports future efforts in both drug discovery and development.

PL07

Enzymatic photocaging of nucleic acids: A strategy for controlling methyltransferase target sites by light

Andrea Rentmeister

Westfälische Wilhelms-Universität, Münster, Germany

Abstract

Methylation of DNA, RNA and proteins constitutes a major regulatory mechanism of biological processes. In nature, *S*-adenosyl-L-methionine (AdoMet) is the cosubstrate of most methyltransferases (MTases) and the main methyl source. Synthetic AdoMet analogues are also accepted by a number of promiscuous MTases or engineered variants.

In this talk, I will show how AdoMet analogs in combination with suitable MTases can be used to transfer photo-cleavable (PC) groups to DNA, RNA and the mRNA 5' cap.[1] This post-synthetic enzymatic modification and the light-induced removal provide methodology for site-specific “writing” and “erasing” marks on long DNA or RNA at natural MTase sites.

The second part of the talk will be dedicated to the enzymatic *in situ* generation of AdoMet analogs with PC-groups from their amino acid precursors using engineered variants of the enzyme methionine adenosyltransferase (MAT).[2] The enzymatic cascade accepting photocaging groups bears potential for future cellular applications.

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PL08

Stealth Fluorescence Labeling for Live-cell Imaging of RNA-based Therapeutics

Marcus Wilhelmsson Professor[1], Tom Baladi[2], Jesper R Nilsson[1], Audrey Gallud[3], Emanuele Celauro[4], Cécile Gasse[5], Fabienne Levi-Acobas[5], Ivo Sarac[5], Dženita Baždarević[6], Marcel R Hollenstein[5], Malin Lemurell[7], Anders Dahlén[2], Elin K Esbjörner[4]

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Abstract

With its multiple roles in biology, RNA has emerged as a promising, versatile drug modality by which previously undruggable diseases may be addressed. The number of approved RNA-based therapeutics including the recent development of RNA-based vaccines against SARS-CoV-2 is still low but the number of clinical trials using these modalities are currently rapidly increasing. Hence, RNA-based therapeutics are central to an ongoing paradigm shift in pharmaceutical industry but are still hampered by the low efficiency of cell delivery by the presently used methodology, *i.e.* lipid nanoparticles (LNPs). To facilitate this potential paradigm shift and to better understand the biology of RNA a more detailed mechanistic comprehension of cellular uptake, endosomal escape and cytosolic delivery of such drugs in combination with elucidation of where and why RNAs are localized to specific cellular compartments at certain time-points is needed. To enable such investigations, we have developed novel non-invasive fluorescence-based methodologies, which we have named stealth fluorescence labeling, for spatiotemporal monitoring of long and short RNA in live cells. Until now many widely used RNA analytical tools involve heavily modified nucleic acids, potentially resulting in loss of ability of the nucleic acid to be correctly recognized and processed by the enzymatic machineries of cells. The fluorescent base analogues (FBAs), which we develop and use for stealth labeling, are internal nucleobase-like fluorophores, which are less functionally perturbing than other external probes and designed to retain the normal base-pairing and -stacking of the target nucleic acid. Recently, we published the first two studies of live-cell fluorescence imaging of biologically relevant RNA modalities (gapmer ASOs and mRNA; mRNA approach exemplified schematically in the Figure) using FBA labels. Importantly, we also demonstrated high FBA tolerability of several enzymatic machineries – RNase H1 for ASOs, polymerases and ribosomes for mRNA – resulting in unaltered biological activity of most FBA-labeled constructs. Altogether, these results suggest that our FBAs are excellent mimics of their corresponding canonical bases and show sufficient brightness to be used in live-cell imaging of biologically critical RNAs in a standard confocal microscopy setup. Therefore, we believe that our FBAs will be beneficial to pharmaceutical industry, clinical laboratories, and academia aiming at improving the understanding of

uptake and endosomal escape mechanisms and allow them to take vital steps towards new and improved delivery strategies for next-generation nucleic acid-based drugs.

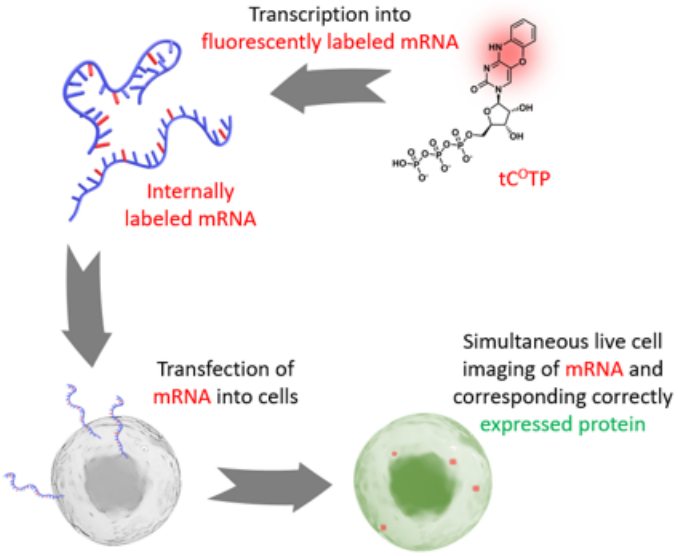


Figure. *General approach of stealth fluorescence labeling of mRNA and its use in live-cell imaging of uptake of mRNA-based therapeutics and translation of corresponding protein.*

PL09

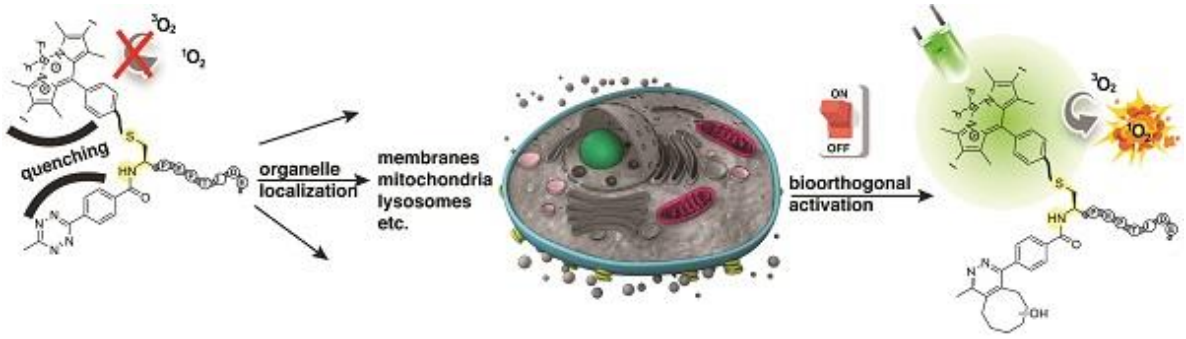
Light as a Tool for Understanding Biological Processes

Greta Linden, Lei Zhang, Lea Albert, Benedikt Heinrich, Olalla Vázquez

Philipps-Universität, Marburg, Germany

Abstract

Light is unsurpassed in its ability to modulate biological interactions. Along these lines, our group strives to design novel photosensitive molecules capable of sensing biological processes and remotely controlling the sophisticated cellular machinery behind relevant disease-related processes. In this talk, we will briefly present our contributions to both fluorophores to study bacterial infection[1] and optoepigenetic photoswitches,[2] and focus on photosensitizers. Indeed, to our knowledge, we first used bioorthogonal reactions to modulate the production of reactive oxygen species. Thus, our novel halogenated BODIPY-tetrazine conjugates only become efficient photosensitizers through the intracellular bioorthogonal inverse electron-demand Diels–Alder reaction.[3] Furthermore, our bioorthogonal approach enabled conditional phototoxicity and subcellular localization simultaneously. Recently, we extend this concept to a modular platform.[4]



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PL10

Development of Sugar-modified Oligonucleotides with Higher Enzymatic Stability Than PS Oligonucleotides

Satoshi Obika PhD

Osaka University, Suita, Osaka, Japan. National Institutes of Biomedical Innovation, Health and Nutrition, Ibaraki, Osaka, Japan

Abstract

Phosphorothioate (PS) linkages play an important role in the development of antisense oligonucleotides, and the *in vivo* stability of oligonucleotides can be dramatically improved by replacing phosphodiester (PO) linkages with PS linkages. Furthermore, the pharmacokinetic profile of PS oligonucleotides is better than that of PO oligonucleotides. However, when n-mer PS oligonucleotides are synthesized, they are obtained as a mixture of 2^{n-1} diastereomers, and there is a concern that PS oligonucleotides may be toxic owing to their non-specific binding to proteins; regarding the former concern, the development of stereoselective PS oligonucleotide synthesis methods is underway and future prospects look promising, and regarding the latter concern, one solution is to reduce the number of PS linkages introduced into the antisense oligonucleotides. However, replacing PS linkages with PO linkages greatly decreases the stability of the antisense oligonucleotide *in vivo* and reduces its activity.

Therefore, we focused on the synthesis of oligonucleotide derivatives with higher enzymatic resistance than that of PS oligonucleotides by introducing a suitable modification into the sugar moiety. First, we designed and synthesized 5′-dialkylated nucleoside derivatives. After several experiments, we successfully synthesized a nucleoside derivative, 5′-CP, in which the hydroxymethyl group at the 5′-position was replaced with a hydroxycyclopropyl group. The nuclease resistance of the obtained oligonucleotide derivatives was superior to that of the corresponding PS-modified oligonucleotides.

We previously reported a bridged nucleic acid, scpBNA, in which the oxygen atom at the 2′-position and the carbon atom at the 4′-position are connected by a cyclopropylene group. Based on this structure, we newly designed and synthesized scpBNA2 with a bulkier bridge structure between the 2′- and 4′-positions. We found that scpBNA2 could be easily introduced into the oligonucleotides with a similar efficiency as that observed for the original scpBNA and other bridged nucleic acids.

In addition, we designed and synthesized BANAs, novel derivatives of hexitol nucleic acid with a bridged structure between the 1′- and 3′-positions, and found that the oligonucleotides containing BANA3, one of BANAs, exhibited high nuclease resistance and good binding affinity to the target RNA.

In this presentation, I would like to demonstrate the design, synthesis, and properties of these three types of nucleic acid derivatives and their *in vitro* and *in vivo* biological activities.

PL11

PNAs with Double Face: Bimodal PNAs as Templates for Supramolecular Assemblies of Nucleic Acids

Krishnan N Ganesh FNA, FASc, FTWAS[1,2], Manoj Kumar Gupta PhD[3], Pramod Bhingaradeve PhD[2]

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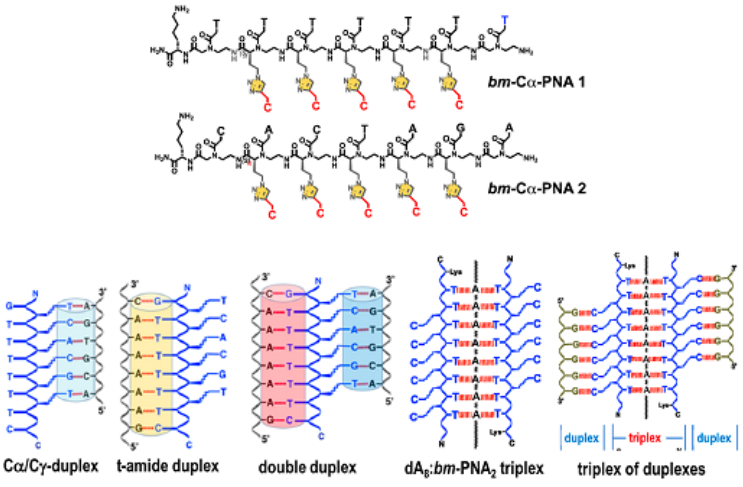
Abstract

Peptide nucleic acids (PNAs) are linear analogues of DNA with a neutral acyclic polyamide backbone that has nucleobases attached via *t*-amide link to a backbone consisting of repeating units of aminoethylglycine. They bind complementary DNA or RNA with sequence specificity to form hybrids that are more stable than the corresponding DNA:DNA/DNA:RNA duplexes. We envisioned new and novel PNA constructs termed "bimodal PNA" (*bm*-PNA) with the design of a second nucleobase linked at C_α or Cγ on backbone via amide/triazole spacer on each unit of *aeg*-backbone. Such *bm*-Cα/Cγ-PNAs with mixed sequences on both sides can form double duplexes by simultaneous binding to two DNAs, one complementary to the base sequence on t-amide side and the other to the bases on the Cα/Cγ-side chain. The duplexes in such *bm*-PNA:DNA_2 ternary complexes exhibit higher thermal stability than the isolated duplexes, indicating synergistic stability effects. Circular dichroism studies showed that assembly can be achieved by either by formation of triplex first and duplex later or vice versa. Bimodal PNAs are first examples of PNA analogues that can form DNA:PNA:DNA double duplexes via recognition by canonical pairing of natural bases. When *bm*-PNAs have homothymine (T7) on the t-amide face, they can form a triplex bm-PNA-T_8:dA_8:T_8-*bm*-PNA triplex and Cα/Cγ-side bases can in turn form duplexes with complementary DNA. This leads to a higher order pentameric assembly of triplex of two duplexes. *bm*-PNAs with homocytosine / homoguanaine sequences on one side can form the corresponding *i*-motif-C4 or G4-tetraplexes, which can subsequently bind 4 starnds of complementary DNA on the other face to form tetra duplexes of a tetraplex. Bimodal PNAs thus open up ways to design programmed higher order fused duplex/triplex/tetraplex supramolecular nucleic acid assemblies, in hitherto unprecedented manner. These have may potential applications in both biology and material science.



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PL12

New conjugates of DNA-boron clusters with two faces

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Abstract

Epidermal growth factor receptor (EGFR) is one of the most appealing molecular targets for anticancer therapy. Based on our previous studies [1,2], conjugates of EGFR antisense DNA oligonucleotides modified with boron clusters [*o*-carborane, $C_2B_{10}H_{12}$; dodecacarborane, $B_{12}H_{12}^{2-}$; and metallacarborane, $[Fe(C_2B_9H_{11})_2]^-$] (B-ASOs) were obtained and tested as potential agents in antisense therapy [3,4]. We also used boron clusters as a platform for generation of new materials [5]. For this, functional DNA constructs conjugated with boron clusters were developed. These B-ASOs, built from 1,2-dicarba-*closo*-dodecaborane linked with two anti-EGFR antisense oligonucleotides, form with their complementary congeners torus-like nanostructures, as shown by atomic force microscope (AFM) and transmission electron cryo-microscopy (cryo-TEM) imaging. Deepened studies carried out on B-ASO's properties [6] have demonstrated that in solution, B-ASOs formed four dominant complexes as confirmed by non-denaturing polyacrylamide gel electrophoresis (PAGE). These complexes exhibited increased stability in cellular medium comparing to the non-modified ASO. Fluorescently labelled B-ASOs localized mostly in the cytoplasm and decreased EGFR expression by activating RNase H. Moreover, the B-ASO complexes altered the cancer cell phenotype and decreased cell proliferation by arresting the cells in the S phase of cell cycle. The 1,2-dicarba-*closo*-dodecaborane-containing nanostructures did not exert an inflammatory response in human macrophages. Finally, as shown by inductively coupled plasma mass spectrometry (ICP MS), these nanostructures effectively penetrated the human squamous carcinoma cells (A431) even without assistance of transfecting agent showing their potential applicability as ASOs and boron carriers in BNCT therapy.

Acknowledgment: This research was financially supported by The National Science Centre in Poland [Grant number 2015/16/W/ST5/00413 for years 2015–2021].

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PL13

Improved Specificity of Conjugate siRNAs Through Chemical Modification

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Abstract

During the lead selection process, a subset of GalNAc-siRNAs does not pass the stringent safety criteria for nonclinical evaluation due to hepatotoxicity in the rat at suprapharmacological doses. Comprehensive mechanistic studies demonstrated that the observed hepatotoxicity can largely be attributed to miRNA-like, hybridization-based off-target effects. To minimize this undesired activity, we developed a novel design strategy termed ESC+, which may utilize a panel of chemical modifications aimed towards enhancing siRNA specificity. These ESC+ designs have translated to significant improvements in therapeutic index in rodents and improved human safety in the clinic.

PL14

New backbone chemistry enhances the drug-like properties of stereopure oligonucleotides in preclinical studies

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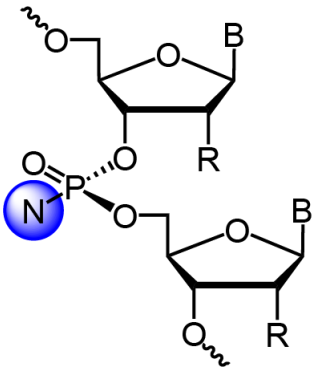
Wave Life Sciences, Cambridge, MA, USA

Abstract

Chemically modified oligonucleotides hold great promise for treating human disease. Using PRISM™, Wave can generate stereopure oligonucleotides—those in which the chiral configuration of backbone linkages is precisely controlled at each position—to target genetically defined diseases. We have expanded our repertoire of backbone chemistry to include chiral nitrogen-containing modifications, called PN linkages. Using phosphoryl guanidine as an example, we provide an overview of the advances we have made that allow us to synthesize stereopure oligonucleotides with chimeric backbone structure. We then apply this chemistry, showing that it improves key properties of stereopure oligonucleotides that act via different mechanisms. For silencing with RNase H, we highlight the impact of PN chemistry by showing >50% RNA knockdown lasting for 6-9 months in neural tissue from preclinical models. For splicing, we show that a PN-containing molecule that promotes exon skipping and restores robust expression of a truncated dystrophin protein extends the median lifespan of mice with a severe dystrophic phenotype from 7 weeks to more than 37 weeks. For RNA editing with ADAR (adenosine deaminases acting on RNA), we show that PN-containing stereopure oligonucleotides support up to 50% transcript editing in the liver of non-human primates. These data highlight the potential for backbone chemistry and stereochemistry to improve the drug-like properties of oligonucleotides.



PL15

In Vivo Therapeutic Genome Editing via Transient Expression of CRISPR/Cas9

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Abstract

Intellia Therapeutics, a leading clinical-stage genome editing company, is developing novel, potentially curative therapeutics using CRISPR/Cas9 technology for *in vivo* and *ex vivo* applications. The *in vivo* programs use our proprietary lipid nanoparticle (LNP) platform to deliver to the liver a two-part genome editing system: a guide RNA specific to the disease-causing gene and messenger RNA that encodes the SpCas9 enzyme, which carries out the precision editing. NTLA-2001 is the first investigational CRISPR therapy candidate to be administered systemically by IV infusion to patients. NTLA-2001 is designed to inactivate the *TTR* gene in liver cells to prevent the production of misfolded transthyretin (TTR) protein which accumulates in tissues throughout the body and causes the debilitating and often fatal complications of transthyretin (ATTR) amyloidosis. Robust preclinical data, showing deep and long-lasting TTR reduction following *in vivo* inactivation of the target gene, supports NTLA-2001's potential as a single-administration therapeutic. Interim Phase 1 clinical data released in June 2021 confirm substantial, dose-dependent reduction of TTR protein following a single dose for six ATTRv-PN patients across two single-ascending dose cohorts of the Phase 1 study by day 28. Treatment with NTLA-2001 led to dose-dependent reductions in serum TTR, with mean reductions of 52% among the three patients in the 0.1 mg/kg dose group, and 87% among the three patients in the 0.3 mg/kg dose group, including one patient with a 96% reduction. For patients with ATTR amyloidosis, we believe these interim results point to NTLA- 2001's potential to halt and reverse their disease with a single dose. This data provides clinical proof-of-concept for human CRISPR gene editing and substantially de-risks Intellia's clinical *in vivo* platform.

PL16

Oligonucleotides Based Approaches for Treatment of Chronic Hepatitis B

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Abstract

Chronic Hepatitis B is the most common chronic viral infection in the world (> 200mln carriers worldwide). We will describe challenges in treatment of CHB arising from unique viral cycle of this pathogen. We will also analyze state of the art in the field by contrasting clinical data from different modalities including Nucleic Acids Polymers, ASO and siRNA oligonucleotides. We will then describe Aligos HBsAg - centric approach toward CHB functional cure focused on advancing our STOPS™ (ALG-010133), ASO (ALG-020572) and siRNA (ALG-12755) oligonucleotides including MOA for STOPS™, utilization of Luxna XNA modifications in our ASO and results of new stabilization chemistry in our siRNA modalities.

PL17

A natural riboswitch scaffold with methyltransferase activity

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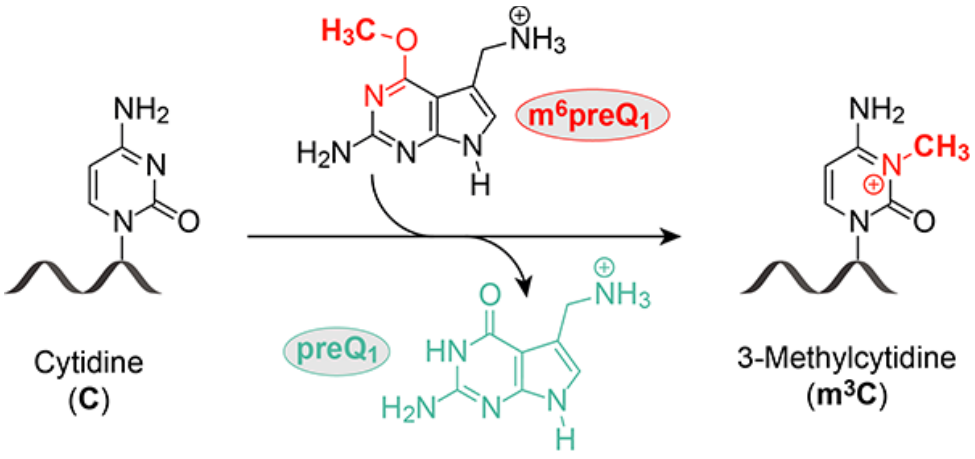
Abstract

Methylation is a prevalent post-transcriptional modification encountered in coding and non-coding RNA. For RNA methylation, cells use methyltransferases and small organic substances as methyl-group donors, such as *S*-adenosylmethionine (SAM). SAM and other nucleotide-derived cofactors are viewed as evolutionary leftovers from an RNA world, in which riboswitches have regulated, and ribozymes have catalyzed essential metabolic reactions. Here, we disclose the thus far unrecognized direct link between a present-day riboswitch and its inherent reactivity for site-specific methylation. The key is O^6-methyl pre-queuosine (m^6preQ_1), a potentially prebiotic nucleobase which is recognized by the native aptamer of a $preQ_1$ class I riboswitch. Upon binding, the transfer of the ligand's methyl group to a specific cytidine occurs, installing 3-methylcytidine (m^3C) in the RNA pocket under release of pre-queuosine ($preQ_1$). Our finding suggests that nucleic acid-mediated methylation is an ancient mechanism that has offered an early path for RNA epigenetics prior to the evolution of protein methyltransferases. Furthermore, our findings may pave the way for the development of riboswitch-descending methylation tools based on rational design as a powerful alternative to *in vitro* selection approaches.



PL18

RNA Functionalization via an Expanded Genetic Alphabet

Stephanie Kath-Schorr Prof. Dr.

University of Cologne, Cologne, Germany

Abstract

Pulsed electron-electron double resonance (PELDOR) is a powerful approach to study the global fold of biomolecules and has been applied to measure nanometer distances in both DNA and RNA. The efficient, site-specific introduction of spin labels, usually nitroxides, into oligonucleotides is a critical step for successful PELDOR experiments.

The synthesis of site-specifically modified long RNA molecules, which cannot entirely be prepared via solid phase synthesis due to its limitations in length, has remained a challenge. We employ a chemo-enzymatic approach by *in vitro* transcription based on an expanded genetic alphabet[1] to incorporate various functional groups into large RNAs at specific positions. For this, unnatural hydrophobic base pairs[1] are introduced into DNA to direct the site-specific introduction of unnatural ribonucleoside triphosphates during RNA transcription. By this approach alkene modifications, in particular norbornene and cyclopropene moieties, are enzymatically incorporated at specific positions into RNA by *in vitro* transcription allowing posttranscriptional labeling via additive-free inverse electron-demand Diels-Alder (iEDDA) cycloaddition[2-4] reactions.[5,6] We demonstrate that site-specific labeling by *in vitro* transcription is feasible for large, naturally occurring RNA molecules with complex foldings.[7] We extended our approach to incorporate nitroxide spin labels site-specifically into RNAs to study their folding by electron paramagnetic resonance spectroscopy.[8] Recent results on site-specific spin labeling of long non-coding RNAs will be presented.[9]

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PL19

Ribozyme gene switches for biosensing and regeneration studies

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Abstract

Chemical induction of gene expression is a important regulatory tool in nature and in biotechnology. In particular, inducible promoters and riboswitches found in nature dynamically control metabolism and cellular communication in response to intracellular and environmental signals. Efforts to expand this natural chemical diversity include the engineering of synthetic switches that incorporate novel RNA aptamers and other functional nucleic acids such as ribozymes. In particular, ribozyme switches that control gene expression using an mRNA degradation mechanism are generalizable to any gene and are poised to be important genetic controllers in animal models due to the lack of immunogenic protein components. This presentation will examine the work of our group in the design, screening, and characterization of ribozyme switches in vitro and in vivo. As one example, our group is applying these switches for spatiotemporally-controlled genetic manipulations in zebrafish as a proof of concept for vertebrate models of regeneration. Furthermore, we are developing switches that can serve as real-time intracellular biosensors for detecting cellular stress such as oxidative stress.

PL20

Rapid synthesis and delivery of antisense oligonucleotides enabled by machine learning

Bradley Pentelute

MIT, Cambridge, MA, USA

Abstract

There are more amino acid permutations within a 40-residue sequence than there are atoms on Earth. This vast chemical search space hinders the use of human learning to design functional polymers. Here we couple supervised and unsupervised deep learning with high-throughput experimentation to drive the design of high-activity, novel sequences reaching 10 kDa that deliver an antisense oligonucleotide to the nucleus of cells. The models in which natural and unnatural residues are represented as topological fingerprints decipher and visualize sequence-activity predictions. The new variants boosted antisense activity by 50-fold, are effective in animals, are nontoxic, and can deliver proteins into the cytosol.

TSRNA IRT

2021 VIRTUAL SYMPOSIUM

AUGUST 26–27

Phosphates Run the World: Chemical Biology and Applications of Nucleosides, Nucleotides and Nucleic Acids

STUDENT-POSTDOC SHORT TALKS

ST01

Enantioselective organocatalytic synthesis of non natural nucleosides by cross aldol addition

Leticia Lafuente PhD, Lautaro G Maidana Student, Mariano Nigro PhD, Adolfo M Iribarren Professor, Elizabeth S Lewkowicz Professor

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Abstract

In recent years, our research group has explored the synthesis and characterization of potential new antiviral agents such as acyclic nucleoside analogs using organocatalyzed aldol additions [1, 2]. While organocatalysis has emerged as a promising area for sustainable asymmetric synthesis [3] and aldol reactions have been widely used for the creation of the β-hydroxylcarbonyl structural motif, which is found in numerous biologically active natural products and biocative molecules, these combined approaches had not been applied to acyclic nucleoside synthesis.

Since not only modifications of the sugar moiety but also the nature of the heterocycle are crucial for the biological activities of nucleosides analogs [4], benzimidazole was tested as a structural mimic of the purine base. The presence of this heterocycle conferes on its derivatives a broad spectrum of biological activities when interacting with DNA, RNA or protein [5]. To synthesize novel benzimidazole-based acyclic nucleosides, we screened a series of commercially available amines such as pyrrolidine, L-proline, L-prolinamide, among others, as organocatalysts in the aldol reaction of oxo-benzimidazole derivative generated by *N*-alkylation of the bicyclic fused ring. Cyclohexanone was chosen as the aldol donor to study both enantioselectivity and diastereoselectivity of the reaction. The desired product was obtained with high stereoselectivity using L-prolinamide as catalyst.

Encouraged by these results, the same synthetic route was carried out using the *N*-alkylated derivatives of cytosine, adenine, uracil, obtaining the corresponding acyclic nucleoside analogs with high stereoselectivity.

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ST02

Triazolo nucleotide analogues as new chemotype for CD73 inhibition

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Abstract

Ecto-nucleotidases CD39 and CD73 play a central role in the control of the extracellular concentration of adenosine, which is responsible for immunosuppression in the tumor microenvironment. In the last decade, CD73 has been validated as a therapeutic target using anti-CD73 monoclonal antibodies and several of them are in clinical development for the treatment of cancer.[i] In the meantime, several independent groups reported the use of small molecules as CD73 inhibitors to block extracellular adenosine production.[ii] Our contribution concerns the design, the synthesis, and the biological evaluation of three series of nucleotide analogues including a triazole moiety.[iii] This multidisciplinary project involved synthetic organic chemistry, enzymology, molecular modeling, and cell-based assays.

Nucleobase replacement consisted in the introduction of a triazole moiety that is generated from 1,3-dipolar cycloaddition. Within, the 3 series of 4-substituted-1,2,3-triazolo-nucleoside analogues studied, the β-hydroxyphosphonylphosphonate ribonucleosides (series 2) proved to be the most interesting one. Two derivatives were able to reverse the adenosine-mediated immune suppression on human T cells and were more potent than the AOPCP, used as a reference CD73 inhibitor in the literature.

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ST03

Impact of Multivalency and Encapsulation of Affinity Reagents and Catalysts

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Abstract

Increasing enzyme efficiency and protease resistance is highly desired for therapeutics such as enzyme replacement therapy (ERT) and for commercial uses such as pet waste degradation and removal. The most common method for increasing enzyme efficiency is mutating key residues in these enzymes. However, this is very time consuming, costly, and a matter of trial and error. In addition, many enzymes do not function properly when key residues are mutated. The most common method for bypassing protease degradation is pegylation. However, pegylation can also lead to an enzyme misfolding and functioning at a lower efficiency. Another method for bypassing protease degradation is encapsulating enzymes. We propose that by encapsulating an affinity reagent with an enzyme, we can sequester the enzyme substrate and place the substrate in close proximity to the enzyme via virus-like particle (VLP) encapsulation, leading to a higher functioning enzyme and protease exclusion. As a model, we are using cocaine as a target substrate. Our affinity agents are various aptamers, single-stranded DNA that is capable of binding a specific substrate, that bind cocaine, and our model enzyme is cocaine esterase. Using three, reported cocaine aptamers, we characterized binding affinities with cocaine via microscale thermophoresis (MST) and melting temperature studies T_m using UV. In addition, we mutated key guanosine residues to inosine to provide a wide range of binding affinities. With this, we generated aptamers with binding affinities between 5μM and 80μM. We also have characterized wild type cocaine esterase using UV detection. We have also co-packaged enzymes and aptamers and compared the kinetics of free enzymes to those of packaged enzymes and co-packaged enzymes and aptamers. We found that encapsulating enzymes in VLPs with and without aptamers increased enzyme activity. Here we introduce a new and generalizable method for increasing enzyme activity.

ST04

Biophysical and biological evaluations of G-quadruplex thrombin binding aptamers stabilized by charge-transfer interactions

Kévan Pérez de Carvasal[1], Claudia Riccardi PhD[2], Irène Russo Krauss PhD[2,3], Domenico Cavasso PhD[2,3], Luigi Paduano Pr.[2,3], Jean-Jacques Vasseur Dir.[1], Michael Smietana Pr.[1], François Morvan Dir.[1], Daniela Montesarchio Pr.[2]

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Abstract

Aptamers are a class of short DNA or RNA molecules which, upon folding into peculiar three-dimensional arrangements, act as specific and high-affinity ligands for relevant targets.[1] One of the most studied aptamer is the thrombin binding aptamer (TBA).[2] This G-rich oligonucleotide interferes with the coagulation cascade, selectively recognizing the fibrinogen-binding exosite I of the thrombin which prevent blood clotting.[3]

In the search for more efficient thrombin binding aptamers,[4] we developed a library of 10 TBA analogues obtained by end-functionalizations with electron-rich 1,5-dialkoxy naphthalene (DAN or D) and electron-deficient 1,8,4,5-naphthalenetetra-carboxylic diimide (NDI or N) moieties known to interact strongly in water.[5] Indeed, we anticipated that when the G-rich oligonucleotide folds into its antiparallel G-quadruplex architecture effective charge-transfer donor-acceptor interactions between DAN and NDI residues will generate a stable pseudo-cyclic structure allowing an accommodation upon binding to thrombin. All doubly modified pseudo-cyclic TBAs were synthesized and characterized by a combination of biophysical techniques.

Our study revealed that these analogues exhibited higher nuclease resistance compared to the parent structure and along with a marked thermal stabilization as evidenced by their increased Tm values. These favorable properties were also associated with an improved anticoagulant activity of the derivative called **TBA-NNp/DDp** exhibiting also 1,3-propanediol (p) at the ends.Our results indicated that charge-transfer interactions represent an efficient approach to improve the aptamer features, while permitting the necessary flexibility needed to efficiently binds the thrombin.

Acknowledgment: This work was supported by University of Montpellier through to a PhD grant.

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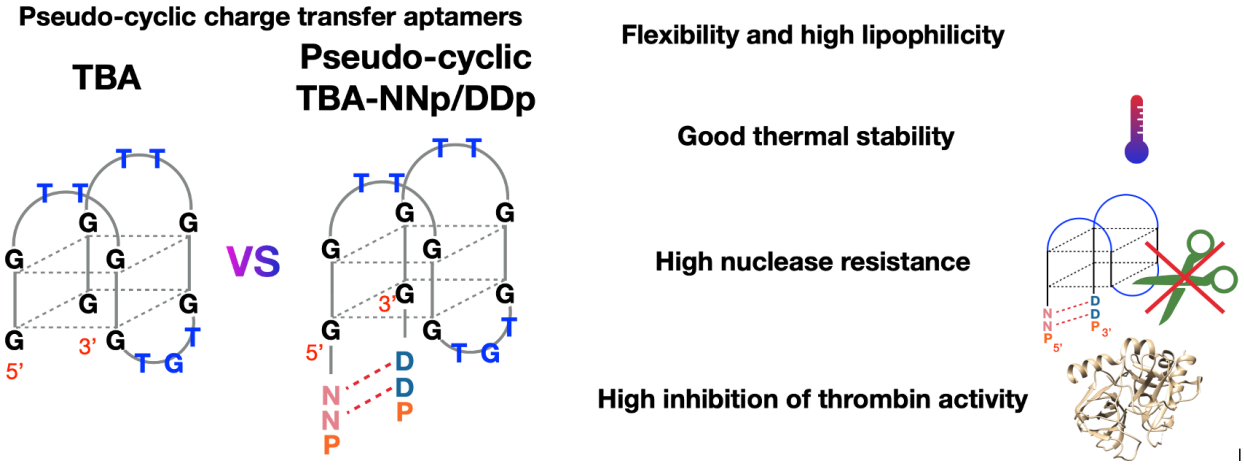

ST05

2'-Fluoroarabinocytosine substitutions promote the exclusive formation of a novel TC_5 dimeric structure with i-motif characteristics

Roberto El-Khoury[1], Miguel Garavís PhD[2], Hala Abou Assi PhD[3], Carlos J González PhD[2], Masad J Damha PhD[1]

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Abstract

Non-canonical i-motif structures have received significant interest in recent years due to emerging evidence for their biological relevance. Studies have illustrated their potential role in regulating the transcription of oncogenes and in maintaining telomere homeostasis.

i-Motifs form from cytosine-rich sequences and consist of two parallel duplexes, intercalated in an antiparallel orientation by hemiprotonated cytosine-cytosine base pairs ($C:CH^+$). Due to their protonation requirement, they are typically more stable at acidic pH *in vitro*.

The first i-motif was discovered in 1993 by Gehring *et al.* and was described as a tetrameric species of sequence $d(TC)_5$.[1] This well-characterized structure has been used as a model i-motif in subsequent studies. We have previously shown that substituting cytosines for 2'-fluoroarabinocytosines (araF-C) could increase the thermal stability of the tetrameric species at pH 5.[2] We have also found that additional araF-C substitutions result in unprecedented i-motif formation at neutral pH. The added stabilization has been attributed to favorable sequential and inter-strand contacts, facilitated by the fluorinated nucleosides.

In this study, we show that araF-C can be used to promote the exclusive formation of a previously undiscovered dimeric $d(TC)_5$ structure. By varying oligonucleotide concentration and annealing conditions, the formation of either the tetrameric or dimeric species can be modulated. To better understand the system at hand, we designed several sequences by varying the positions of araF-C substitutions. We then characterized dimer and tetramer structure and stability through native polyacrylamide gel electrophoresis (PAGE) and spectroscopic techniques, including circular dichroism, UV-Vis, and NMR spectroscopy. Our results show that the novel araF-C-rich dimeric species is a kinetic product with i-motif characteristics that forms at moderate oligonucleotide concentration under rapid snap-cool annealing conditions.

This study highlights a novel role for araF-C in the modulation of i-motif structure and is useful in further understanding and characterizing i-motif folding. Future studies will focus on better understanding the added i-motif stability imparted by araF-C substitution through additional NMR characterizations.

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ST06

Investigating the impact of epitranscriptomic modifications on RNA ensembles

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Abstract

Studies over the past decades have emphasized the dynamic nature of RNA structures showing that they do not adopt a single conformation but rather an ensemble of conformations that have different populations and that interconvert on timescales ranging between picoseconds and seconds. In addition to the dominant ground state (GS) secondary structure, the dynamic ensemble includes low-abundance short-lived RNA conformations referred to as 'excited states' (ESs) that form on the micro- to-millisecond timescale and possess alternative secondary structures. Despite being low-populated and short-lived, ESs have been detected with the use of NMR R1rho relaxation dispersion (RD) and chemical exchange saturation transfer (CEST) experiments. ESs have been shown to play critical roles in the folding and regulatory functions of RNAs and are increasingly considered to be attractive drug targets.

Recently, post-transcriptional modifications have been shown to play essential roles in the biological functions of coding and non-coding RNAs with more than 100 modified RNA nucleosides identified to date. These modifications can have a profound effect on RNA stability, translation, splicing, and RNA-protein interactions. Some post-transcriptional modifications, such as N^6-methyladenosine, primarily exert their biological activity by recruiting reader proteins. However, for other modifications, such as 2'-*O*-methyl (Nm), no reader proteins have been discovered to date. We hypothesize that these modifications affect biological processes by altering the RNA ensemble.

To investigate whether epitranscriptomic modifications have the potential to redistribute the RNA ensemble, HIV-1 TAR was chosen as a model system since it is one of the few RNAs for which a detailed and comprehensive ensemble is available. Our studies show that Nm preferentially stabilizes alternative secondary structures in which the Nm-modified nucleotides are paired, increasing both the abundance and lifetime of a low-populated short-lived ES by up to 10-fold. The extent of stabilization increased with the number of Nm modifications and was also dependent on Mg^{2+}. On the contrary, m6A destabilized the TAR ES relative to the GS by ~2 kcal/mol. Therefore, our results suggest that epitranscriptomic modifications could alter the biological activities of modified RNAs by redistributing their secondary structural ensembles as well as establish the utility of modifications as tools for the discovery and characterization of RNA excited state conformations.

ST07

Efficient Post-Synthetic Preparation of Oligonucleotide Conjugates *via* One-pot Diselenide-Selenoester Ligation-Deselenization/Alkylation

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Abstract

The modification of oligonucleotides is necessary in order to capitalize on their therapeutic potential. This entails the requirement for a breadth of strategies aimed at *in vivo* chemical stability, as well cell-targeting and cell-penetrating abilities. Peptide oligonucleotide conjugates (POCs) are an important class of oligonucleotide in this regard. The chemical linkages commonly utilized for this type of conjugate, however, have limitations in terms of their stability and/or synthesis. We report the first systematic assessment of POC synthesis using a diselenide selenoester ligation (DSL)-deselenization strategy, which rapidly generates a robust amide linkage. This approach utilizes a novel selenide phosphoramidite to construct a diselenide-bridged oligonucleotide dimer by solid-phase synthesis, which is then ligated to a C-terminal selenoester peptide. Deselenization, post-ligation, efficiently provides the desired POC in the same pot, under mild conditions. The diselenide handle was also shown to be highly versatile for fluorescent labelling, as well as for alkylation and subsequent micelle formation (lipid-oligonucleotide conjugates). It follows that adapting a DSL-deselenization strategy for the synthesis of POCs is not only efficient and high yielding, but allows for valuable late-stage functionalization. Furthermore, evidence is provided for the “deconjugation” of the prepared micelles, hypothesized to proceed through a selenoxide elimination pathway.

ST08

Lipid-oligonucleotide conjugates incorporating long-chain sulfonyl phosphoramidate groups: synthesis and biological properties

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Abstract

Widespread application of therapeutic oligonucleotides is hampered by their often inefficient cellular uptake and poor bioavailability. Conjugates of antisense oligonucleotides and small interfering RNAs with neutral lipids have improved transfection ability and extended half-life in the blood stream with beneficial impact on therapeutic activity *in vivo*.

We describe herein a set of new DNA and RNA derivatives, which incorporate one to four lipophilic *N*-(sulfonyl)-phosphoramidate groups, *viz*., dodecylphenyl or hexadecyl groups at internucleotidic positions near the 3'- or 5'-end (see figure). The oligonucleotides were obtained by automated solid-phase synthesis using Staudinger reaction with the corresponding sulfonyl azide to introduce the modifications, and characterized by RP-HPLC, PAGE and MALDI-TOF MS as described previously [1–3].

Oligodeoxynucleotides containing one or two lipophilic groups next to the 5'-end were not cytotoxic up to 20 uM for human RAW 264.7 or Mϕ cells, and were taken up by the cells much more efficiently than the unmodified counterpart. As shown by confocal microscopy, the oligonucleotides localized in the cytosol mainly in endosomes. Quantities of internalized oligonucleotide depended on the type of modification and the cell line. Oligonucleotides with hexadecyl groups were taken up better than the same with dodecylphenyl groups, and the ones carrying two lipophilic groups penetrated into cells more efficiently than those containing only one group [4].

Modified siRNAs containing two lipophilic groups next to the 3'-end of the passenger strand or four groups at the 3'-ends of both strands were also obtained. A known sequence targeting repulsive guidance molecule a (RGMa) was used. The siRNAs with two groups showed similar gene silencing activity in PK-59 cells as unmodified anti-RGMa siRNA, whereas siRNAs containing four modifications in both strands were less active. The siRNA with two hexadecyl groups was the best silencer [4].

This work was funded by RSF grant No. 19-74-30011 (cytotoxicity, confocal microscopy, flow cytometry, dynamic light scattering), RFBR grant No. 18-515-57006 (siRNA synthesis), the Ministry of Science and Higher Education of the Russian Federation, project No. FSUS-2020-0035 to Novosibirsk

State University (oligodeoxynucleotide synthesis), and Japan Medical Research Foundation grant No. 2018JP002 (biological activity).

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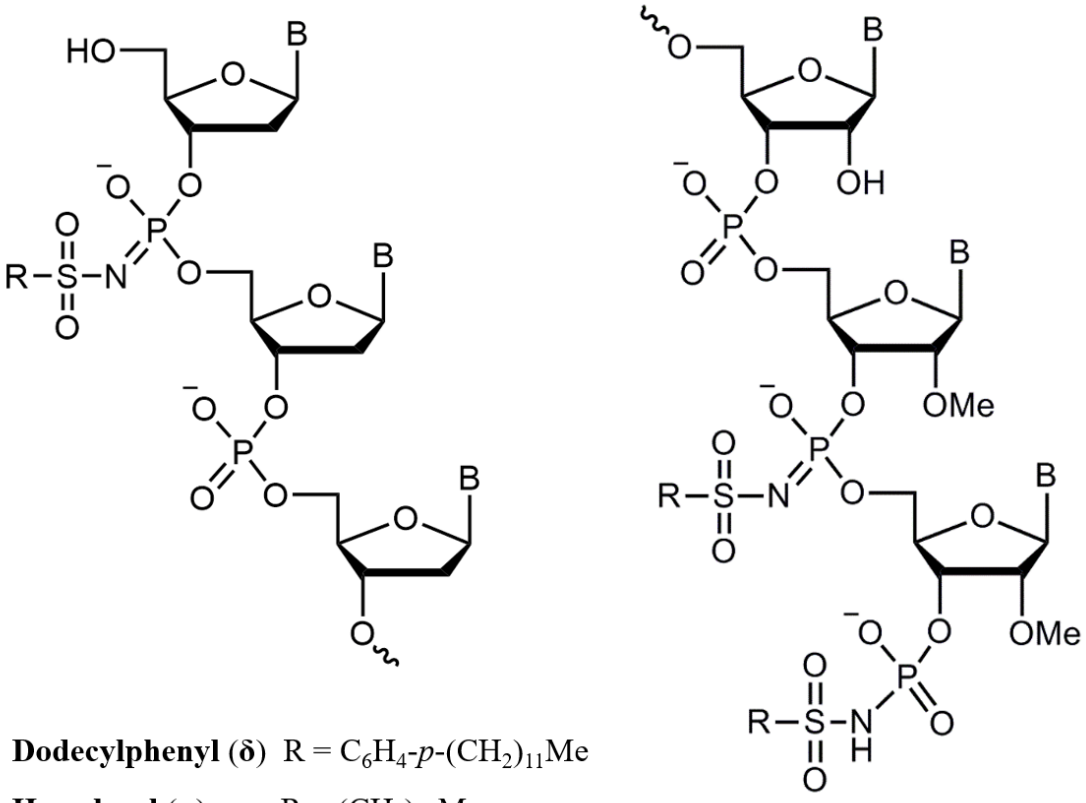

Dodecylphenyl (δ) R = C_6H_4-*p*-$(CH_2)_{11}$Me

Hexadecyl (η) R = $(CH_2)_{15}$Me

ST09

Regulation of circRNA expression in cell via RNA binding small molecule

Takeshi YAMADA Ph. D., Lu Ni M. Sc., Asako MURATA Ph. D., Kazuhiko NAKATANI Ph. D.

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Abstract

Circular RNA (circRNA) is a long single-stranded RNA with a covalently linked 5' and 3' termini, which commonly stems from the exons. They are generated via an alternative form of splicing reaction: back-splicing. This is usually promoted by the formation of a hairpin structure, facilitated by base-pairing between two introns flanking the circularizing exon. We demonstrate an RNA-binding small molecule can upregulate the expression of circRNAs in cellular environment, via promoting the base-pairing between the flanking introns, by engineering of a model construct where two introns have reverse-complementary sequence, and are responsive to a synthetic small molecule naphthyridine carbamate dimer (**NCD**). The results illustrate the feasibility of direct regulation of circRNAs at the biogenesis level using small molecules, which may provide a novel approach in studying of circRNA functions.

ST10

5'-cap modified oligoribonucleotides with *S*-adenosyl-L-methionine analogs mimicking RNA 2'*O*-methylation transition state as original inhibitors against Dengue and SARS-CoV-2 RNA 2'*O*-methyltransferases

Rostom Ahmed-Belkacem PhD[1], Priscila Sutto-Ortiz PhD[2], Joris Troussier M.S.[1], Adrien Delpal M.S[2], Etienne Decroly PhD[2], Bruno Canard PhD[2], Jean-Jacques Vasseur PhD[1], Françoise Debart PhD[1]

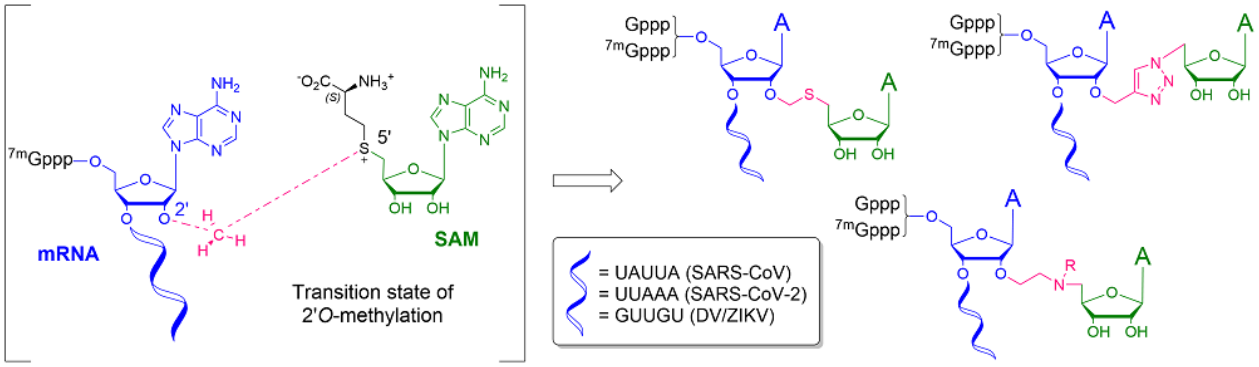
[1]University of Montpellier, Montpellier, France. [2]Aix-Marseille University, Marseille, France

Abstract

Our research aims at studying and targeting viral RNA methyltransferases (MTases) which play a crucial role by catalyzing the methylation of the RNA cap structure (GpppN1) using *S*-adenosyl-L-methionine (SAM) as the methyl donor. This cap at 5'-end of mRNAs is essential for their translation into proteins. Viral mRNAs are mainly methylated at the nitrogen in position *N*7 of cap-guanosine and at the 2'*O*-position of the N1 residue (adenosine for Dengue, Zika and SARS-CoVs) (^{7m}GpppA$_m$). These methylations are essential for RNA stability, protection against innate immune system and stimulation of the translation into viral proteins. Small-molecule RNA MTases inhibitors as SAM analogs have already been described but they show inadequate selectivity due to the high homology of the SAM binding domain of various MTases including human RNA MTases.

To overcome this lack of selectivity, we developed another approach with modified cap-oligoribonucleotides (ORNs) containing bisubstrate nucleoside analogs mimicking the transition state of the 2'*O*-methylation of the RNA with each substrate (**Figure 1.**) SAM analogs were attached to ORNs *via* various linkers containing S, N atoms or a triazolyl ring. The coupling SAM/ORN was achieved either before ORN elongation on solid support by synthesizing the corresponding dinucleoside phosphoramidite that was incorporated into ORN or post-ORN elongation using Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) reaction. Several 5'-end modified ORN with sequences figuring the 4 or 6 first nucleotides of mRNA in SARS-CoV-2 and Dengue/Zika viruses were synthesized.

Interestingly, preliminary screening showed significant inhibitions in the low micromolar range of NS5 (Dengue) and nsp16 (SARS-CoV-2). These modified ORNs will be evaluated as antiviral inhibitors in infected cells. Furthermore, the higher stability of the binary complex ORN-SAM analog / 2'-*O*-MTase might favor the co-crystallization and increase its crystal resolution.



ST11

Simple chemical approaches to introduce 2,6-diaminopurine and 2-aminoadenine conjugates into oligonucleotides

Mimouna Madaoui PhD, Dhrubajyoti Datta PhD, Muthiah Manoharan PhD

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Abstract

The 2,6-diaminopurine (DAP) nucleobase can form Watson-Crick base pairs with thymine in DNA and uracil in RNA. These pairs are stabilized by three hydrogen bonds resulting in improved thermodynamic duplex stability relative to base pairing with adenine. Previously reported strategies for synthesis of DAP building blocks for oligonucleotide synthesis require multiple steps and have exocyclic $-NH_2$ protecting group related issues. We have developed a post-synthetic strategy using the 2-fluoro-6-amino-adenine as the key nucleobase intermediate to make DAP-containing oligonucleotides. The synthesis of this new building block, which has been produced with different ribose modifications (e.g., R = H, F, OMe), its incorporation during solid-phase oligonucleotide synthesis, and deprotection using ammonia to obtain oligonucleotides containing DAP will be presented. Furthermore, this building block has also been used to enable post-synthetic conjugation of various ligands. The strong electronegative 2-fluoro makes the 6-NH_2 of adenine totally inert, and simple aromatic nucleophilic substitution of 2-fluoro makes reaction with NH_3 or RNH_2 feasible at 2-position.

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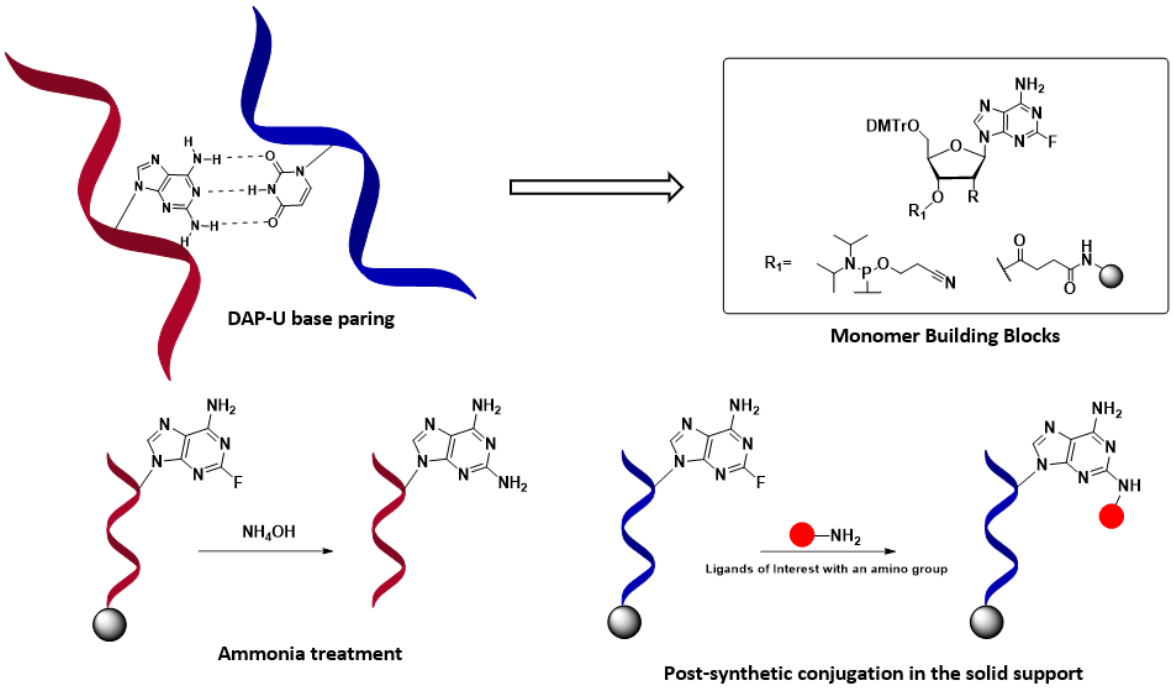

DAP-U base paring
Monomer Building Blocks
DMTrO
R1=
NH4OH
Ammonia treatment
Ligands of Interest with an amino group
Post-synthetic conjugation in the solid support

ST12

Multi-functionalized gold nanoparticles for the delivery of gapmer oligonucleotides to target mutant p53 cancer cells.

Eduardo García-Garrido (Organic Chemistry PhD student), Marco Cordani, Álvaro Somoza

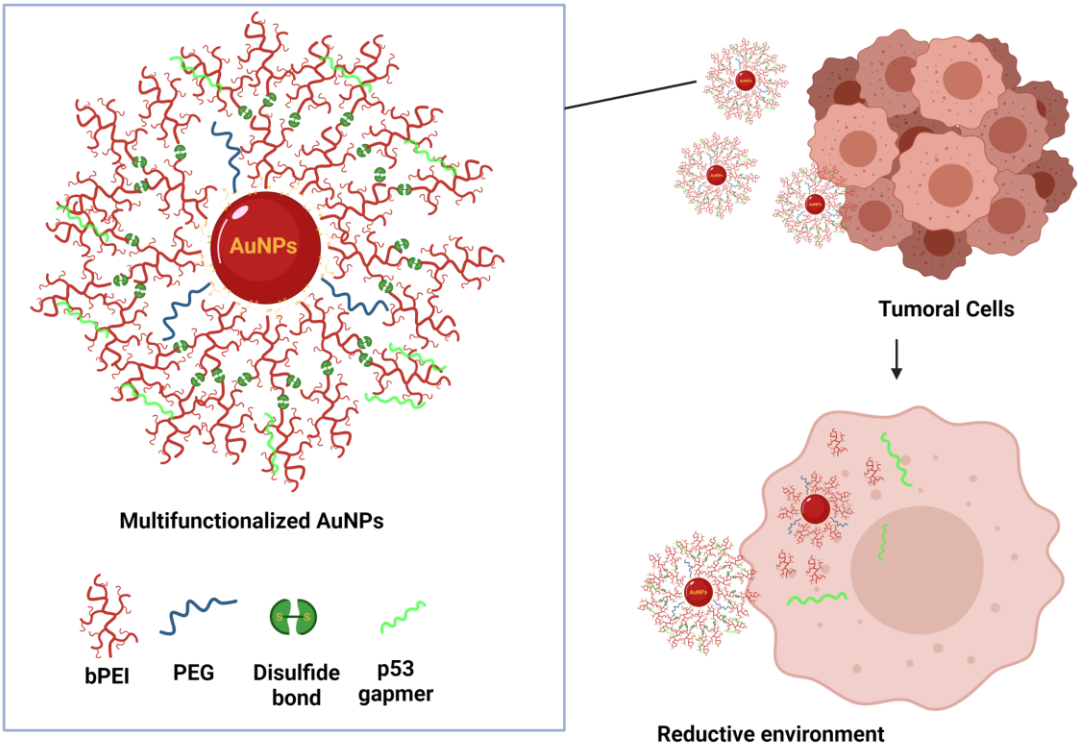
IMDEA Nanociencia, Madrid, Madrid, Spain

Abstract

TP53 is the most frequently inactivated tumor suppressor gene in tumors, which is mutated in over 50% of human cancer types. The loss of its tumor suppressor functions leads to the accumulation of genomic alterations culminating in cancer progression. In addition, some mutant p53 (mutp53) proteins may acquire oncogenic functions. To prevent cancer development, therapeutic oligonucleotides have been explored to downregulate mutp53 protein levels. However, their use is limited by the delivery process, which needs to ensure the stability of the oligonucleotides till the target cell is reached to provide optimun therapeutic effect. In this regard, AuNPs have been employed as carriers of nucleic acids due to their low toxicity, high biocompatibility, and easy to tailor surface. In this regard, the addition of different molecules can tune their properties, including water solubility, blood circulation time, and pharmacokinetics, which can improve the delivery efficiency.

In this work, we have developed a new nano-formulation consisting of a combination of a polymeric mixed layer of polyethylene glycol (PEG) and branched polyethylenimine (bPEI) (low molecular weight) and layer-by-layer assembly of bPEI through a sensitive linker. bPEI is one of the most popular non-viral transfer agents of nucleic acids due to its cationic characteristics. However, the efficiency is stretchily correlated to its geometry and molecular weight, directly affecting its cytotoxicity. Low molecular weight bPEI are much less toxic, but the efficiency of condensing the nucleic acids usually gets affected. However, the developed nanoformulation presents the same efficacy as a transfer agent of higher molecular weight bPEI, but with reduced toxicity. Additionally, the introduction of a sensitive linker consisting of a disulfide bond, to produce the polymeric layer assembly, help the release of the nucleic acids in the presence of reductive agents as found in the tumoral environment.

This novel nano-structures have been used as carriers of gapmers to target mutated p53. The delivery of the gapmers has led to a significant reduction of the expression of the mutp53 protein and cell viability. Notably, they also were able to reduce the chemoresistance to gemcitabine (GEM) in breast and pancreatic cancer cells carrying mutp53 proteins through the regulation of signaling pathways involved in cell growth and apoptosis.


AuNPs
Multifunctionalized AuNPs
bPEI
PEG
Disulfide bond
p53 gapmer
Tumoral Cells
Reductive environment

ST13

Promoter Recognition and Processive RNA Polymerization by a Clamping RNA Polymerase Ribozyme

Razvan Cojocaru PhD, Peter J. Unrau PhD

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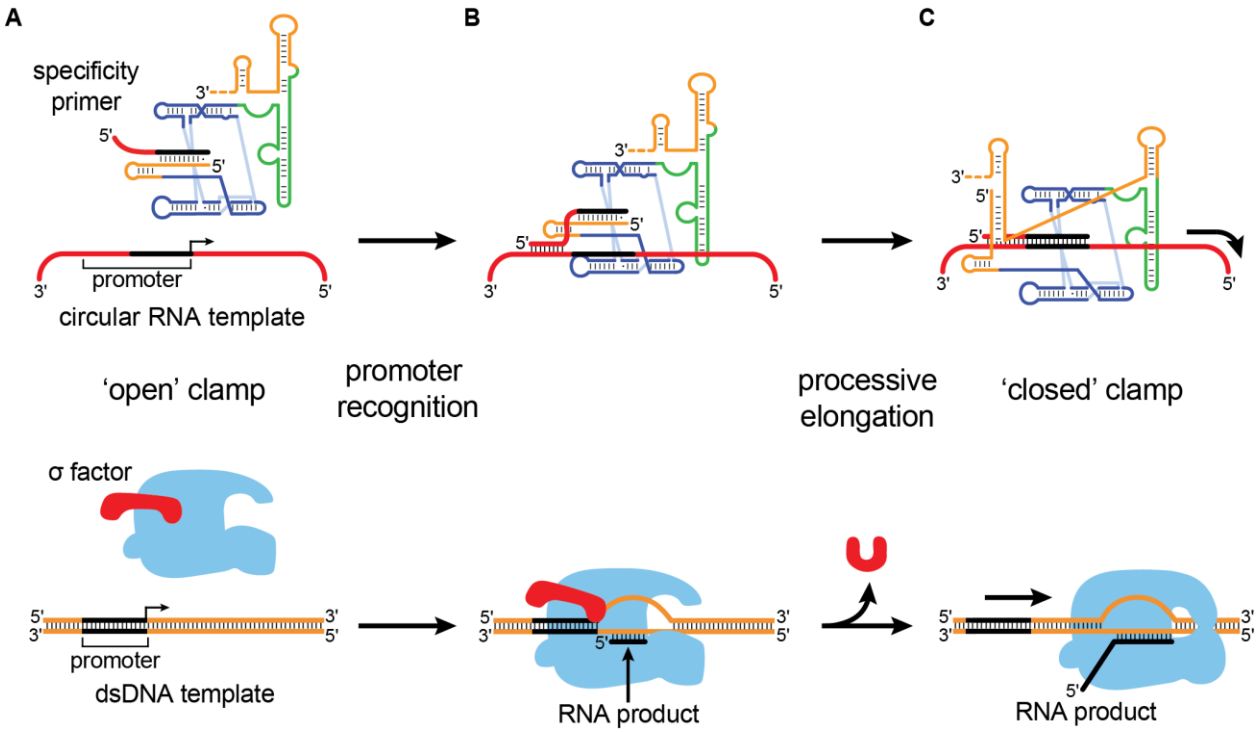
Abstract

The hypothesized “RNA World” proposes that the early evolution of life began with RNAs that can serve both as carriers of genetic information and as catalysts. Later in evolution, these functions were gradually replaced by DNA and enzymatic proteins in cellular biology. Arguably, the greatest limitation to exploring this hypothesis is the lack of a true RNA replicase capable of processive and general replication of RNA. Where the most current evolved variants of the class I ligase RNA polymerase ribozymes achieve processivity by direct hybridization to RNA templates or primers, we have recently implemented a complex selective strategy that screened ~10^{13} pool variants to isolate a processive polymerase ribozyme that is able to use an RNA clamp domain that mimics many of the mechanisms of modern protein DNA-dependent polymerases.

This clamping polymerase resembles in many ways a bacterial DNA dependent RNA polymerase (Fig. 1, bottom), taking advantage of hybridization mechanics by first partially hybridizing to a sigma factor like specificity-primer to form an 'open' clamp complex (Fig. 1A). This 'open' clamp form is able to search for and recognize a specific single-stranded RNA promoter. When found, the specificity-primer is displaced from the clamp onto the template (Fig. 1B), triggering a structural rearrangement to a 'closed' clamp form, gripping the template. This allows the ribozyme to couple the force generated from NTP incorporation to slide along the template as it extends the specificity-primer, enhancing its polymerization rate and processivity (Fig. 1C).

Depending on which specificity-primer it was formed with, the 'open' clamp complex shows promoter selectivity even in mixtures of promoter-templates. Subsequently, when correctly assembled, the 'closed' clamp complex results in more than one order of magnitude increase in extension, synthesizing duplexes of 50-107 bp in size. Additionally, the selected polymerase ribozyme can also synthesize part of its own specificity-primer which facilitates subsequent promoter specificity. The creation of a ribozyme polymerase that is self-priming, capable of promoter recognition and that has integrally coupled processivity helps demonstrate how RNA polymerase ribozymes could have preferentially recognized their own genomes and replicated specific gene targets in a primordial “RNA World”, functioning much like modern protein polymerases in gene expression.


A
B
C
specificity primer
3'
5'
5'
promoter
3'
5'
circular RNA template
'open' clamp
promoter recognition
processive elongation
'closed' clamp
σ factor
promoter
dsDNA template
RNA product
RNA product

ST14

Click and cut: Enzymatic production of targeted DNA scissors.

Brionna McGorman[1], Nicolo Zuin Fantoni[2], Afaf H. El-Sagheer[2], Tom Brown[2], Andrew Kellett[1]

[1]Dublin City University, Dublin, Ireland. [2]University of Oxford, Oxford, United Kingdom

Abstract

There is an increasing demand for the development of novel targeted drugs for the treatment of cancer and other diseases. One family of antigene therapeutics are short single stranded oligonucleotides that can hybridise to a specific sequence of double-stranded DNA forming a triple helical structure.[1] These triplex forming oligonucleotides (TFOs) can block transcription and inhibit protein production. Therefore, this antigene strategy is appealing for the field of targeted therapeutics. In this work a primer extension (PEX) protocol (Figure A) was applied to enzymatically synthesise chemically modified TFO hybrids. Click chemistry was employed to generate a library of artificial metallo-nuclease (AMN)-dNTP hybrids, which were then incorporated into the TFO strand by a DNA polymerase. To our knowledge, this is the first example of the enzymatic production of AMN-TFO hybrids. Through polyacrylamide gel electrophoresis (PAGE) and real-time quantitative PCR (qPCR) it was demonstrated that the AMN-TFO hybrid can selectively damage a DNA sequence of interest, while other *off*-target sequences remain intact (Figure B).[2,3] These results indicate that nuclease-TFO hybrids can overcome problems associated with non-targeted inorganic therapeutics and represent a potentially new class of gene knockout agents.

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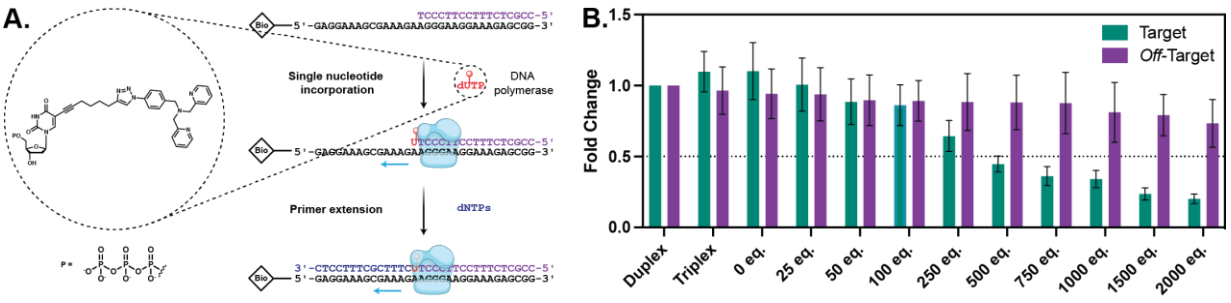

TSRNA IRT

21 VIRTUAL SYMPOSIUM

AUGUST 26–27

Phosphates Run the World: Chemical Biology and Applications of Nucleosides, Nucleotides and Nucleic Acids

POSTER ABSTRACTS

P01

Efficient alkene–alkene photo-cross-linking reaction on the flipping-out field in duplex DNA

Ahmed Mostafa Abdelhady, Kazumitsu Onizuka PhD, Sayaka Yajima, Kei Ishida, Eriko Mano PhD, Fumi Nagatsugi PhD

Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, Sendai, Miyagi, Japan

Abstract

Many enzymes acting on a nucleobase enable a specific chemical reaction by flipping the target base out of the helix and taking the base into an active site (Fig. 1A). For example, RNA editing enzymes flip the target base out of the helix, take the base into an active site, and convert adenine and cytosine to inosine and uracil by accelerating the hydrolysis of the amino group of adenine and cytosine. Similarly, artificial nucleic acids to induce the base flipping out are expected to create the specific field for chemical reactions, leading to a basis for the new functions (Fig. 1B).

In previous study, we reported a novel type of base flip-inducing oligodeoxynucleotide (ODN) and alkyne-alkyne photo-crosslinking reaction using two 3-arylethynyl-5-methyl-2-pyridone nucleotides.[1] In this study, we developed the base flip-inducing ODNs with alkene-type phenyl (Ph) or anthracenyl (An) base, which are 5-methylpyridone derivatives linked to aromatic compound (Ph or An) with an alkene linker at a C3 position. Both Ph and An bases flipped the complementary base on DNA and RNA and stabilized the flipped-out structure by the stacking interaction. In addition, a rapid photo-crosslinking was realized by taking advantage of the formed specific reaction field. The Ph-Ph combination provided a cross-linked product in a high yield by only a 10-sec photoirradiation (Fig. 1C). The highly efficient reaction enabled forming a crosslinked product even when using the duplex with a low T_m value. This will be helpful to make short strand cross-linking. Our alkene-alkene photo-cross-linking is prospective to be a new candidate to form crosslinked DNAs in addition to our previous alkyne-alkyne photo-cross-linking. Further efforts are underway toward exploring the unique biological and nanomaterial functions of the flipped-out cross-linked nucleic acids. All these results will elaborate in this presentation.

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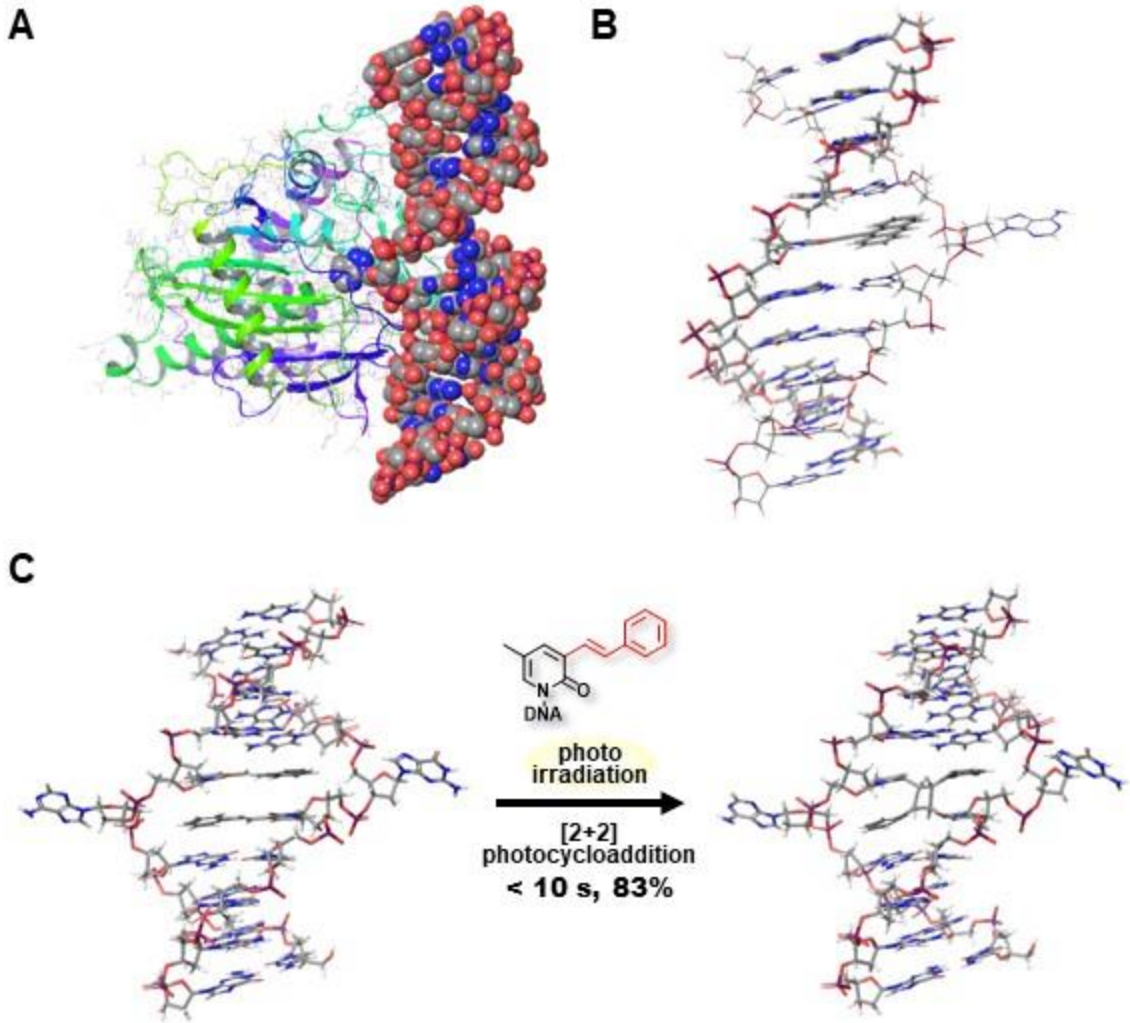

Fig. 1. Molecular design of the base flip inducing ODN. (A) Base flipping by human ADAR2 (PDB: 5ED1). (B) Base flipping by alkyne-type ODN, (C) Alkene-alkene photo-cross-linking reaction.

P02

Synthesis of new cyclic dinucleotide analogues (CDNs) modified by *N*-acylsulfonamide linkages

Romain Amador PhD Student, Jean-Jacques Vasseur PhD, Guillaume Clavé PhD, Michael Smietana PhD

IBMM, Univ Montpellier, CNRS, ENSCM, Montpellier, France

Abstract

Cyclic dinucleotides (CDNs) have been recognized as second messengers in eukaryotes [1] and reported as triggers for the immune response to pathogens. Indeed, CDNs could activate the stimulator of interferon genes (STING) protein, leading to the induction of cell defending type I-interferon pro-inflammatory cytokines [2] that will limit the infection of neighboring cells. Therefore, targeting the STING protein with CDN analogues depicts an interesting strategy to regulate the immune system and to study their therapeutic potential (e.g. bioactivity, cell penetration and nuclease resistance). In this context, several modifications of the nucleobases (mostly purine derivatives), the 2' or 3' hydroxyl groups of ribose, and the internucleoside phosphate group of CDN were investigated.[3]

Among the possible chemical modifications of the phosphodiester linkage group, the *N*-acylsulfonamide motif appears to be an interesting alternative. Indeed, this moiety is known to be a good mime of the phosphate group [4] while being neutral. It also presents an additional H-bonds acceptor site [4] that can interact within the active site of the STING protein.

The sulfo-click reaction is an efficient click reaction between a thioacid and a sulfonyl azide that leads to the formation of an *N*-acylsulfonamide derivative.[5],[6] Its exploitation in the context of nucleoside chemistry was previously reported by our group demonstrating bioorthogonal, quantitative and fast bioconjugaisons to nucleoside derivatives in biocompatible conditions.[7] Following this previous work, herein we present an original and efficient convergent synthesis of CDN exhibiting an *N*-acylsulfonamide internucleoside linkage using the sulfo click reaction. In this poster we will present the synthesis of these new derivatives which are obtained in 13 steps.

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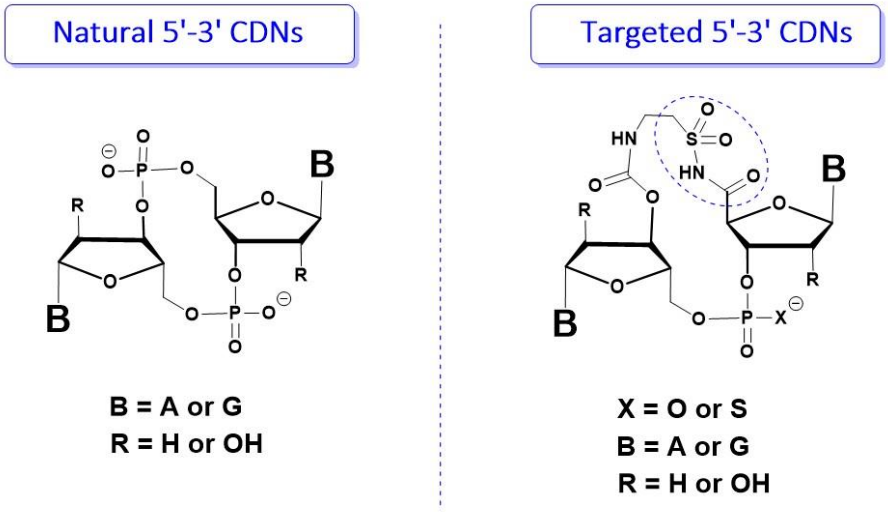

Figure : Comparison between a natural cyclic dinucleotide structure and the intended target.

P03

Fluorescent nucleotide-based probe as a tool for high-throughput screening of decapping enzyme inhibitors: targeting viral decapping enzyme D9.

Marcelina Bednarczyk[1,2], Renata Kasprzyk[2,3], Jagoda Starek[1], Jessica K Peters[4], John D Gross[4], Joanna Kowalska[1], Jacek Jemielity[2]

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Abstract

The stability of mRNA is an important factor affecting gene expression. mRNA levels in cells depend on the rate of its production and decay. The degradation of messenger RNA could be initiated by the decapping process during which the unique, protective cap structure is removed from the 5' end of target mRNA. Some viral proteins (e.g. from poxviruses) also possess this hydrolytic activity. For instance, D9 enzyme from Vaccinia virus is a Nudix hydrolase that recognizes m^7G cap structure in mRNA and cleaves its pyrophosphate bond between α and β phosphates to release m^7GDP. The cleavage activates host mRNA degradation process which leads to the shutdown of host protein synthesis [1].

Here, we present a simple, high-throughput, fluorescence-based assay to identify new, potent inhibitors of decapping enzymes with pyrene-labelled m^7G nucleotide as an activity probe. We used this approach to monitor the fluorescence intensity changes during enzymatic reactions on a 96-well plate reader in the presence of various small molecule compounds as potential inhibitors of viral D9 enzyme (Figure 1). We screened our in-house library of nucleotide-derived compounds as well as commercially available LOPAC®1280 library consisting mostly of drug-like molecules. The most potent compounds from the screening experiments were then verified on longer RNA substrates to confirm their inhibitory properties and characterized for their binding affinity, selectivity to the target by lower-throughput but more accurate biophysical methods.

This work was supported by grant from the Foundation for Polish Science (TEAM/2016-2/13).

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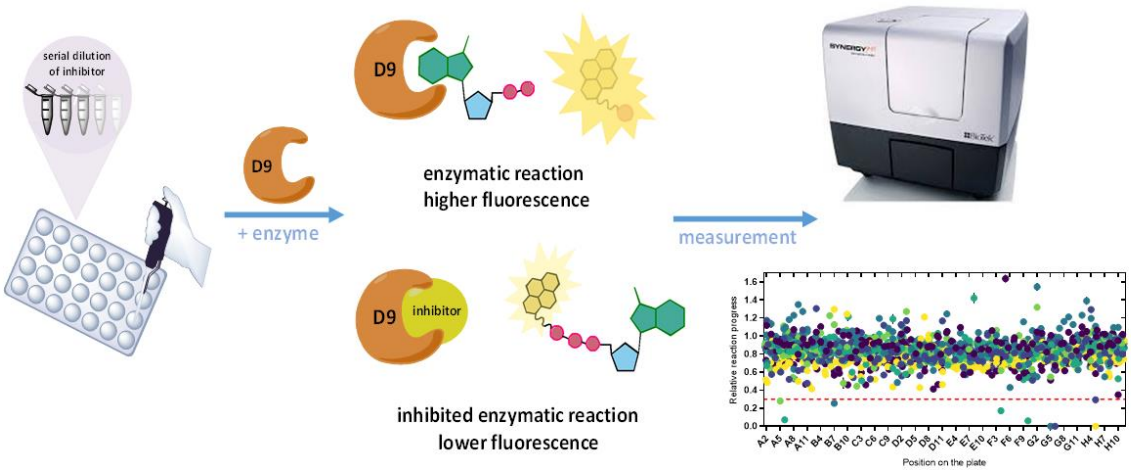
serial dilution
of inhibitor
D9
+ enzyme
D9
enzymatic reaction
higher fluorescence
measurement
D9
inhibitor
inhibited enzymatic reaction
lower fluorescence
Relative reaction progress
Position on the plate

P04

Probing structure and sequence effect of DNA i-motifs structures by native ion mobility mass spectrometry

Sanae Benabou Zdaou PhD[1,2,3], Valérie Gabelica PhD[1,2,3]

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Abstract

Cytosine-rich DNA sequences can form the i-motif structure, consisting of parallel-stranded duplexes held together by intercalated base pairs. The *in vitro* formation of i-motifs has been demonstrated by different classical spectroscopic techniques. Recently, the first direct evidence for its *in vivo* presence in human cells and control regulatory functions has been found. This structure is not only interesting from a biophysical and biomedical point of view, but also for their potential application in analytical chemistry or nanotechnology. Ion mobility spectrometry coupled to native mass spectrometry (IMS-MS) can be used to study i-motif structures because of its multiple advantages: it allows to isolate the molecules of interest from the solvent, depurinated DNA, etc. and study its intrinsic properties.

In this work, the topology of 34mer i-motif structures was studied in solution and in native mass spectrometry (produced from physiological ionic strength and with minimal internal energy input) by using ion mobility mass spectrometry (IMS-MS) to assess the ion shape. The gas-phase structures depend on the charge state. The lowest charge states have the inconvenience of being compact whatever the starting structure and ion activation conditions[1]. In this work, supercharging agents such as m-NBA were used as additives solution in electrospray ionization IMS-MS to increase the charge states of DNA i-motif at physiological ionic strength. As i-motif formation is favored at acidic pH, we investigate the ESI-IMS-MS behavior of i-motif structures at pH ~5.6 as well pH~7.1 for additional control.

The results show that at low collision energy, the mobility of ions having high charge states can directly indicate the solution folding into an i-motif structure. Moreover, the low charge states keep a memory of the electrospray charging process and they are related to the compactness of the gas-phase structures, but to reveal this memory, pre-IMS ion activation is necessary: folded structures need a higher voltage to unfold in the gas-phase. Multivariate data analysis approaches based on soft-modeling multivariate curve resolution methods was applied to pre-IMS data to obtain quantitative information such as the transition voltage. The combination of IMS and chemometric tools enable the interpretation of processes involving i-motif structures. In addition, we discuss also the effect of base composition on i-motif structures and its controls in gas-phase.

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P05

Characterization of Commercial Fluorescent DNA-Intercalating Dyes: Probing the Transcription-Induced B/Z-Transition

Hayley Bennett, Alyssa McAdorey, Tony Yan*

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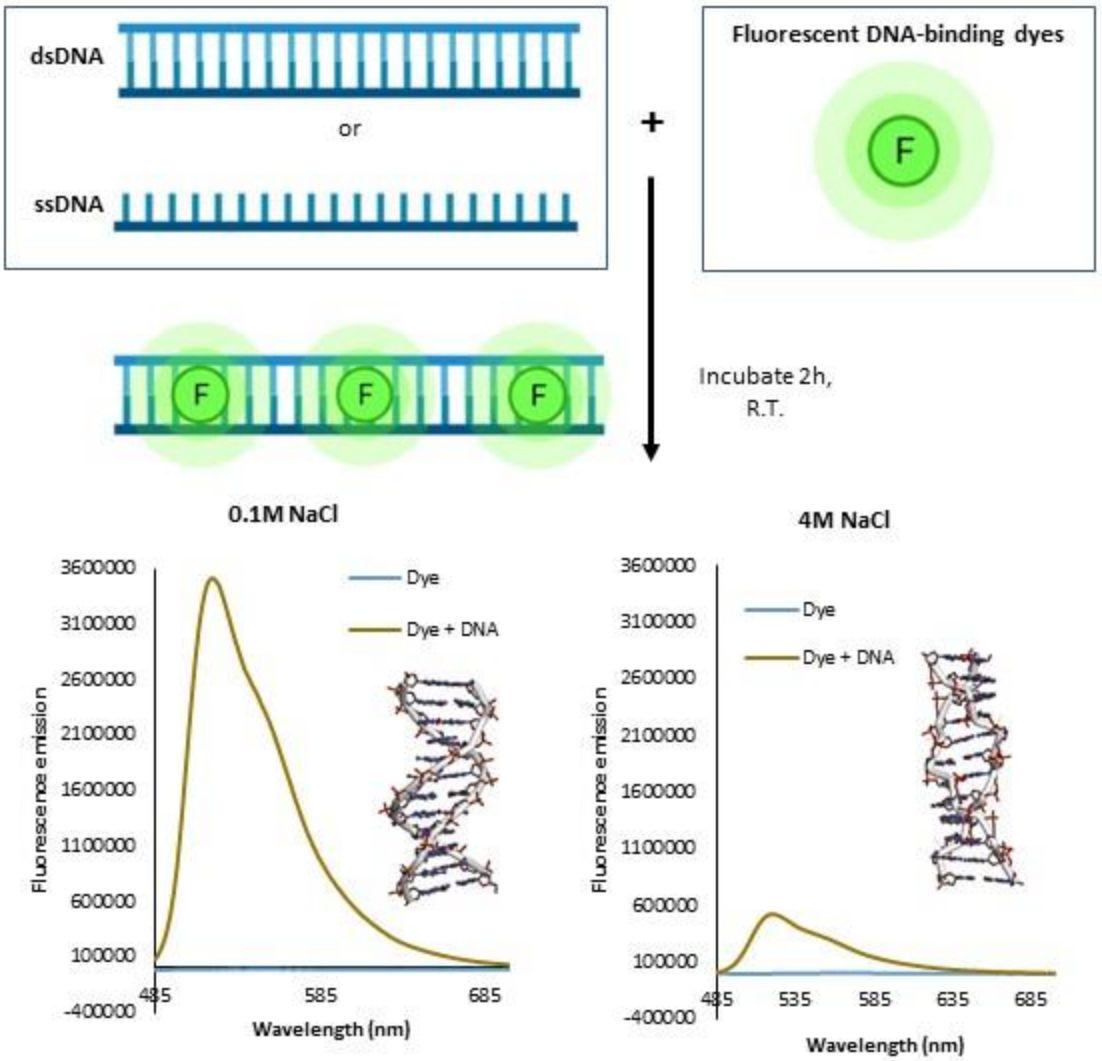
Abstract

Multiple conformations of DNA are recognized today. A-, B-, and Z- form DNA are distinguishable by a key set of structural features, including but not limited to their handedness, sugar pucker, groove width and depth, helical diameter, rise per base pair, and distance between phosphates. Although B-DNA is dominant under biological conditions, left-handed Z-DNA has been extensively studied over the last half-century to better understand the biological relevance of this unique conformation, and to elucidate the mechanism of the of B-Z DNA transition. Z-DNA is a high-energy form of DNA which can be induced and stabilized by factors such as high salt concentrations, Z-DNA binding proteins, and negative supercoiling. One biological process in which Z-DNA has been recognized is during transcription, wherein DNA sequences behind the site of transcription are subject to negative supercoiling which may support Z-DNA formation if sequence requirements are met. Given the potential for Z-DNA formation at this point, ways to dynamically explore this transcription-induced B-Z DNA transition are of interest. To this end, commercially available fluorescent DNA-binding dyes which may probe DNA under different conformations were analyzed. This current work outlines characterization of commercially available fluorescent DNA binding dyes as they interact with DNA under conditions which support either B- or Z-DNA conformations. Multiple classes of DNA-intercalating dyes have been explored – the phenanthridine-based ethidium bromide and propidium iodide, the cyanine-based SYBR Green I and SYBR Safe, and the bis-intercalating cyanine-based EvaGreen. Dyes were characterized according to their fluorescence emission spectrum upon incubation with DNA under low salt (supporting B-DNA formation) and high salt (supporting Z-DNA formation) conditions. Suitable dyes will be used for *in vivo* fluorescence microscopy studies to dynamically monitor transcriptional-induced transition of B-DNA to Z-DNA.


dsDNA
or
ssDNA
+
Fluorescent DNA-binding dyes
F
F
F
F
Incubate 2h,
R.T.
0.1M NaCl
Dye
Dye + DNA
Fluorescence emission
3600000
3100000
2600000
2100000
1600000
1100000
600000
100000
-400000
485
585
685
Wavelength (nm)
4M NaCl
Dye
Dye + DNA
Fluorescence emission
3600000
3100000
2600000
2100000
1600000
1100000
600000
100000
-400000
485
535
585
635
685
Wavelength (nm)

P06

Real-time Monitoring of Human Guanine Deaminase Activity by an Emissive Guanine Analog

Marcela S Bucardo, Yu Wu, Paul T Ludford, Yao Li, Andrea Fin, Yitzhak Tor

UCSD, La Jolla, CA, USA

Abstract

Guanine deaminase (GDA) deaminates guanine to xanthine. Despite its significance, the study of human GDA remains limited compared to other metabolic deaminases. As a result, its substrate and inhibitor repertoire are limited, and effective real-time activity, inhibitory, and discovery assays are missing. Herein, we explore two emissive heterocyclic cores, based on thieno[3,4-*d*]pyrimidine (th**N**) and isothiazole[4,3-*d*]pyrimidine (^{tz}**N**), as surrogate GDA substrates. We demonstrate that, unlike the thieno analog, th**G**$_{N}$, the isothiazolo guanine surrogate, ^{tz}**G**$_{N}$, does undergo effective enzymatic deamination by GDA and yields the spectroscopically distinct xanthine analog, ^{tz}**X**$_{N}$. Further, we showcase the potential of this fluorescent nucleobase surrogate to provide a visible spectral window for a real-time study of GDA and its inhibition.

P07

Synthesis and Evaluation of Functionalized Oligonucleotides as Serine Protease Mimics

Crystalle Chardet Master[1], Sandra Serres Master[1], Béatrice Gerland PhD[2], Corinne Payrastre PhD[1], Jean-Marc Escudier PhD[2]

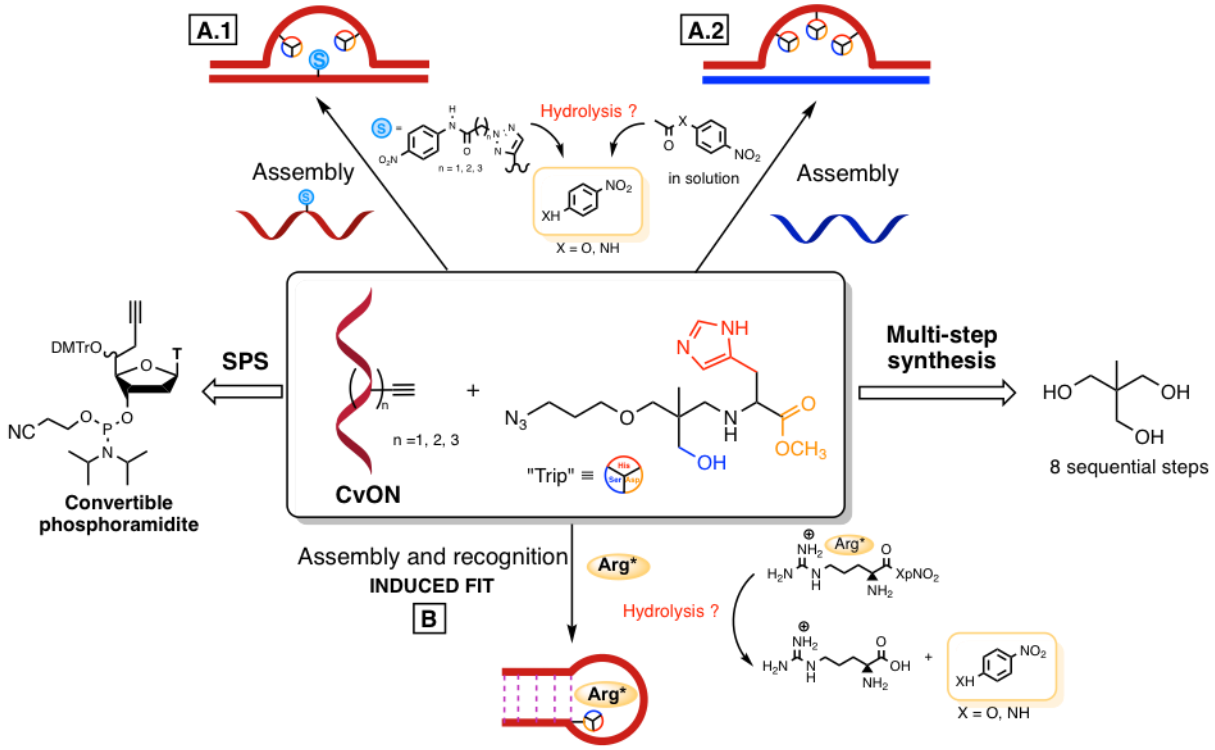
[1]Paul Sabatier University, Toulouse, France. [2]CNRS, Toulouse, France

Abstract

This project aims at the rational design of functionalized oligonucleotides (FuON) covalently modified to expand their catalytic repertoire towards biomimetic reactions to give access to protease-like DNA catalysts. In particular, the goal is to design nucleic mimics capable of performing the hydrolysis of the amide bond which is realised in Nature by serine proteases thanks to a catalytic triad of three cooperative amino acids, serine (Ser), histidine (His) and aspartate (Asp). A library of functionalized oligonucleotides was obtained using the phosphoramidite approach to introduce at precise coordinates along the oligonucleotide backbone amino acid side chains-like residues. These residues were conjugated on an oligonucleotide using "click" chemistry as one tri-functionalized azide building block ("**Trip**"). This approach involves the use of **convertible nucleotides**, bearing an alkyne group introduced during the solid phase synthesis.

With carefully designed sequences and thanks to oligonucleotides ability to self-assemble, the obtained FuON were arranged into flexible secondary structures (such as bulges or hairpins) by the use of a pertinent modified (or not) complementary strand. The stability of these modified structures was then evaluated using T_m studies to optimize the sequences length and the positions of introduced modifications. The catalytic properties of those DNA catalysts were then evaluated on chromogenic ester or amide substrates, presented either on the complementary strand of the FuON (A.1) or free in solution to study turn-over possibility (A.2).

We are also working on aptamer sequences in order to ally recognition and catalytic properties to construct an aptazyme able to perform specific hydrolysis of a fluorogenic amide or ester substrate (B). In order to create the most efficient aptazyme, we first currently studied, using circular dichroism, the impact of the position and the size of the introduced catalyst on the recognition of the substrate. Finally, we are currently studying the hydrolytic properties of the most promising functionalized aptazymes.


A.1
A.2
Hydrolysis ?
in solution
Assembly
Assembly
X = O, NH
SPS
DMTrO
NC
Convertible
phosphoramidite
n =1, 2, 3
CvON
"Trip" ≡
Multi-step
synthesis
8 sequential steps
Assembly and recognition
INDUCED FIT
B
Arg*
Hydrolysis ?
Arg*
X = O, NH

P08

β-Substituted acyclic nucleosides phosphonates as potent antimalarials

Thomas Cheviet PhD[1], Manon Lagacherie[2], Sharon Wein RI[3], Rachel Cerdan Pr.[3], Suzanne Peyrottes PhD[1]

[1]IBMM / University of Montpellier, Montpellier, France. [2]IRD, Montpellier, France. [3]LPHI-DIMNP / University of Montpellier, Montpellier, France

Abstract

Malaria is the most lethal parasitic disease (more than 400 000 deaths per year worldwide) and the resistance phenomena against current treatments, such as chloroquine or artemisinin (ACTs), strengthens the urgency to find new ones.[1] With the aim to overcome resistance, novel targets are studied, and among them the purine metabolism pathway of the parasite (*Plasmodium falciparum)* has received a particular interest these last years.[2] Indeed, unlike mammals, *Plasmodium* parasite has only one tool to produce its genetic materials from purine derivatives: the salvage pathway, which consist in the use of purine metabolites originating from the infected erythrocyte.[3]

Herein, we will report syntheses and biological activities of novel series of ANPs, highlighting among them a very promising lead with significant antimalarial activity *in vitro* against asexual blood stages (in the nanomolar range) and efficacy *in vivo* (*Plasmodium*-infected mice).[4]

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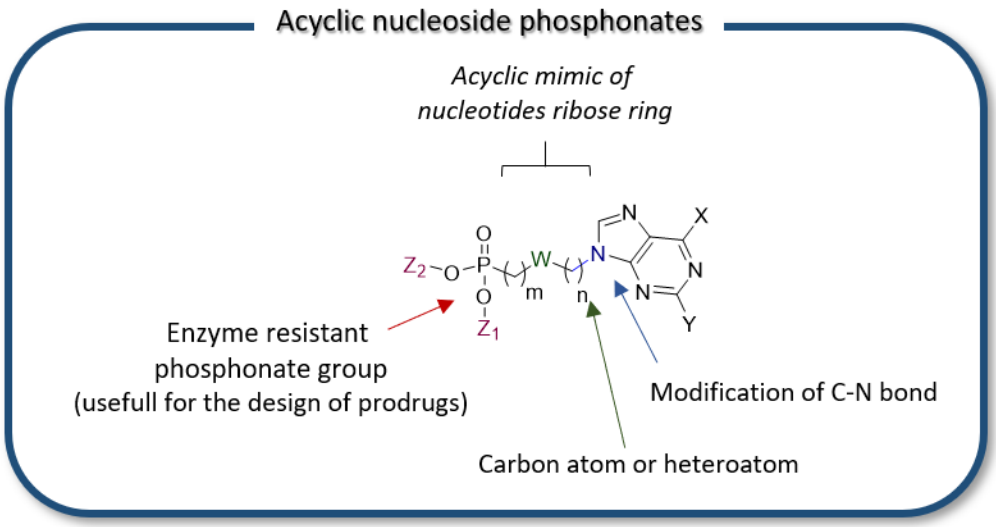
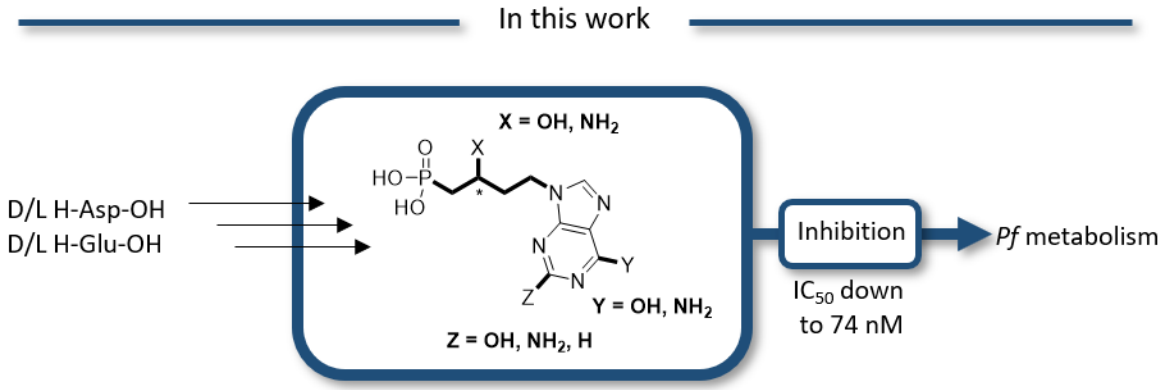

Acyclic nucleoside phosphonates
Acyclic mimic of
nucleotides ribose ring
Enzyme resistant
phosphonate group
(usefull for the design of prodrugs)
Modification of C-N bond
Carbon atom or heteroatom


In this work
D/L H-Asp-OH
D/L H-Glu-OH
X = OH, NH2
Y = OH, NH2
Z = OH, NH2, H
Inhibition
IC50 down
to 74 nM
Pf metabolism

P09

Synthesis of Triazole-Linked SAM-Adenosine Conjugates: Functionalization of Adenosine at N-1 or N-6 Position without Protecting Groups

Dylan Coelho, Colette Atdjian, Laura Iannazzo, Mélanie Ethève-Quelquejeu, Emmanuelle Braud

Paris University, Paris, France

Abstract

RNAs undergo numerous post-transcriptional modifications regulating their fate and function at the cellular level. Among these modifications, methylation at the N6 position of adenosine (m^6A) in mRNA is crucial for RNA metabolism, stability, and other important biological events. Methylation of the N1 position is found mainly in ncRNA and mRNA. This rearrangement plays an essential role in the stability and fidelity of translation. These modifications are installed respectively by m^6A and m^1A methyltransferase RNAs (rMtase). m^6A rMtases and m^1A rMtases catalyze the transfer of the methyl group of the *S*-adenosyl-*L*-methionine (SAM) cofactor following an SN_2 mechanism to the N6 or N-1 position of adenosine, respectively. In humans, the deregulation of m^6A rMTase activity is associated with many diseases including cancer, neurological or metabolic diseases.[(a)] However, structural data are missing to elucidate the RNA recognition process and the molecular mechanism involved in methyl transfer. Here, we report the synthesis of new SAM-adenosine conjugates containing a triazole linker branched at the N-1 or N-6 position of adenosine by Copper(I)-Catalyzed Alkyne-Azide Cycloaddition (CuAAC). Two synthetic strategies were followed using protected or not protected alkyne and azido partners. The molecules described here were designed as potential bisubstrate analogues for the m^6A and m^1A RNA MTases that could be used for structural studies.[(b)]

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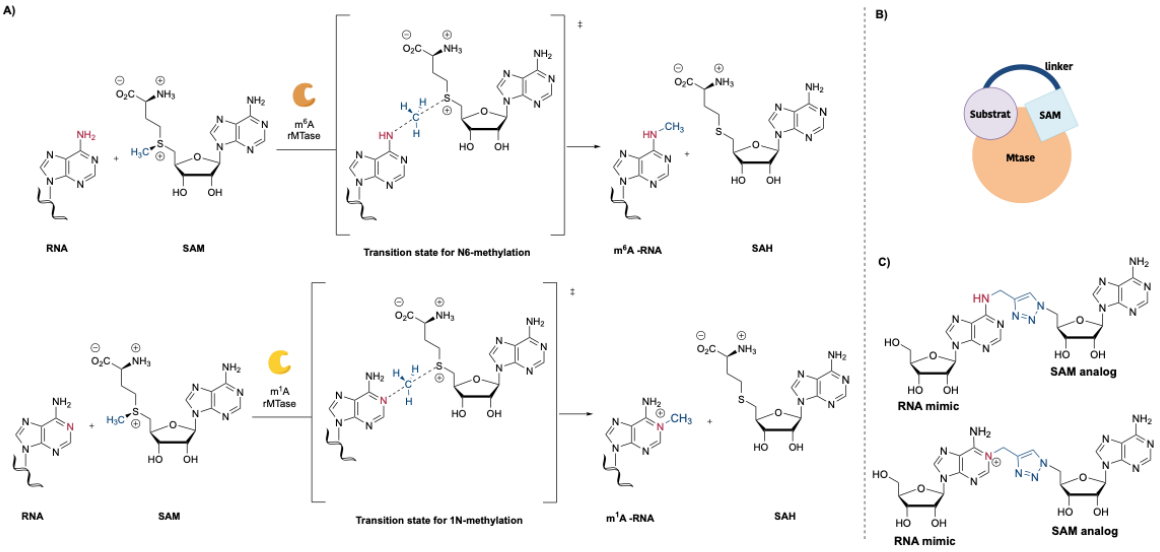

Schema. A) rMTases-catalyzed methylation of adenosine at the N-6 and N-1 positions. B) General structure of bisubstrates C) Structure of SAM-adenosine conjugates with a 1,2,3-triazole linker.

P11

Multiplexing ligands through click chemistry at the anomeric site of sugars for oligonucleotide conjugation

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Abstract

Copper-assisted azide alkyne cycloadditions (CuAAC)[1-3] are versatile, and we have used this approach to functionalize a 1' pentose-azide scaffold with the goal of functionalizing oligonucleotides with multiple ligands. The 1' pentose-azide scaffold was reacted with various multivalent alkynes to produce scaffolds with mono-, bi-, tri-, and penta-valent ligation sites. As ligands, we have used mono- and tri-valent carbohydrates, vitamins, lipids, PEGs, amino acids, small molecules, peptides, polyamines, fluorophores and biotin. The α- and β-anomers were separated after the CuAAC reaction to enable creation of a larger library of conjugates from the same scaffold. Reactions were performed in solution as well as on solid supports. Regioisomeric conjugates can be derived using ruthenium-assisted click chemistry (RuAAC)[4].

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3. Perrone, D.; Marchesi, E.; Preti, L.; Navacchia, M. L. *Molecules* **2021**, *26*, 3100.

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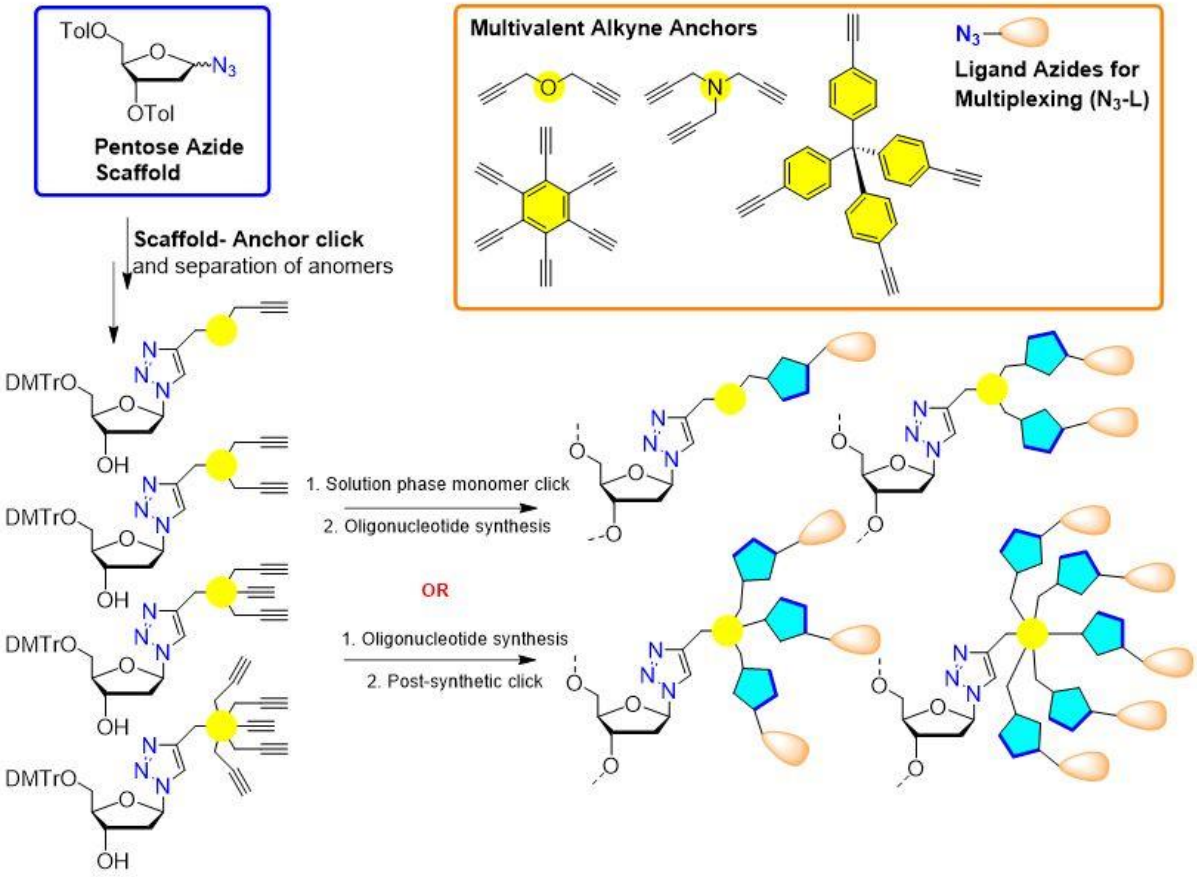
TolO
O
N3
OTol
Pentose Azide Scaffold
Scaffold- Anchor click
and separation of anomers
Multivalent Alkyne Anchors
O
N
N3
Ligand Azides for Multiplexing (N3-L)
DMTrO
OH
1. Solution phase monomer click
2. Oligonucleotide synthesis
OR
1. Oligonucleotide synthesis
2. Post-synthetic click

P12

RNA ligation towards mono and dually labeled RNAs

Anaïs Depaix Dr.[1], Agnieszka Mlynarska-Cieslak M. Sc.[1], Marcin Warminski Dr.[1], Pawel J. Sikorski Dr.[2], Jacek Jemielity Prof. Dr.[2], Joanna Kowalska Dr.[1]

[1]Division of Biophysics, Faculty of Physics, University of Warsaw, Warsaw, Poland. [2]Centre of New Technologies, University of Warsaw, Warsaw, Poland

Abstract

Labeled RNAs are valuable probes for the investigation of RNA function and localization. However, methods to achieve RNA labeling remain challenging, especially for mRNA. Here, we developed an improved method for the 3'-end labeling of in vitro transcribed RNAs. We first synthesized novel adenosine 3',5'-bisphosphate analogs modified at the N6 or C2 position of adenosine with an azide-containing linker, fluorescent label, or biotin. These adenosine derivatives were then assessed as substrates for RNA labeling using T4 RNA ligase 1, either via direct ligation of already labeled nucleotide, or via a two steps procedure – ligation of azide derivative followed by post-enzymatic strain-promoted alkyne-azide cycloaddition (SPAAC). All analogs were substrates for T4 RNA ligase. Interestingly, the efficacy of the two procedures were linker dependent, and analogs containing bulky fluorescent labels or biotin showed better overall labeling yields via direct ligation than via post-enzymatic SPAAC. We applied this method to various *in vitro* transcript RNAs and successfully obtained 3'-mono labeled uncapped RNAs and NAD-capped RNAs, as well as 3',5'-dually fluorescently labeled m^7GpppA_m-capped RNA and mRNA.[1] The dually labeled RNA_{35} has already been used as a tool to study the decapping machinery in P-bodies.[2] Besides, the highly homogenous dually labeled mRNA was translationally active and enabled fluorescence-based monitoring of decapping.[1] This method provides an easy access to various functionalized mRNA-based probes.

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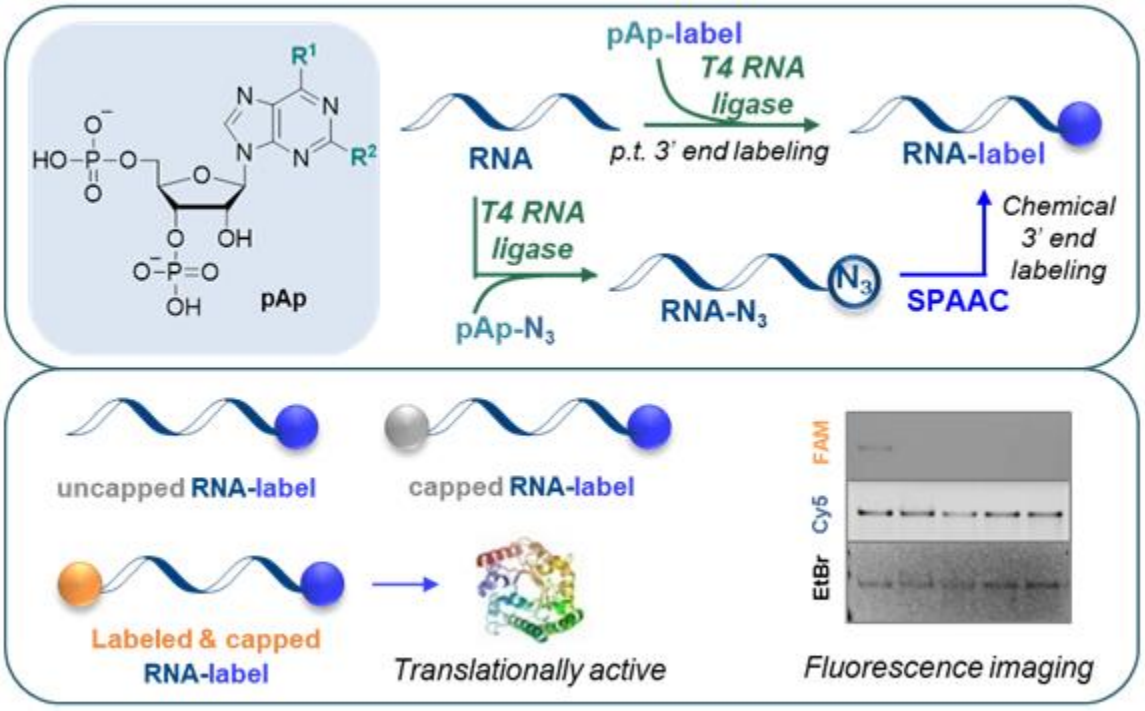
2. R. W. Tibble, A. Depaix, J. Kowalska, J. Jemielity and J. D. Gross *Nat. Chem. Biol.* **2021**, *17*, 615–623.

The project was financially supported from the National Science Centre (2015/18/E/ST5/00555 and 2019/33/B/ST4/01843).


pAp-label
T4 RNA ligase
RNA
p.t. 3' end labeling
RNA-label
T4 RNA ligase
pAp-N3
RNA-N3
N3
SPAAC
Chemical 3' end labeling
pAp
uncapped RNA-label
capped RNA-label
Labeled & capped RNA-label
Translationally active
FAM
Cy5
EtBr
Fluorescence imaging

P13

Development of a fluorescent aptamer-based assay for RNA degradation studies

Mateusz Fido[1], Katarzyna Grab[1], Anaïs Depaix[2], Tomasz Śpiewla[1], Adam Mamot[1,2], Marcelina Bednarczyk[1,2], Jacek Jemielity[2], Joanna Kowalska[1]

[1]Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Warsaw, Poland. [2]Centre for New Technologies, University of Warsaw, Warsaw, Poland

Abstract

Aptamers are nucleic acid molecules designed in a process called SELEX (systematic evolution of ligands by exponential enrichment) to bind specific molecules. One such example is the "vegetable" aptamer Broccoli, emitting light upon binding its ligand and creating a fluorescent complex. In this work, we created RNA fluorescent probes using this aptamer, and applied them to study decapping enzymes.

Natural RNAs differring in sequences and functions differ also in the structures of their 5' ends. For example, prokaryotic RNAs are usually triphosphorylated, whereas eukaryotic mRNAs are 5' capped. The eukaryotic 5' cap is a vital structure for mRNA turnover, gene regulation, and nuclear transport. Therefore, cap recognizing proteins are of great interest to RNA research and RNA-related technologies. Cap degradation enzymes play a key role in RNA degradation by mediating the cap removal process. Thus far, over 20 human decapping proteins have been discovered, alongside with different canonical and non-canonical cap structures, such as FAD, NAD and dephosphoCoA (1). However, their mutual relationships have not been fully characterized.

Here, we sought to develop a fluorescent assay distinguishing 5' monophosphorylated RNAs from 5' capped RNAs, which could serve as a universal method to study various cap structures and decapping enzymes. To that end, we employed RNA aptamer probes binding DFHBI-1T, alongside Xrn1 5'-exonuclease, which specifically degrades RNA 5'-monophosphates. We have identified conditions that enable differentiation of capped and uncapped RNAs based on the fluorescence intensity of the Xrn1-treated sample, and then demonstrated how this method can be applied to study decapping enzymes and their inhibitors.

This work is supported by the National Science Centre (NCN) [2018/31/B/ST5/03821].

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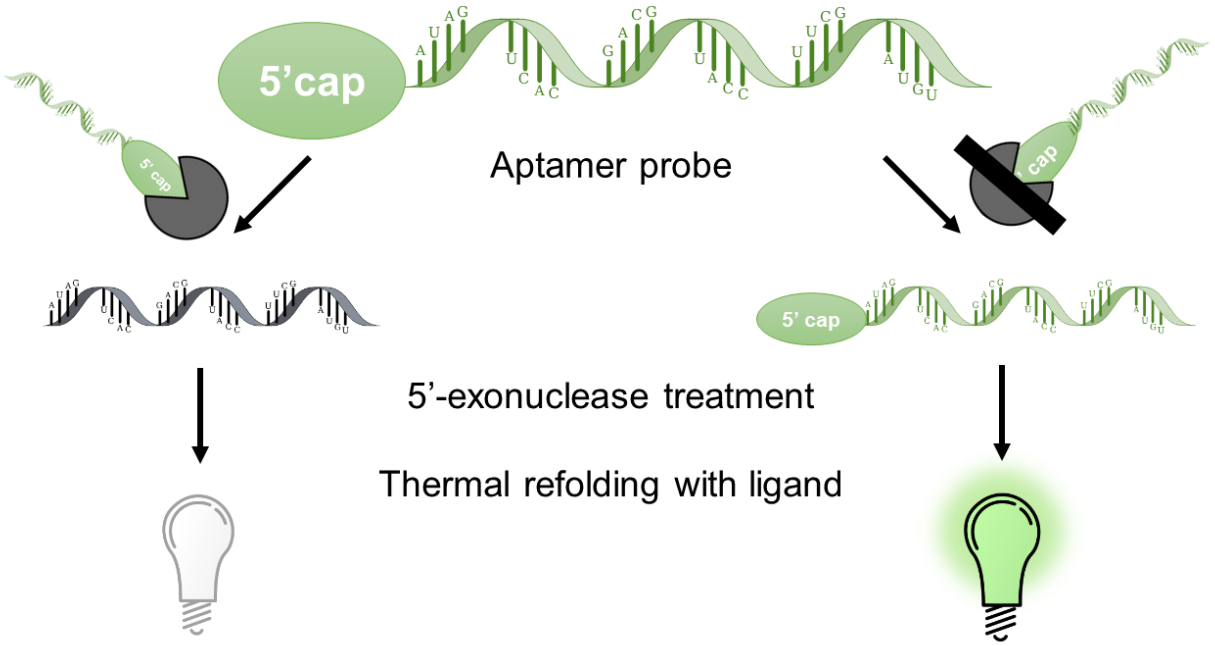

5’cap
Aptamer probe
5’ cap
cap
5’ cap
5’-exonuclease treatment
Thermal refolding with ligand

P15

Elimination of off-target effect of siRNA by chemical modification

Masayuki Fujii PhD[1], Yasuo Shiohama PhD[2], Masaaki Hisano Bc[1], Maika Sonokawa Bc[1], Yojiro Kotake PhD[1]

[1]Kindai University, Iizuka, Fukuoka, Japan. [2]University of The Rykyus, Saibaru, Okinawa, Japan

Abstract

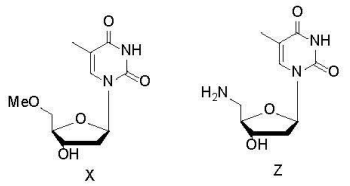
In the present study, we investigated RNA interference (RNAi) efficiencies of siRNAs bearing 5'-O-methylthymidine (X) and 5'-aminothymidine (Z) at 5'-end of the strands.

siRNA3 and siRNA4 bearing U at the 5'-end of the sense strand and X or Z at the 5'-end of the antisense strand showed completely no RNAi efficiencies and that siRNA9 and siRNA13 bearing U at the 5'-end of the antisense strand and X or Z at the 5'-end of the sense strand showed completely equivalent RNAi efficiencies with unmodified siRNA1.

The results can be interpreted that the methyl group of X or the ammonium group of Z strongly affected on the strand selection and that the U-sense strand was selected as a guide strand for siRNA3 and siRNA4 to show no RNAi efficiencies and the U-antisense strand was selected as a guide strand for siRNA9 and siRNA13 to show equivalent RNAi efficiencies to unmodified siRNA1.

These results strongly suggested that the sense strand modified with X or Z at the 5'-end will not be selected as a guide strand in RISC and eliminate an off-target effect of the sense strand.

Ant-EGFP siRNAs (214-234)
sense; 5'-RACGGCAAGCUGACCCUGAag-3'
antisensne; 5'-RCAGGGUCAGCUUGCCGUAgg-3'
R = U, T, X, Z



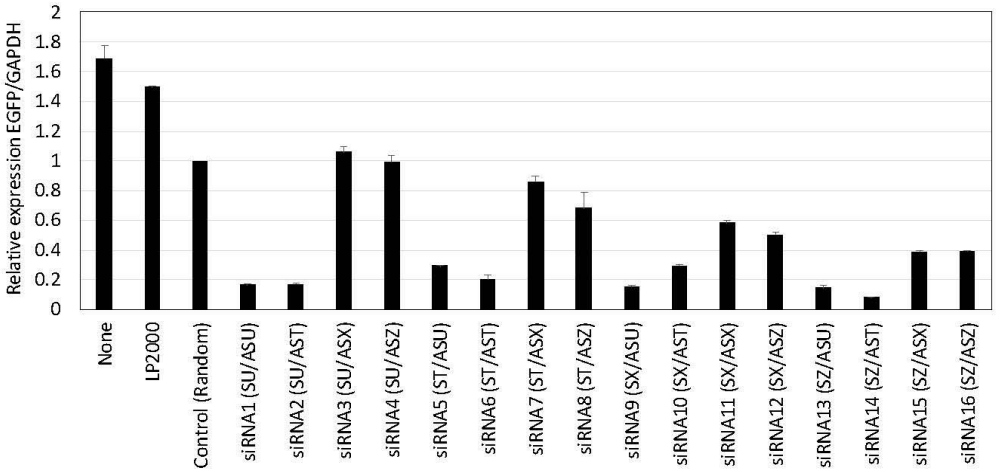


Figure 1. Silencing EGFP mRNA by 5'-Modified siRNA
[siRNA] = 100 nM, transfected by Lipofectamine 2000 HeLa(5 x 10^4 cells /well, 10 % FCS/MEM), 5% CO_2, 37°C, 24h

P16

Structural characterization of long repeated RNA associated with ALS

Miguel Garavís PhD.[1], Halle Barber BSc[2], Mohini Ramkaran PhD[2], Carlos González PhD[1], Masad J Damha PhD[2]

[1]Instituto de Química Física Rocasolano - CSIC, Madrid, Spain. [2]McGill University, Montreal, Canada

Abstract

The most frequent genetic cause of Amyotrophic Lateral Sclerosis (ALS) is the massive expansion of the 5'-GGGGCC-3'/3'-CCCCGG-5' sequence in the intron of the gene *C9ORF72*[1,2]. Many efforts have been dedicated to decipher the mechanisms by which this repeat expansion contributes to the disease. Although not completely understood, the most prominent mechanism suggested is the toxicity mediated by bidirectional transcription of RNA containing expansions of the repeated motif.

The structural characterization of these RNAs has a doubtless importance given their great potential as therapeutic targets to combat ALS. Most structural studies carried out on these repeated sequences focus on DNA and RNA oligonucleotides containing a few units of the sense and antisense repeated motif. Those reports show that these oligonucleotides can fold into non-canonical structures such as hairpins, G-quadruplex and i-motifs[3].

Here we present the structural study of sense and antisense RNAs containing up to sixteen repeats of the expandable motif found in *C9ORF72* gene. Thus, we describe the structural scenarios given by RNA molecules more representative of the ones associated with the disease under different conditions.

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P17

Mechanistic aspects of the BH_3 group substitution and modification in boranephosphonates

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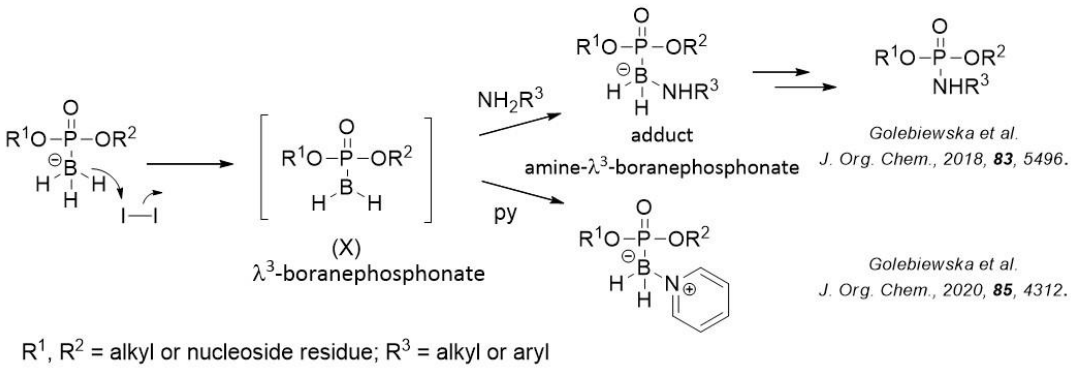
Abstract

Nucleoside boranephosphonates emerged as an important class of nucleotide analogues due to their important biological potential, which make them useful tools in designing new therapeutics and medicinal diagnostics. Modifying the borane moiety (-BH_3) of boranephosphonates is so far very little explored area of the chemistry of these compounds.

Our research on reactivity of the borane group of nucleoside boranephosphonates under oxidative conditions with a wide spectrum of *N*-nucleophiles provided a deeper insight into the chemistry of this class of compounds. Using a stereochemical correlation analysis we found that boranephosphonate diesters react with 1° and 2° amines in the presence of iodine to afford the respective phosphoramidates with a total inversion of the configuration at the phosphorous centre. Further mechanistic study of the reaction of boranephosphonates with 3° and heteroaromatic amines that affords stable products with the P-B-N structural motif, let us to propose a general mechanism for the reactivity of boranephosphonate with *N*-nucleophiles. A crucial step of this mechanism implies formation of a tricoordinated borane intermediate X with an empty p orbital (Scheme 1) that is captured by the amine present in the solvent cage leading to the final *B*-modified boranephosphonates (3° and heteroaromatic amines) or to unstable aminoborane-phosphonates (1° and 2° amines) that after collapsing to the H-phosphonate diesters and further oxidation afforded the corresponding phosphoramidate diesters.

Exploring second generation of nucleotide analogues containing phosphoranesulfen- or phosphoraneselenamides as new structural motives 2018/31/N/ST5/03589



Scheme 1. General mechanistic pathway of the reactivity of boranephosphonate with N-nucleophiles.

P18

Towards miRNA sensors based on SWCNTs and oligonucleotides.

Arturo González-Camunas[1], Luc Chavignon[2], Christophe Blanc[2], Eric Anglaret[3], Alvaro Somoza[1]

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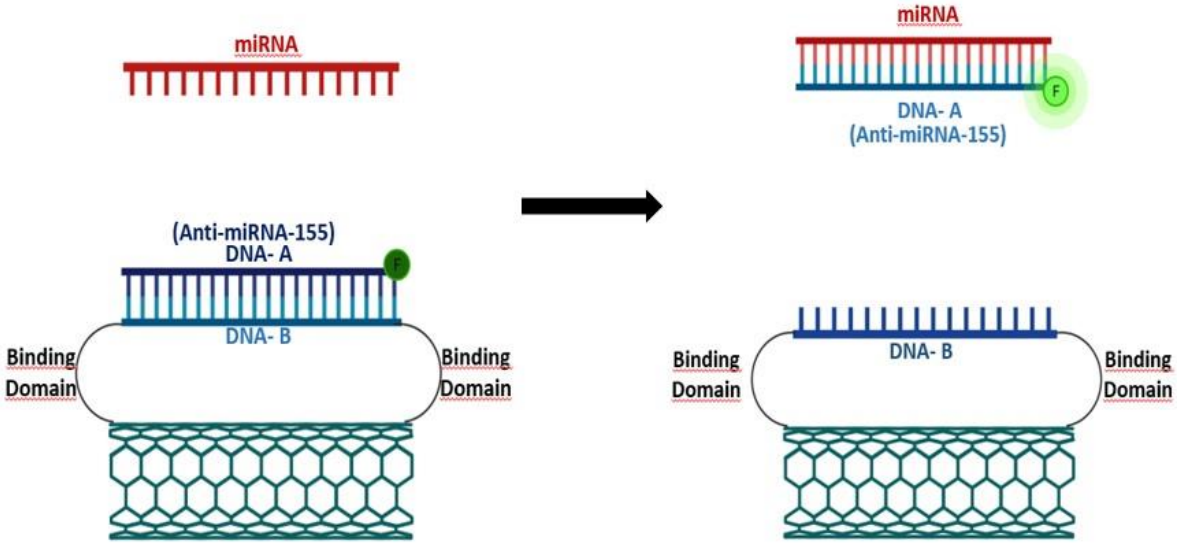
Abstract

MicroRNAs are small RNAs that regulate the expression of their target genes by reducing the expression of the proteins encoded in the genes by repressing translation and / or degradation of their microRNAs. MicroRNAs play a fundamental role in virtually all cellular processes, including development, function, and survival. Recent studies have established microRNAs as critical regulators of immune tolerance and autoimmunity. These findings have revealed miRNAs as potential new therapeutic targets for the treatment of multiple diseases in the future.

The objective of this project is to develop sensor for the detection of multiple microRNAs involved in pancreatic cancer. This idea arises from the fact that changes in the expression of these biomarkers have been found in various diseases (cancer, liver damage and heart disease), with alteration of their levels in plasma, serum, urine and saliva.

The sensor to be designed transduces the hybridization of small DNA and RNA oligonucleotides in carbon nanotube photoluminescence spectral changes. We will determine its mechanism of action through experiments and molecular dynamics simulations that will offer a competitive response to local dielectric and electrostatic factors.

The preliminary experiments have been focused on the optimization of the interaction between the SWCNT and oligonucleotides. The results obtained so far reveal that surfactants must be used to achieve a homogeneous dispersion of SWNTs, and efficient interaction between the two type of structures. Also, the preliminary optimization has revealed that the concentration of oligonucleotides used should be in the nanomolar range.


miRNA
(Anti-miRNA-155)
DNA- A
F
DNA- B
Binding
Domain
Binding
Domain
miRNA
F
DNA- A
(Anti-miRNA-155)
DNA- B
Binding
Domain
Binding
Domain

P19

Specificity profiling of decapping enzymes using HTS aptamer - based probes

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Abstract

Messenger RNA (mRNA) is a natural molecule that plays the key role in the process of protein biosynthesis. The hydrolysis of the phosphate bridge in the cap structure, known as decapping, has a major impact on mRNA stability, translation process and cell homeostasis in general [1]. A number of decapping enzymes (DE) have been identified over the last few years, however, their exact biological roles and interrelations are still under debate. Molecular tools to visualise the activity of DE in vitro and living cells during the decapping process, could be useful in understanding their functions and determining their exact substrate preferences.

In this project, we aimed to use high-throughput screening (HTS) method based on aptamer probes to profile the specificity of several DE. A few of natural and synthetic analogues of cap were synthesised and subsequently incorporated into RNA by in vitro transcription. The mRNA modified at the 5' end and containing the aptamer sequence was purified using reversed-phase high-performance liquid chromatography. The fluorescence assay consisted of three main steps: enzymatic degradation of the capped mRNA, refolding of the mRNA strand with a fluorogenic ligand and fluorescence intensity measurement of the resulting complex using a microplate reader. An additional confirmation of the efficiency of the method was the detection of residual non-degraded RNA using boronate affinity gel electrophoresis. All data obtained from the experiments allowed us to determine substrate preference of the selected DE and the developed and optimised method can be applied in further studies on the activity of cap-degrading enzymes.

This study was supported by the National Science Centre, Poland, Grant No. 2018/31/B/ST5/03821.

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P20

Telomerase-Driven Telomeric DNA Modification in Cancer Cells Leads to Efficient Induction of cGAS-mediated Innate and Adoptive Immune Responses.

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Abstract

Telomeres and telomerase in cancer cells are highly attractive targets for specific anti-tumor therapy, since telomerase is almost universally expressed in cancer cells, but not in the majority of normal counterparts.

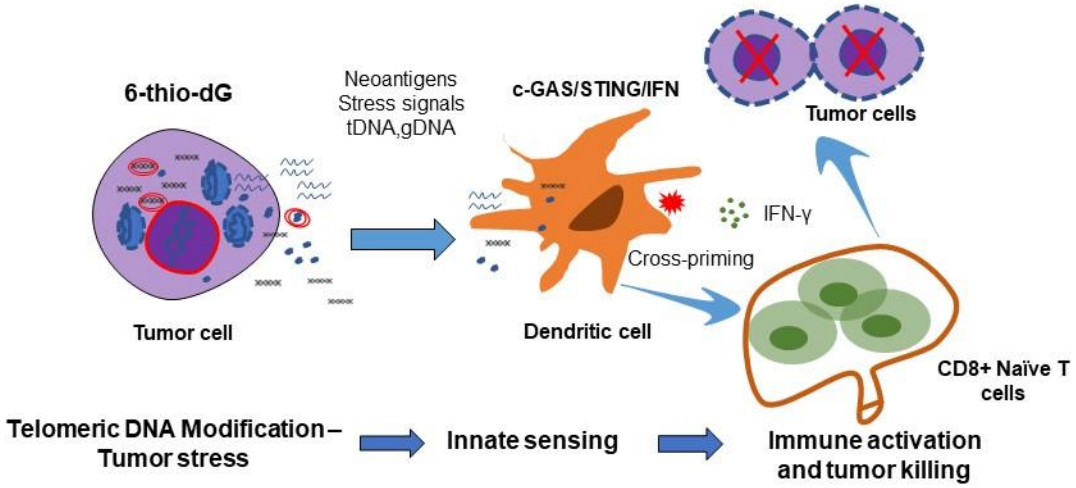
In this presentation we summarize an unexpected, yet promising, functional property of a modified nucleoside - 6-thio-2'-deoxyguanosine (6-thio-dG; THIO), as a potential anticancer agent with a unique mechanism of action. *In vitro* and *in vivo*, THIO is readily converted into the corresponding 5'-triphosphate, which is a substrate for mammalian telomerase. Incorporation of this compound, by telomerase, into *de novo* synthesized cancer cell telomeres leads to a fast induction of DNA damage responses and massive apoptosis. Importantly, the cancer cell death occurs in the telomere length-independent manner. Moreover, THIO treatment leads to generation chromosomal bridges and, eventually, to the formation of cytosolic micronuclei structures, containing cGAS/IFN-I pathway neo-adjuvants - the *de novo* modified telomeric DNA fragments. In addition to activation of innate immunity (*i.e.,* cGAS pathway and NK cells), these *in situ* produced neo-adjuvants are exported extracellularly and then sensed by host dendritic cells, resulting in an enhanced cross-priming and tumor-specific T- cell (both CD4+ and CD8+) activation.

Interestingly, treatment with 6-thio-dG overcomes resistance to checkpoint blockade (by aPD-1 or aPD-L1 agents) in advanced *in vivo* cancer models, leading to profound anticancer effects, and to potent induction of tumor type specific long-term anticancer memory in mice. Thus, *in vivo* cancer curative activity was observed in murine syngeneic models of colorectal (MC-38) and lung (LLC) cancers, when THIO was used in *sequential combination* with aPD-L1 agent (atezolizumab). Combinations with other immune checkpoint inhibitors (*i.e.,* aPD-1: cemiplimab; pembrolizumab) were also highly effective.

In summary, our findings demonstrate the importance of cancer cell telomeric DNA structural and functional integrity, as well as therapeutically attractive opportunity to induce stress, increase innate sensing and adaptive antitumor immunity *via "cancer cell self-produced"* chemical modification of telomeres.

Supported in part by CA070907 to JWS

6-thio-dG (THIO) Induces DNA Damage, Replication Stress, Cytosolic DNA Formation, Immune Signaling, and the Recruitment of Effector T Cells



P21

Controlled Monofunctionalization of Molecular Spherical Nucleic Acids on a Buckminster Fullerene Core

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University of Turku, Turku, Turku, Finland

Abstract

An azide-functionalized 12-armed Buckminster fullerene has been monosubstituted in organic media with a substoichiometric amount of cyclooctyne-modified oligonucleotides. Exposing the intermediate products then to the same reaction (i.e., strain-promoted alkyne−azide cycloaddition, SPAAC) with an excess of slightly different oligonucleotide constituents in an aqueous medium yields molecularly defined monofunctionalized spherical nucleic acids (SNAs). This procedure offers a controlled synthesis scheme in which one oligonucleotide arm can be functionalized with labels or other conjugate groups (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, DOTA, and Alexa-488 demonstrated), whereas the rest of the 11 arms can be left unmodified or modified by other conjugate groups in order to decorate the SNAs' outer sphere. Extra attention has been paid to the homogeneity and authenticity of the C60-azide scaffold used for the assembly of full-armed SNAs.

P22

Structural Modifications to Internal Oligoguanidinium Transporter Tune Endosomal Escape for Enhanced Cytosolic Delivery

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Abstract

Oligonucleotide therapeutics for the treatment of genetic diseases have been optimized to high specificity and low toxicity but unsuccessful non-viral delivery has marred the prospects of many promising candidates such as RNA or morpholino based antisense therapy. We have reported an amphipathic Internal Oligoguanidinium Transporter (IGT) that successfully delivered Nanog targeting antisense Morpholino in MCF7 cells with efficient gene silencing and chemo sensitization with Taxol in presence of serum[1]. The transfection and toxicity profiles of the asymmetric aminopiperidine containing IGT depend on the head group which led us to further modulate the properties with biologically relevant groups. High transfection, low toxicity and high endosomal escape were the determining factors in our quest for the ideal IGT from a set of twenty analogues, all of which were designed rationally with various structural and functional group modifications. Major variations involved exploration of benzyl containing head group analogues since benzyl group is known for helping in the diffusion across endosomal membranes. We also investigated the role of cholesterol (Chol) in IGT backbone and how its enhanced lipophilicity balances the overall properties of amphiphilic IGT. Initial sorting from the set was based on transfection, MTT based toxicity assays and preliminary imaging in live cells to determine the extent and diffusivity of the internalized IGT followed by 3-dimensional co-localization studies with LysoTracker red to converge to the most efficiently escaping IGTs. The two IGTs selected on the basis of similar safety window of operation (difference between maximum transfection dose and IC_{50}), Chol and PF Cbz (pentafluorobenzyl), successfully delivered a pro-apoptotic domain peptide, KLAKLAKKLAKLAK in four different cancer cell lines, PC3, HepG2, MCF7 and A549 and induced apoptosis as an example of efficient endosomal escape since the KLA peptide itself does not enter the cells. We also performed detailed inhibitor analysis with few analogues and found that changing the head group of IGT significantly changes the dynamics of entry. With morpholino delivery and peptide delivery, we proved that IGT is a versatile delivery vehicle that can be designed judiciously and utilized for all types of cargo.

J. Kundu, P. Banerjee, C. Bose, U. Das, U. Ghosh and S. Sinha: Internal Oligoguanidinium Transporter: Mercury Free Scalable Synthesis, Improvement of Cellular Localization, Endosomal Escape, Mitochondrial Localization and Conjugation with Antisense Morpholino for NANOG Inhibition to Induce Chemosensitisation of Taxol in MCF-7 Cells. *Bioconj. Chem.* **2020,** *31* (10), 2367–2382 and *US 10,919,857 B2,* ***date of patent Feb 16, 2021***

P23

Site-directed spin labeling of oligonucleotides with short-tethered trityl radicals

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Science Institute, University of Iceland, Reykjavik, Iceland

Abstract

It is essential to study the structure and dynamics of nucleic acids to gain deeper understanding of their function. One valuable technique for that purpose is electron paramagnetic resonance (EPR) spectroscopy. Since biomacromolecules are commonly diamagnetic, the incorporation of radical centers at specific sites is required for EPR studies. This is referred to as site-directed spin labeling (SDSL). Nitroxide radicals are commonly used for this purpose, but trityl radicals, such as Finland trityl and its derivatives, possess many advantages. Due to their long electron relaxation time (T_M), Pulsed Electron-Electron Double Resonance (PELDOR) distance measurements (distance range of 2-10 nm between two radical centers) can be performed, even at room temperature.[1] Furthermore, their single-line EPR spectra opens the possibility to use lower microwave power[2] and their high persistence under reducing conditions enables their use for *in-cell* studies.[3] Although, trityls have been utilized to investigate nucleic acids, their usage is still limited due to several drawbacks related to a tedious synthesis. So far, trityls have mostly been limited to end-labeling and conjugation at internal sites by long and flexible tethers, which can lead to inaccurate distance measurements. Here we show the synthesis and the incorporation of short-tethered trityl radicals into internal positions of DNAs and RNAs forming a stable, covalent linkage between the oligonucleotide and the radical. The modified trityls involve an easy and efficient synthesis to generate several short-tethered, stable trityl radicals for bioconjugation by both alkylation and Cu-catalyzed click chemistry.

Acknowledgment: This work was supported by a grant from the Icelandic Research Fund (206708) and a postdoctoral fellowship from the DFG (414196920).

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P24

Bioinspired Preorganized Peptide-Nucleotide Nanofibers

Simone I.S. Hendrikse[1,2], Chelsea R Forest[2], Sally L Gras[1], Pall Thordarson[2], Albert C Fahrenbach[2], Amanda V Ellis[1]

[1]School of Chemical and Biomedical Engineering, The University of Melbourne, Melbourne, Australia.
[2]School of Chemistry, University of New South Wales, Sydney, Australia

Abstract

In nature, fibrous assemblies continuously assemble and disassemble to fulfill a variety of functions, including providing structural support and driving cell motility. Both natural and synthetic mimics1,2 have been inspired by these hierarchically complex structures and the essential tasks they perform. By creating synthetic mimics, control over structure, and hence, function can be achieved. Moreover, new capabilities, not achievable in nature, can be realized. For example, the Tobacco Mosaic Virus (TMV) forms helical cylindrical rod-like fibrous assemblies with very high persistence lengths.3,4 The highly infectious RNA of the virus is coated by so-called coat proteins, that protect the virus from enzymatic degradation, keeping the virus stable for several years within infected tobacco leaf products, e.g., infected cigars. The assembly of virion TMV includes synergistic effects between RNA and already aggregated coat proteins (i.e., disks or small helices). In the absence of RNA, protein modules are intrinsically disordered, whereas upon RNA binding, a highly organized hybrid structure is obtained with extensive intermolecular interactions occurring both laterally and axially, including salt-bridges.

Fascinated by the hybrid self-assembled structure of RNA with coat proteins in virion TMV, we developed peptide-nucleotide hybrid polymers that also form rigid rods (Fig. 1). Our peptide-nucleotide rods are formed as a consequence of lateral non-covalent interactions between peptide sequences and feature pendant mononucleotides, assembling to form rigid rods in aqueous solution. Despite having similar peptide sequences, installing distinct mononucleotides at the periphery of the polymers induced slight differences in helicity, as was confirmed with circular dichroism, and in structural morphology as confirmed with AFM, TEM and SAXS. Adenine mononucleotide functionalized polymers displayed a higher tendency to bundle as compared to thymidine and uracil functionalized polymers. RNA also induced increased helicity compared to DNA mononucleotides. Complementary binding with an artificial complementary mimic and canonical RNA and DNA suggested transient weak interactions between chains. These polymers will serve as templates for the non-enzymatic synthesis of DNA and RNA.

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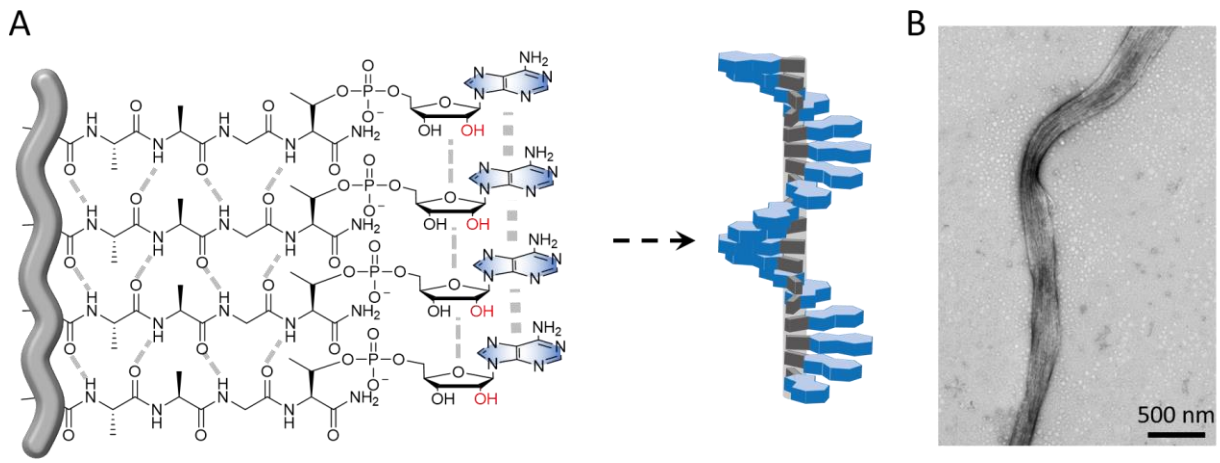

A
B
500 nm

P25

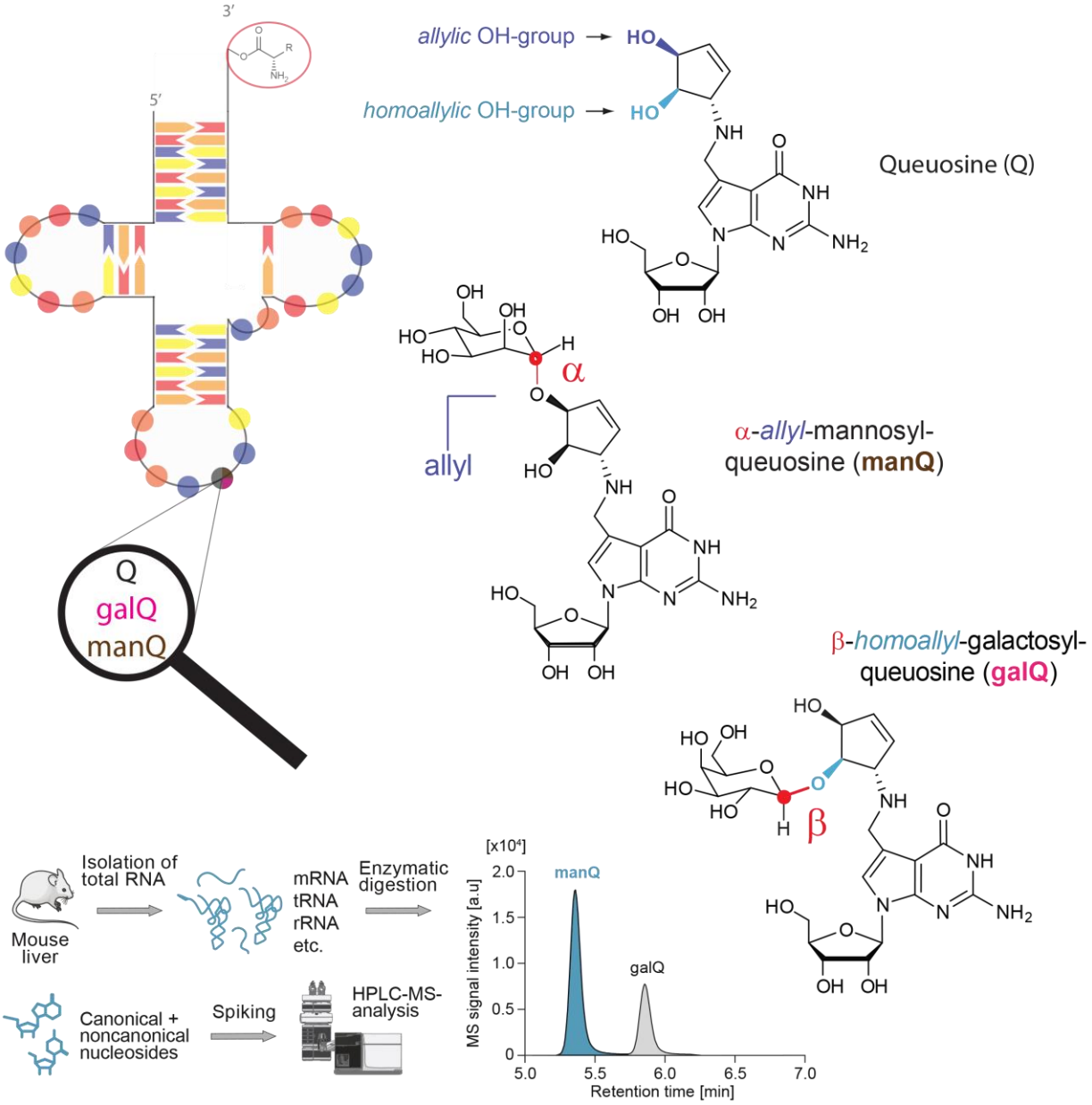
Structure elucidation of the natural queuosine derivatives galactosyl-queuosine (galQ) and mannosyl-queuosine (manQ) by total synthesis

Markus Hillmeier M.Sc., Mirko Wagner Dr., Peter Thumbs Dr., Eva Korytiakova M.Sc., Markus Müller Dr., Thomas Carell Prof. Dr.

LMU, Munich, Germany

Abstract

Queuosine is a non-canonical nucleoside that is found in many prokaryotic and eukaryotic species. It is located at the wobble position of certain tRNAs, where it replaces guanosine in GUN anticodons. Queuosine has been reported to directly influence protein biosynthesis via altered preferences for synonymous codons and it has a general effect on translational speed. In this regard, Queuosine derivatives have recently also been discovered to have a potential medical use as drugs for treatment of diseases associated with mitochondrial dysfunction, such as Parkinsons' or Alzheimers' disease. In addition to Queuosine itself, several further modified derivatives have been identified, including glutamyl-Queuosine (GluQ) in prokaryotes and galactosyl- (galQ) and mannosyl-Queuosine (manQ) in higher vertebrates including humans. The function of these derivatives is still unknown until today. For galQ and manQ, a chemical structure has been reported derived from NMR studies of material isolated from tissue. In a first attempt to confirm these structures and in order to synthesize standards for LC-MS experiments, we confirmed the originally proposed beta-homoallyl-structure for galQ via total synthesis and LC-MS co-injection experiments with natural material. Regarding manQ however, we found to our surprise, that the natural manQ differs from the originally proposed β-homoallyl-structure. By synthesizing and co-injecting all possible isomers we could prove, that manQ does indeed have an α-allyl-connectivity, therefore not only differing in stereochemistry from galQ, but also regarding the regiochemistry. This interesting finding leaves the question, why the yet unknown glycosyltransferases, which add galactose or mannose to the queuosine scaffold have different constitutional preferences. The total synthesis of both derivatives as MS-standards presented here will allow to gain deeper understanding of the biological mechanisms behind these highly complex RNA-modifications.


3′
5′
Q
galQ
manQ
allylic OH-group
homoallylic OH-group
Queuosine (Q)
α-allyl-mannosyl-queuosine (manQ)
allyl
β-homoallyl-galactosyl-queuosine (galQ)
Isolation of total RNA
Mouse liver
mRNA
tRNA
rRNA
etc.
Enzymatic digestion
Canonical + noncanonical nucleosides
Spiking
HPLC-MS-analysis
[x10⁴]
MS signal intensity [a.u]
manQ
galQ
Retention time [min]

P26

Development of Fluorescent Thymidine Analog, ^{diox}T and Its Utilization in FRET.

Shingo Hirashima MD[1], Soyoung Park PhD[1], Hiroshi Sugiyama Prof.[1,2]

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Abstract

Fluorescent nucleoside is a versatile tool in chemical biology thanks to characteristics of fluorescence such as high sensitivity and environmental responsiveness. Since the report of Stryer and his co-worker on 2-aminopurine, which has been widely used as a fluorescent adenosine analog, various kinds of fluorescent nucleosides have been reported. We developed a fluorescent thymidine analog, ^{diox}T.[1] It showed a relatively higher quantum yield than the other isomorphic and expanded thymidine/uridine analogs. Contrary to a general tendency, the quantum yield of ^{diox}T was still high even in double-stranded DNA (upto 0.20). In our latest study, also showed potential as a turn-on fluorescent probe to visualize binding of a transcription factor.

We applied the thymidine analog to FRET (Förster resonance energy transfer).[2] Recently, several groups including us have constructed distance- and orientation-dependent FRET pairs in DNA or RNA duplexes. In traditional FRET assays, bulky fluorophores tend to be connected to target molecules via flexible linkers. In this case, unexpected interaction by the fluorophores is concerned and orientation dependency is lost due to free rotation of the fluorophores. In our study, we used ^{diox}T as a donor and tC[3] as an acceptor. Experimental FRET efficiency showed periodical change as a number of base pairs between donor and acceptor increased, which demonstrated orientation-dependency. This FRET system was evaluated further by comparing experimental FRET efficiency with the theoretical value.

Furthermore, we incorporated our FRET pair into the nucleosome. Dynamic behavior of nucleosomes has received much attention from an epigenetic viewpoint. We prepared a DNA with 145nt containing the donor or acceptor by solid-phase synthesis. After construction of a nucleosome, FRET was observed by steady-state fluorescence spectroscopy.

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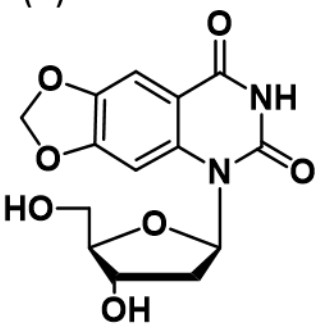
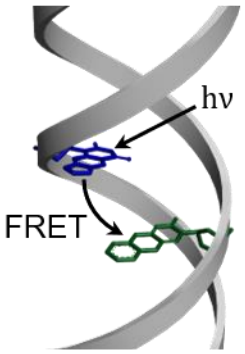
(a)

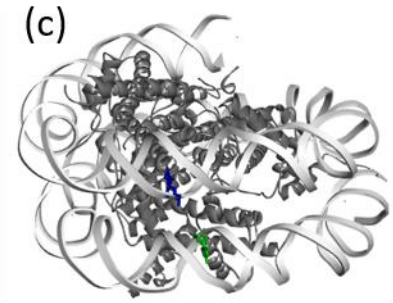


λ_{abs} = 325 nm
λ_{em} = 380 nm
Φ = 0.36
(in water)

(b)



(c)



(a) Chemical structure of the fluorescent thymidine analog, ^{diox}T
(b) Schematic illustration of our FRET pair in double-stranded DNA.
(c) Schematic illustration of nucleosome containing our FRET pair.

P27

Tropylium Derivatives as New Entrants that Sense Quadruplex Structures

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Abstract

G-quadruplex (G4) is the most well-known noncanonical conformation of DNA involved in diverse pharmacological and biological contexts. G4 ligands have been extensively developed as molecular probes and tumor therapeutic reagent candidates. They have been used to detect the presence of G4s and identify their biological roles. Currently used ligands are commonly aromatic, planar, and electron deficient for effective interaction with quadruplex-stranded DNAs. We recognized that tropylium cations possess the aforementioned features of effective quadruplex ligands. In this study, we prepared tropylium derivatives to validate their binding affinity with G4 and i-motif. Titration against various DNA sequences revealed gradual changes in the UV–vis spectra of the tropylium derivatives. Furthermore, ITC measurement provided evidence of interaction between quadruplex structures and tropylium derivatives. Distinct from the conventional G4 ligand skeletons (porphyrins, polyacenes, thioflavins, etc.), we propose tropylium derivatives as new structural motifs and successfully demonstrate its quadruplex-specific binding abilities. Our ligands possess the following advantages: small size (MW. ~300), facile synthesis (two steps with excellent yield), and high solubility in aqueous conditions. Given the prevalence of use of G4 ligands in therapeutic and detection purposes, the potentials of these tropylium derivatives are underway using cell studies.

P28

Nucleoside analogues with a seven-membered sugar ring: synthesis and structural compatibility with DNA in DNA:RNA hybrids.

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Abstract

Oxepane nucleic acid (ONA) elicits RNase-H mediated degradation of ONA/RNA hetero duplex.[1] However, low duplex stability of ONA/RNA limits its application in gene silencing approaches. Herein we describe the synthesis of a new series of 7-membered ring (oxepane) thymine nucleosides (OxT), their conversion to phosphoramidites derivatives and their use in solid-phase oligonucleotide synthesis to yield chimeric OxT-DNA and OxT-RNA strands. The different regioisomeric OxT phosphoramidites allowed for positional variations of the phosphate bridge (3'-7', 4'-7', 5'-7' linkages) and assessment of duplex stability when the oxepane nucleotides were incorporated in dsDNA, dsRNA and DNA:RNA hybrids. We found that OxT units destabilized RNA: RNA duplexes. However, little or no destabilization was observed when 3'-7'- and 4'-7'-linked OxT units were incorporated in the DNA strand of DNA:RNA hybrids ($D T_m = \sim 0$), a remarkable result considering the dramatically different structure of oxepane vs 2'-deoxynucleosides.

Figure. Construction of functionalized ONA nucleosides and oligonucleotides carried out in the present work

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P29

STAUDINGER TRACELESS REACTION FOR BIOCONJUGAISON OF 3'-end of RNA

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Abstract

Aminoacylated tRNAs are well known to be used as amino acid donors in protein synthesis by the ribosome. In addition, they also serve as substrates by diverse enzymes in non-ribosomal peptide synthesis (NRPS).[ref1] To investigate this aspect and the Non ribosomal functions of the tRNA, a reliable bioconjugaison reaction for the synthesis of suitable 3'-modified tRNA is highly desired.

A first challenge for RNA conjugation is the design of chemical groups that are compatible with RNA stability. A second challenge is the choice of a chemoselective reaction for the post-functionalization step. Reactions for RNA modification must be site-selective and require control over both chemo- and regioselectivity. This selectivity must also be realized in conditions required to prevent denaturation: aqueous media, low to ambient temperature, and at or near neutral pH.

Among bioconjugaison reactions targeting non-native functional groups,[ref2] Staudinger-type reactions[ref3] are particularly interesting for the creation of amide bond. A "trace-free" version of the Staudinger ligation, was reported in 2000 by Raines[ref4] and Bertozzi. This methodology has been used for the modification of polysaccharides, peptides or proteins, but no example report the modification of RNA by this strategy.

Herein, we describe a post-synthetically traceless Staudinger ligation for the modification of tRNA. Optimization of the reaction conditions will be presented on a model dinucleotide and 8 substituted-phosphines will be considered.

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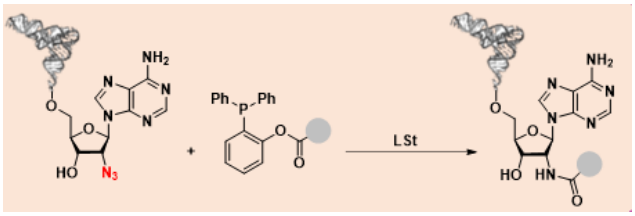

P30

A new method for the synthesis of oligonucleotide conjugates with amide bond via the modified phosphate group by the Staudinger reaction

Kristina Klabenkova[1,2], Ekaterina Burakova[1,2], Sergey Bizyaev[1], Alesya Fokina[1,2], Dmitry Stetsenko[1,2]

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Abstract

Synthetic oligonucleotide derivatives capable of interfering with biological RNA targets via antisense or RNAi mechanism have gained widespread recognition as promising 'smart' therapeutics. The most widely used DNA or RNA analogs are the ones with modified phosphate groups such as, e.g. phosphorothioate oligonucleotides, due to their relatively facile synthesis. However, most oligonucleotide derivatives exhibit insufficient cellular and tissue uptake, and unfavorable biodistribution and pharmacokinetics, which slows down their transition to clinical practice. One of the possible ways to solve the delivery problem is oligonucleotide conjugation with various transport molecules such as, e.g. polyamines, which can improve cellular uptake of an oligonucleotide by the attachment of positively charged side-chain groups.

Earlier, we have shown that the Staudinger reaction between an electrophilic organic azide and the internucleotidic phosphite triester can be an efficient and straightforward method for the preparation of phosphate-modified DNA and RNA analogs [1-3] as well as for pinpoint chemical modification of oligonucleotides [4]. In this work, we have synthesized pentafluorophenyl (1a) and 4-nitrophenyl (1b) esters of 4-carboxybenzenesulfonylazide and employed the compounds for the introduction of an activated carboxyl group into oligonucleotides at the internucleotidic position via Staudinger reaction. After the treatment with an excess of primary alkylamine, removal of protective groups and cleavage from the polymeric support, a series of oligonucleotide conjugates containing an amide bond, including a polyamine-linked, were obtained in good yield (Fig. 1).

Fig. 1. Preparation of oligonucleotide conjugates with various primary amines through modified phosphate group via Staudinger reaction followed by amide bond formation. Abbreviations: DMTr – 4,4'-dimethoxytrityl group; Bp or B – N-protected or unprotected heterocyclic base.

The work was supported by the Russian Foundation for Basic Research (projects Nos. 18-515-57006, 18-29-08062, 18-29-09045 and 20-04-60433) and the Ministry of Science and Higher Education of the Russian Federation (project of Novosibirsk State University No. FSUS-2020-0035).

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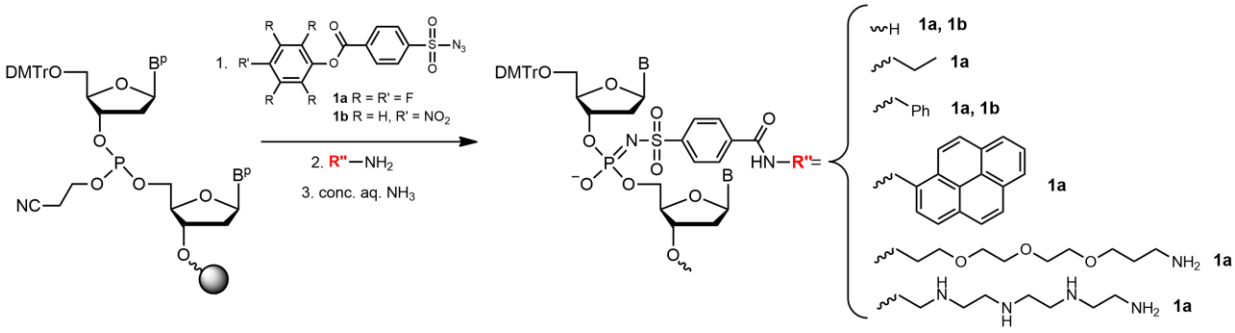

P31

Structural requirements for photo-induced RNA-protein cross-linking

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Abstract

RNA-protein interactions play a fundamental role in many biological processes and their perturbations lead to cellular dysfunction and diseases. Therefore, identifying the RNA targets of RNA binding proteins (RBPs) is crucial to understand the molecular mechanisms of RBP-mediated diseases. State-of-the-art methods often deploy UV-induced RNA-protein cross-linking to detect target transcripts bound by proteins followed by sequencing or mass spectrometry. UV light forms a covalent bond between RNA and protein and produces complex mixtures of unidentified amino acid/nucleic acid adducts, often in a low yield.

Although it is known that cross-linking often occurs at specific positions in RBP complexes, our understanding of the process is crude. So far a method to pinpoint the exact location of an amino acid-nucleotide cross-link, is missing and subsequently, there has been little progress in recent decades on our understanding of the underlying chemistry of cross-linking. Therefore, we used a novel isotopic labelling strategy and tandem mass spectrometry to systematically probe the influence of nucleotide, position and amino-acid on cross-linking outcomes in a complex of the RBFOX alternative splicing factor bound to its consensus hepta-ribonucleotide. From this analysis, we were able to pin-point cross-linking at three nucleotides in the complex, all of which were stacked onto phenylalanines. Moreover, this stacking feature was also needed in order for *neighbouring* amino-acids along the peptide backbone to cross-link. This suggests that it is a general requirement for RNA-protein cross-linking, thereby helping to explain the seemingly random cross-linking events that are typically observed.

We anticipate that our findings will facilitate the interpretation of RNA-protein cross-linking data and thereby accelerating advances in exploring RNA-binding selectivity of RBPs.

P32

Amide-modified RNAs for CRISPR-Cas9 technology

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Abstract

Recent developments in antisense, RNA interference (RNAi) and, most recently, CRISPR-Cas9 technologies continue driving the interest in modified oligonucleotides. Our group has been developing amide linkages as replacement of phosphates in RNA. Recently, our group demonstrated that siRNA bearing amide linkages at certain positions were more active, and most importantly can suppress the off-target effects from the undesired passenger strand while promoting the desired guide-strand targeted mRNA degradation.

We recently extended our amide-modified RNA studies to CRISPR-Cas 9 technology, our approach and initial data will be presented here. This presentation will discuss the synthesis of new amide-dimer phosphoramidites and the solid-phase synthesis of modified 36-mer crRNAs carrying amides at pre-defined positions of the RNA backbone. The effect of amide-modified crRNAs on the efficiency of introducing dsDNA breaks in the HPRT and VEGF-A regions in cells using CRISPR-Cas9 technology will also be presented.

P33

Synthesis and evaluation of novel triazole trinucleotide cap analogs

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Abstract

The 5' end of mRNA is terminated by a unique structure called cap. Cap consists of positively charged 7-methylguanosine linked to first transcribed nucleotide via 5',5'-triphoshate bridge and plays a crucial role in many intracellular processes. Cap is involved in mRNA maturation, transport, and turnover and protects mRNA against premature degradation by 5'-exoribonucleases. It also plays a significant role in the initiation of protein expression[1]. These facts make it an interesting subject for chemical modification. Cap analogs are commonly used as reagents to modify mRNA 5' end for research and therapeutic purposes.

Despite many applications of mRNA 5' cap, the development of novel cap analogs is still limited due to challenges associated with their synthesis. The standard synthetic approach based on phosphorimidazolide chemistry is sometimes inefficient and time-consuming. We have recently proposed a novel class of cap analogs bearing a triazole modification at 5'5'-triphosphate bridge which were synthesized by fast and efficient copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC)[2]. Some of these analogs had interesting biological properties, but the efficiency of their incorporation into mRNA (capping efficiencies) was not optimal[2, 3]. Independently, we have shown the RNA capping efficiency can be improved using trinucleotide cap analogs, such as m^7GpppA_mpG[4].

Here, we aimed to combine these two promising technologies by developing trinucleotide cap analogs that can be synthesized using fast and efficient CuAAC reaction. As a result, we present a novel class of trinucleotide cap analogs carrying triazole modification within the 5',5'- triphosphate bridge. Dinucleotides bearing an azido group at the 5' position were obtained using solid-phase synthesis and linked with 7-methylguanosine 5'-triphosphate analogs carrying an alkyl group attached to terminal phosphate using CuAAC reaction. Overall, we obtained 13 novel trinucleotide cap analogs, whose structures were confirmed by nuclear magnetic resonance. The cap analogs were first incorporated into short RNA via in vitro transcription (IVT) to assess capping efficiency. Then, the analogs were incorporated into mRNA encoding *Gaussia Luciferase* via IVT to determine their translation efficiencies in Rabbit Reticulocyte System and JAWS II cells.

This work was supported by the Foundation for Polish Science (TEAM/2016-2/13)

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P34

Phosphoramidate linkage approach for molecular barcoded transcript analysis

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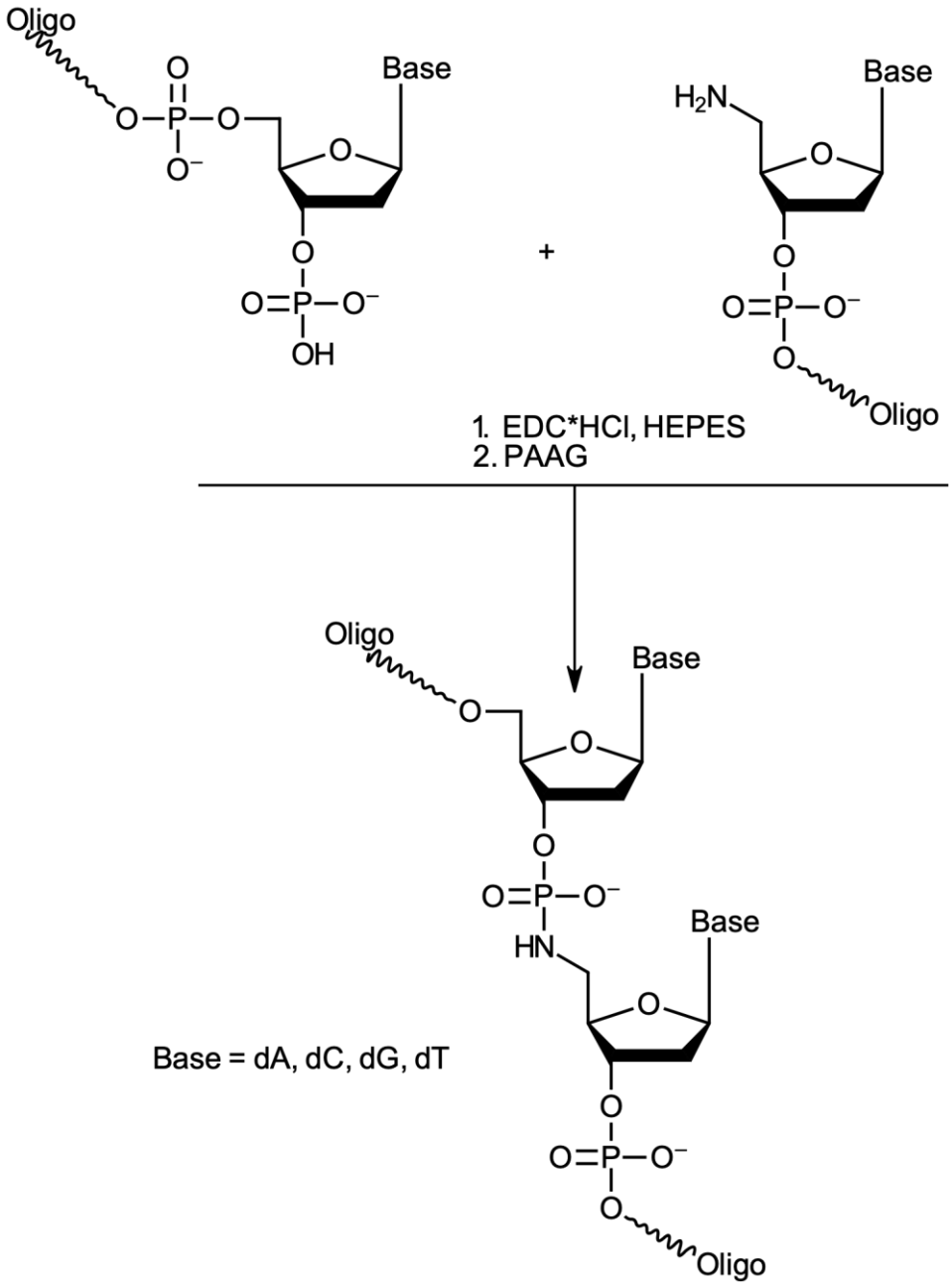
Abstract

Substrate-fixed oligonucleotides (ONs) are being used in molecular genetic analysis, including the single cell level. Synthesis of long ONs with a barcoded tag (>60 nucleotides) is complicated due to the reduced efficiency of the attachment reaction of subsequent nucleotide at the 3'-end. In addition, a technically unresolved condition for the use of such ONs is the possibility of attaching several sequences, linked by a single physiological function, to the substrate.

We developed a new way to synthesize long ONs with the option of attaching any sense sequence by the formation of a phosphoramidate inter-nucleotide bond. Splint-ON and ONs modified with phosphate and amino groups were synthesized. Synthesis of the oligonucleotide chain was performed using standard phosphoramidites. The 5'-Hydroxyl ON was functionalized to the 5'-amino product using 5'-Amino-dT-CE Phosphoramidite (GlenResearch, USA). Formation of the phosphate derivative at the 3'-end was performed utilizing 3'-Phosphate CPG. Deblocking was performed in ammonia solution at 55°C for 5 hours.

The ONs were mixed in a molar ratio (1 part Ph-ON and 2 parts NH_2-ON) to perform a phosphoramidate bond formation reaction involving HEPES in NaCl solution. EDC*HCl was used as a catalyst. Initially, the reaction mixture was heated to 85°C for 5 min and slowly cooled to room temperature for 1 hour. After adding the catalyst, the reaction was carried out at 10°C for 2 hours. The reaction mixture was purified using PAGE. The obtained product could not be determined using Maldi-TOF because the addition of hydroxypicolinic acid (matrix) causes hydrolysis of the phosphoramidate bond resulting in the initial products. The quality of the ON isolated with PAGE was determined in an experiment on cDNA synthesis of the human T-cell receptor alpha chain gene. The efficiency of cDNA production was assessed by agarose gel electrophoresis. The polymerase is shown not to detect differences in the altered structure of the inter-nucleotide bond, and cDNA synthesis is successful. The developed technique will be used in the immobilization of OH on a solid substrate according to the method we described earlier [Kozlov et al, 2019]. Our approach will allow us to remove restrictions on the length of the seminal OH and thus incorporate molecular barcodes for a significant number of analyzed cells without loss of assay accuracy. Attaching different oligonucleotides to a single substrate will create new opportunities for the determination of functional markers that play an important role in the development of immune-dependent pathology.

Acknowledgments: The reported study was funded by RFBR, project number 19-33-90076.


Oligo
Base
H2N
Base
+
1. EDC*HCl, HEPES
2. PAAG
Oligo
Base
Base
HN
Base = dA, dC, dG, dT
Oligo

P35

Nucleoside 5'-*O*-Selenophosphates: synthesis, properties and activity towards cancer cells.

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Abstract

Selenium is an essential trace element, and it is suggested that selenium compounds are beneficial in the prevention and treatment of cancer [1]. Their effect is dose-dependent: at low concentrations (about 55 µg/day) selenium is provided as necessary element in various physiological processes, while at high doses (>400 µg/day) it may exert toxic effects. The mechanism of the chemotherapeutic effect of selenium against cancer is not fully understood. Selenium compounds (selenite, SeMet, MeSeCys, and CysSeSeCys) supplied in the diet and drinking water significantly differ in their metabolic pathways, but their common metabolite has been widely identified as hydrogen selenide (H_2Se). All selenium drugs exhibiting antitumor activity can be divided into two groups: generators of hydrogen selenide and methylselenol. For this reason, the study of existing and new selenium-containing drugs with antitumor activity should be directed to those molecules. Excess of hydrogen selenide can be toxic to cells as it is easily oxidized and can lead to the production of reactive oxygen species (ROS) with toxic effects [1]. On the other hand, H_2Se accumulation can lead to reductive stress instead of oxidative stress and result in autophagy-induced cell death [2].

In this study, we present a new selenium derivative, 2'-deoxyguanosine-5'-*O*-selenophosphate (dGMPSe), which has been synthesized and used to test the potential anticancer activity of this class of compounds. Using the MTT viability assay, we found that dGMPSe was toxic to HeLa cancer cells at low micromolar concentrations at 12, 24, and 48 hours. Moreover, this cytotoxicity was related to the release of H_2Se in the cells upon entry of dGMPSe. For this purpose, we used a fluorescent probe capable of detecting hydrogen selenide *in vitro* and in cells. We also demonstrated that deoxyguanosine 5'-*O*-selenophosphate (dGMPSe) undergoes hydrolysis catalyzed by the enzyme Hint1 with the release of H_2Se, analogous to the transformations of nucleoside phosphorothioates described previously [3]. Further studies on the mechanism of the toxic effect of dGMPSe through the release of H_2Se are in progress.

This research was financially assisted by CMMS PAS Statutory Funds, Poland and support from PAN funds for conferences.

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P36

Design, Synthesis and Characterization of Fluorescent Molecular Rotor-containing Thymidine Analog

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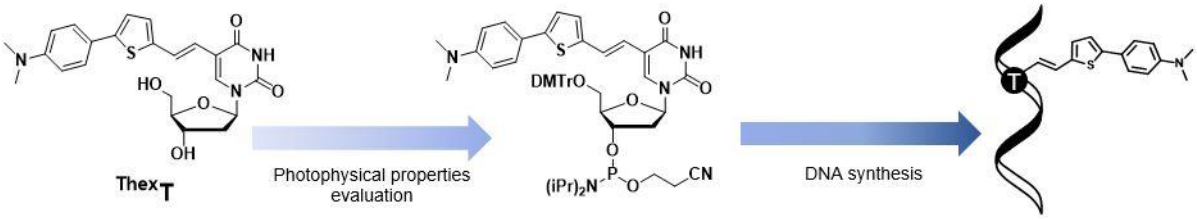
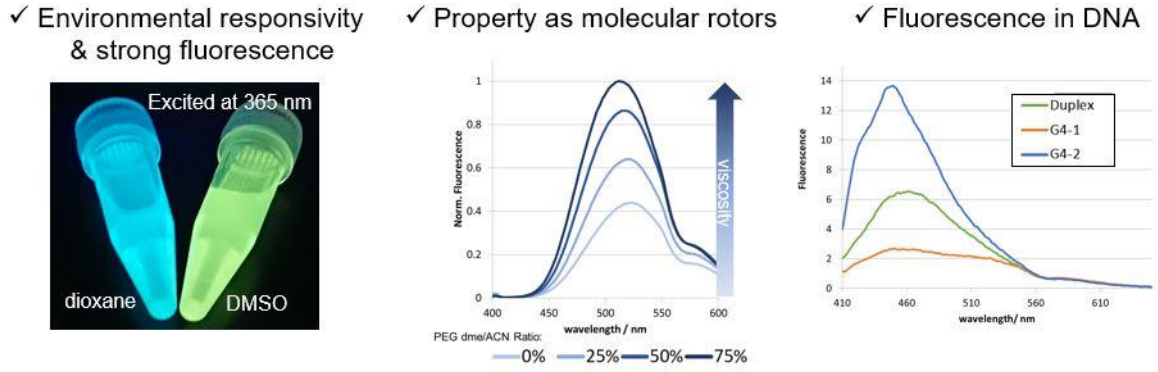
Abstract

With the advances in synthetic molecular machinery, a variety of molecular rotors that reveal distinguishable features affected by external conditions have been developed. Among them, fluorescent molecular rotors have been actively applied to the biological system as biosensors owing to their sensitivity and usability. The advantages of emissive molecular rotors could be further amplified when merged with nucleic acid bases because of their minimal perturbation in DNA/RNA oligonucleotides. In this study, we devised a fluorescent nucleobase analog, ^{Thex}**T**, that has the property of a molecular rotor by introducing a chromophore composed of thiophene and dimethylaniline into 5-position of thymidine via a carbon-carbon double bond. It was straightforwardly synthesized from 5-Iodo-2'-deoxyuridine, and its property could be controlled by the introduction of functional groups. Generated ^{Thex}**T** monomer has absorption at 401 nm and emission at 522 nm (in DMSO). It exhibited a highly viscosity responsive fluorescence increase. In addition, we introduced ^{Thex}**T** into guanine-rich DNA that can associate to form quadruplex structure to investigate its utility as a molecular rotor-based fluorescent nucleotide. Interestingly, ^{Thex}**T**–containing oligonucleotides showed remarkable fluorescent spectra changes by the formation of G-quadruplex structure. These results support the potential of ^{Thex}**T** as the target-specific molecular rotor sensor for biological application.





P37

The Guiding Role of 2'-5'-Linked Nucleic Acids in 'Origin of Life' Puzzle

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Abstract

The 'RNA World' hypothesis considers RNA as catalytic and informational molecule at the 'Origin of Life'. The RNA synthesis under simulated pre-biotic conditions always yielded RNA with mixed 3'-5'-/2'-5'-linkages. As the 2'-5'-linkages are accommodated in RNA:RNA duplexes[1] as well as in the secondary structures of ribozymes[2] and aptamers,[2,3] it was suggested that the mixed 2'-5'-/3'-5'-RNA could be present at the beginning of life which evolved slowly to 3'-5'-ssRNA.[2] What is still unknown is why the evolved ssRNA would replicate to give exclusive 3'-5'-RNA duplexes in the absence of any guiding principle. The 'RNA World' hypothesis does not address this central unsolved puzzle.

We propose answer to this bewildering puzzle[4] and substantiate it with experimental results from the literature and our own work.[5] It is known in the literature that 'although 2'-5'-linked RNA can bind to cRNA, geometrical requirements do not allow its binding to cDNA'.[1] This calls for a revisit the 'Origin of Life' scenario. At the 'Origin of Life', the milieu possibly contained both DNA and RNA precursors and the chemistry required to form oligomers. The survival of long enough DNA might have been highly likely as compared to other possible mosaic 2'-5'/3'-5'/mixed-RNA/DNA. The survived DNA would allow the DNA-templated RNA synthesis, with specific 3'-5' linkages in RNA, because of the instability of template:primer DNA:RNA duplex containing 2'-5'-RNA-linkages. The stability of DNA:RNA duplex is largely compromised with the increasing number of 2'-5'- linkages in RNA compared to that of RNA:2'-5'/3'-5'RNA duplex. The stability of the template:primer duplex is known to be the most valuable attribute for primer extension even under simulated prebiotic conditions. Further, unlike 'dead-end' RNA:RNA duplexes in the 'RNA World', the duplex DNA:RNA thus formed would be amicable for separation into ssDNA and ssRNA due to highly stable secondary RNA structures, leaving DNA template alone for further RNA synthesis. Such ssRNA might be transiently stable and possess catalytic activity and lead evolution of RNA catalysis.[2] DNA to RNA to proteins could have been the 'Central Dogma of Life' even at its origin.

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P38

Introducing self-penetrating morpholino antisense oligonucleotides: Synthesis and Evaluation of gene silencing properties

Jayanta Kundu Ph.D, Ujjal Das Ph.D, Chandra Bose Thesis submitted, Jhuma Bhadra Ph.D, Ujjwal Ghosh M.Sc, Surajit Sinha Prof.

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Abstract

Abstract: Phosphrodiamidate Morpholino Oligonucleotides (PMOs) are one of the most promising 3rd generation antisense agents which are routinely used for regulation of Gene's functions through steric blocking mechanism. These antisense agent is very much target specific, water soluble and nuclease resistant. Eteplirsen and Golodirsen[1], PMO based drugs, developed by Sarepta Therapeutics, have been approved by FDA for the treatment of Duchenne Muscular Dystrophy. However, their delivery is a major concern.

To overcome this limitation, a self-transfecting GMO-PMO or PMO-GMO chimeras has been reported for the first time where GMO[2] stands for guanidinium morpholino oligonucleotides which linked either at the 5´- or 3´-end of PMOs.

Such chimeras were synthesized on solid support where GMO was incorporated via the formation of thiourea followed by the treatment with MeI then methanolic ammonia solution. PMO was synthesized using our previously reported procedure[3]. GMO not only facilitates cellular internalization of such chimeras but also participates in Watson-Crick base pairing during gene silencing in ShhL2 cells when designed against m*Gli1* and compared with scrambled GMO-PMO where mutations were made only to the GMO part. GMO-PMO-mediated knockdown of *no tail* gene resulted no tail-dependent phenotypes in zebrafish model at high stages which was previously unachievable by regular PMO. Its application was extended to target NANOG gene, a transcription factor involved in cancer stem cell proliferation. Its inhibition influences on the expression of other cancer related proteins and the respective phenotypes in breast cancer cells.[4] To the best of our knowledge, this is the first report on the self-transfecting antisense reagents since the discovery of T. C. Bruice's DNG and most effective among the all cell-penetrating PMO reported till date expected to solve the longstanding problem of PMO delivery. In principle, this technology could be useful for the inhibition of any target gene without using any delivery vehicle and should have applications in the fields of antisense therapy, diagnostic and nanotechnology area.

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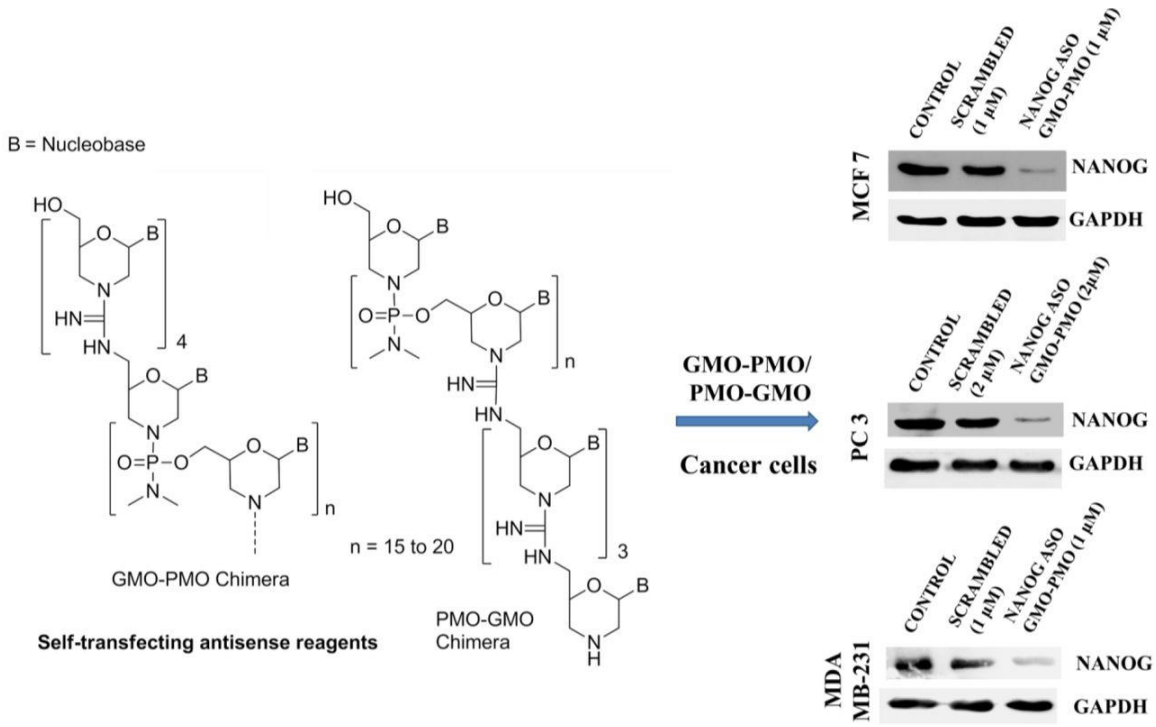
Image



P39

Study of B→Z-DNA transition by NMR spectroscopy

Quanjian Li, Andrei Solodinin, Gautrais Angeline, Samuel Ollivier, Tony Yan

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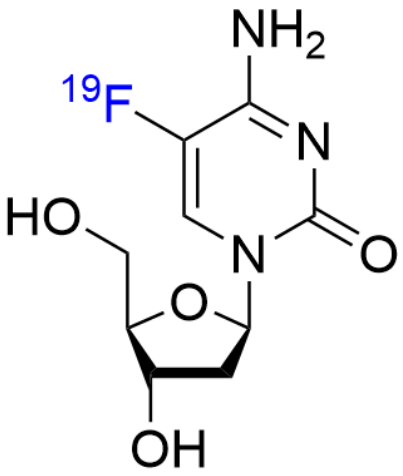
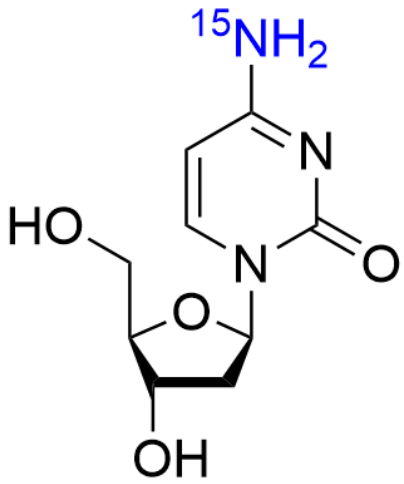
Abstract

Nearly half a century after the discovery of Z-DNA, the mechanistic aspects of Z-DNA formation and biological significance of this unique DNA duplex remain poorly understood. In this work, 5-fluoro-2'-deoxycytidine and 4-^{15}N-2'-deoxycytidine were synthesized for incorporation into d(CG) repeat sequences to study the processes involved in the B→Z-DNA transition induced by elevated salt concentrations using ^{19}F or ^{15}N NMR spectroscopy. Our preliminary results suggested that this transition is initiated at terminal ends, leading to unwinding of duplex at internal position as salt concentration increases.



5-fluoro-2'-deoxycytidine



4-^{15}N-2'-deoxycytidine

P40

Preclinical characterization of antagomiR-218 as a potential treatment forMyotonic Dystrophy

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[1]Department of Genetics and Interdisciplinary Research Structure for Biotechnology andd Biomedicine (ERI Biotecmed), University of Valencia, Valencia, Spain. [2]Translational Genomics Group, Incliva Helath REsearch Institute, Valencia, Spain. [3]Arthex Biotech, Catedrático Agustín Escardino 9, Paterna, Spain. [4]Joint Unit Incliva-CIPF, Valencia, Spain. [5]Institute for Bioengineering of Catalonia, Barcelona, Spain. [6]The IISLAFE Health Research Institute, Valencia, Spain. [7]Biodonostia Health Research Institute, San Sebastián, Spain

Abstract

Myotonic Dystrophy (DM1) is a rare neuromuscular disease caused by the expansion of unstable CTG repeats in a non-coding region of the DMPK gene. CUG expansions in mutant DMPK transcripts fold into hairpins that sequester MBNL1 proteins in ribonuclear foci, being depletion of MBNL1 a chief contribution to disease symptoms such as muscle weakness and atrophy, and myotonia. Thus, the upregulation of endogenous MBNL1 levels may compensate for the sequestration. We have demonstrated that antisense oligonucleotides against miR-218 boost expression of MBNL1 and rescue phenotypes in disease models. Here we provide a preclinical characterization of an antagomiR-218 molecule using the HSA LR mouse model and patient-derived myoblasts. In HSA LR , antagomiR-218 rescued molecular and functional phenotypes in a dose-dependent manner, showed a good toxicity profile, and lasted some 15 days after a single subcutaneous injection. In muscle tissue, the antagomiR rescued the normal subcellular distribution of Mbnl1 and did not alter the percentage of myonuclei containing CUG foci. In patient-derived cells, antagomiR-218 improved defective fusion and differentiation and rescued up to 34% of the gene expression alterations found in the transcriptome of patient myoblasts. Importantly, miR-218 was found upregulated in DM1 muscle biopsies, which makes this miRNA a relevant therapeutic target.

P41

A Dual Enhancement Strategy for In Vivo Metabolic Labeling of Vinyl Nucleosides

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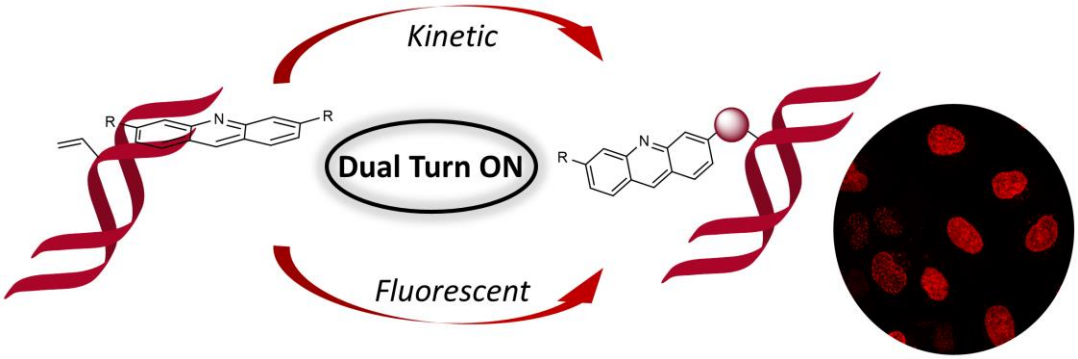
Abstract

Metabolic Labeling of nucleic acids is a powerful technique to decipher the timing and location of DNA synthesis *in vivo*. The technique has been a cornerstone for cell cycle analysis, but also been extended to be used for viral detection and the elucidation of drug resistance mechanisms. In general, a nucleoside of interest is functionalized with a synthetic handle, which is small enough to conserve the original biological function, but also allows for chemoselective detection and visualization of the nucleoside post incorporation. Unfortunately, most bioorthogonal methodologies were originally designed for proteins or carbohydrates and can therefore not be easily translated towards native DNA *in vivo* due to its tightly packed, double stranded form. To address this issue, we developed **PINK** (**P**robe for **I**maging **N**ucleoside Al**K**ene Groups), whose tetrazine-functionalized structure is based on the widely used DNA intercalator acridine orange. PINK intercalates nucleic acids with high affinity (K_D = 3.6 µM), which induces a close proximity between the tetrazine moiety and terminal alkene groups of vinyl-nucleosides and thus allows for bioorthogonal click reaction to occur in double stranded DNA. Importantly, this templated reaction proceeds 60.000-times (590 M^{-1} s^{-1} vs. 0.01 M^{-1} s^{-1}) faster compared to its single molecule level counterpart. In addition to this kinetic increase, the reaction also proceeds in a highly fluorogenic manner (around 20-fold), as PINK's reactive moiety effectively quenches fluorescence prior to the reaction. PINK is highly cell permeable, which enables efficient live-cell labeling of vinyl-modified DNA across different cell lines and nucleoside derivatives. The fluorogenic approach allows for no wash conditions in this regard, while low micromolar concentrations are sufficient for excellent, bright signal. Notably, the cellular viability and DNA metabolism is not affected by this labeling approach at accurately chosen concentrations, so that dynamic, long term-processes such as mitotic divisions can be visualized by time-lapse confocal imaging.



P42

Thiocoumarin Caged Nucleotides: Synthetic Access and Their Photophysical Properties

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Abstract

Photocages have been successfully applied in cellular signaling studies for the controlled release of metabolites with high spatio-temporal resolution. Commonly, coumarin photocages are activated by UV light and the quantum yields of uncaging are relatively low, which can limit their applications in vivo. Here, syntheses, the determination of the photophysical properties, and quantum chemical calculations of 7-diethylamino-4-hydroxymethyl-thiocoumarin (thio-DEACM) and caged adenine nucleotides are reported and compared to the widely used 7-diethylamino-4-hydroxymethyl-coumarin (DEACM) caging group. In this comparison, thio-DEACM stands out as a phosphate cage with improved photophysical properties, such as red-shifted absorption and significantly faster photolysis kinetics.

P43

Simple chemical approaches to introduce 2,6-diaminopurine and 2-aminoadenine conjugates into oligonucleotides

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Abstract

The 2,6-diaminopurine (DAP) nucleobase can form Watson-Crick base pairs with thymine in DNA and uracil in RNA. These pairs are stabilized by three hydrogen bonds resulting in improved thermodynamic duplex stability relative to base pairing with adenine. Previously reported strategies for synthesis of DAP building blocks for oligonucleotide synthesis require multiple steps and have exocyclic $-NH_2$ protecting group related issues. We have developed a post-synthetic strategy using the 2-fluoro-6-amino-adenine as the key nucleobase intermediate to make DAP-containing oligonucleotides. The synthesis of this new building block, which has been produced with different ribose modifications (e.g., R = H, F, OMe), its incorporation during solid-phase oligonucleotide synthesis, and deprotection using ammonia to obtain oligonucleotides containing DAP will be presented. Furthermore, this building block has also been used to enable post-synthetic conjugation of various ligands. The strong electronegative 2-fluoro makes the 6-NH_2 of adenine totally inert, and simple aromatic nucleophilic substitution of 2-fluoro makes reaction with NH_3 or RNH_2 feasible at 2-position.

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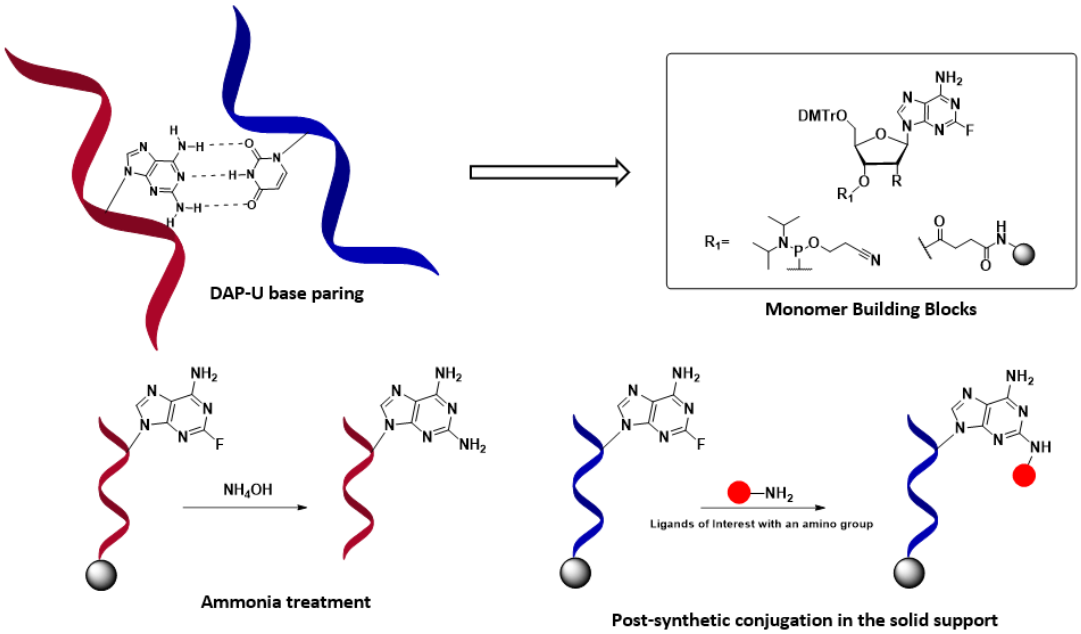

P44

Synthesis of cyclic dinucleotides as modulators of STING, a pivotal protein in immunity and diseases

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Abstract

STING protein is a unique and pivotal protein of cGAS-STING signaling pathway [1]; its modulation is involved in immunity and STING is considered as a new attractive target to treat infections [2] and cancers [3]. The cyclic dinucleotide (CDN) 2',3'-GMP-AMP (cGAMP) is the endogenous agonist of STING with known antiviral activities [4] and has served as lead for new CDNs development, such as ADU-S100, (Figure 1) [5-7]. In fact, main limitations of cGAMP are inherent to its physical properties e.g. instability regarding hydrolases and charged linkages. Neutral cGAMP analogues that feature better cellular penetrability and resistance facing hydrolysis are still needed.

Herein, we report the design and synthesis of two cGAMP analogues with a triazole and an unsaturated chain as new 3',3'-internucleotide linkages. The convergent synthesis involves as key-step (1) a Cu(I)-catalyzed azide-alkyne cycloaddition and (2) a macrocylisation *via* a ruthenium-catalyzed ring-closing metathesis. The nucleobases were introduced under Vorbrüggen conditions affording original dimeric-like CDNs analogues. All synthesized compounds were evaluated to determine their activity as STING agonist or antagonist by measuring type I interferon induced secretion IP-10 in macrophages; some of them displayed an interesting biological activity which will be presented.

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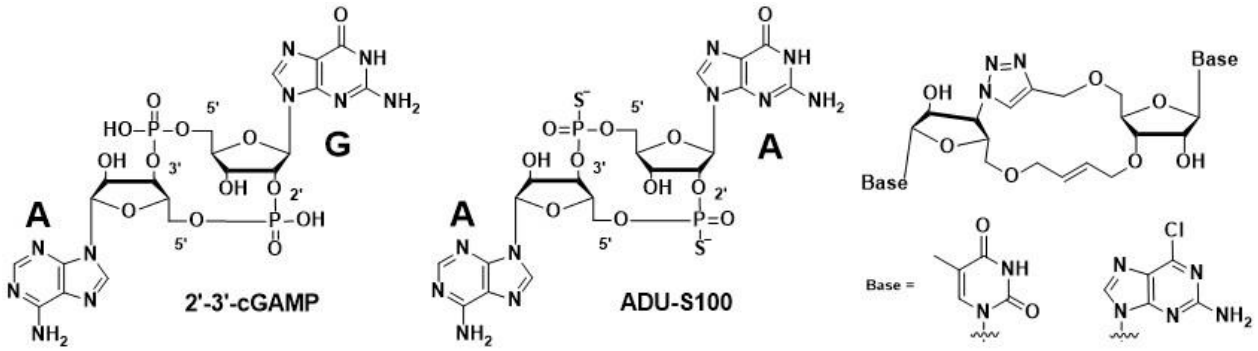

Figure 1. Cyclic dinucleotides modulators of STING and targeted compounds

P45

Enzyme-free modification of mRNA 3’ end – fluorescent labeling and translation *in vivo*

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Abstract

Current development of RNA-based technologies depends on our ability to detect, manipulate, and modify RNA molecules.[1] Functionalized and labelled RNA molecules are invaluable tools to study RNA function in complex biological systems. Despite numerous advances, efficient and selective covalent modification of long RNA molecules still poses a challenge.[2] Here, we report an enzyme-free method for direct modification of RNA 3’ end.[3] We found that *N*-substituted ethylenediamine derivatives are excellent reagents for reductive amination of periodate-oxidized 3’ terminal ribose in RNA, opening the way to selective conjugation of RNA with fluorescent dyes, biotin, and other functionalities. Using this method, we obtained fluorescently labelled or biotinylated RNAs varying in length (from 3 to 2000 nt) and carrying different 5’ end structures (including 5’ capped mRNAs). High reaction yields, ranging from 70% up to quantitative, were directly assayed using HPLC analysis of crude reaction products. The method is scalable as demonstrated by labelling of GFP-coding mRNA at a 0.5 mg scale. The 3’-end labeling was combined with 5’ strain-promoted azide-alkyne cycloaddition (SPAAC)[4] labeling to afford a one-pot and site-specific method of RNA dual labeling with yields exceeding 50%. The method was harnessed to obtain RNA fluorescence resonance energy transfer (FRET) probes, which enabled monitoring the activity of several RNA hydrolases in real-time. The dually labelled mRNAs showed high translation activity in cultured cells and in zebrafish embryos, which combined with their detection by fluorescent methods and scalability of the synthesis, opens new avenues for the investigation of mRNA metabolism and the fate of mRNA-based therapeutics.

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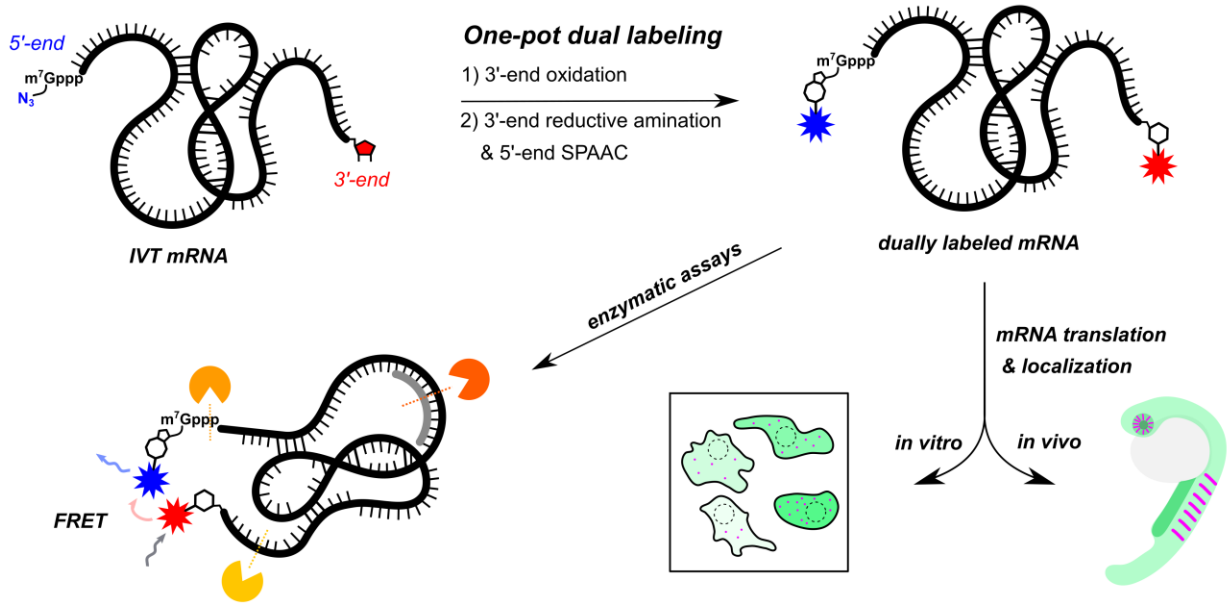

P46

Native chemical ligation of peptide thioester and oligonucleotide via 2´-*O*-modification

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Abstract

Native chemical ligation (NCL) is one of the most powerful tools to connect two large molecules. To apply NCL reaction for peptide-oligonucleotide conjugation in the minor groove, we synthesized a 2'-O-{*N*-[*N*-(*S*-*tert*-butylthiocysteinyl)aminobutyl]carbamoylethyl} (CysBCE) ribothymidine derivatives. The NCL reaction of synthesized oligonucleotides having CysBCE modifications with a peptide thioseter proceeded smoothly even when the CysBCE modification was in the middle of the oligonucleotide sequence. We checked the duplex stability by UV melting experiments and confirmed the destabilization effect by the peptide conjugation was only average –1.2°C per peptide. We believe CysBCE could be a useful scaffold for peptide conjugation in the minor groove.

P47

Direct Access to unique C-5'-acyl modified nucleosides through a Pd-catalyzed Cu(I)-mediated thioester-boronic acid coupling

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Abstract

While sugar modifications on C-1', C-2' and C-3' positions in nucleosides have been widely studied and have resulted in several active antiviral [1], antibacterial [2] and antitumoral [3] compounds, the functionalization of the C-5' position has been neglected and initially avoided due to the importance of the 5'-OH position for nucleoside incorporation[2c] for DNA and RNA synthesis. However, the C-5' position offers the potential uncovering of novel biological activities. In the context of the COVID-19 pandemic, the importance and appeal of finding new biologically active nucleosides has grown. Herein, we describe a straightforward cross-coupling reaction between nucleoside 5'-carbothioates with boronic acids (also known as the Liebeskind-Srogl reaction) for the preparation of C-5' acyl nucleosides [4]. The procedure is rapid, efficient and orthogonal to a wide variety of functional groups due to its mild, baseless, conditions. All of these qualities enable the diversification of the C-5' position which was successfully implemented on pyrimidine nucleosides in both DNA and RNA series, producing a wide-ranging substrate scope containing more than 25 examples with good to excellent isolated yields. This Pd-catalyzed, copper-mediated cross-coupling provides a convenient and direct approach for the preparation of a distinct library of potentially bioactive modified nucleosides, and additional transformation is possible through the conversion of the C-5' ketone.

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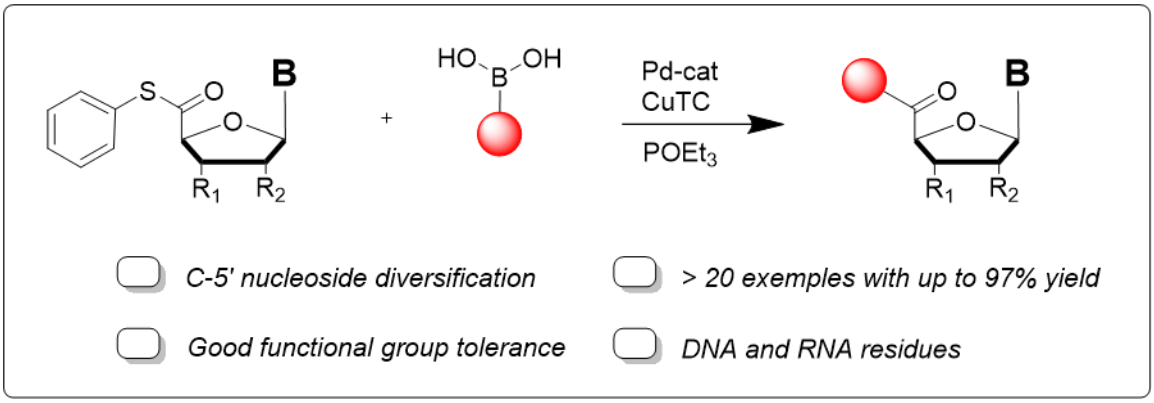

- *C-5' nucleoside diversification*
- *> 20 exemples with up to 97% yield*
- *Good functional group tolerance*
- *DNA and RNA residues*

P48

miR-4443 is a small regulatory RNA independent of the canonic microRNA biogenesis pathway

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Abstract

The human small RNA miR-4443 is functionally involved in several types of cancer and in the biology of the immune system, downstream of insulin and leptin signaling. Next generation sequencing evidence and structural prediction suggest that miR-4443 is not produced via the canonical Drosha – Exportin 5 – Dicer pathway of microRNA biogenesis. We tested this hypothesis by using qRT-PCR to measure miR-4443 and other microRNA levels in HCT-116 cells with Drosha, Exportin 5 and Dicer knockouts, as well as in the parental cell line. Neither of the knockouts decreased miR-4443 levels, while the levels of canonical microRNAs (miR-21 and let-7f-5p) were dramatically reduced. Previously published Ago2-RIP-Seq data suggests limited incorporation of miR-4443 into RISC, in agreement with the functional studies. The miR-4443 locus shows conservation in primates but not other mammals, while its seed region appears in additional microRNAs. Our results suggest that miR-4443 is a Drosha, Exportin 5 and Dicer - independent, non-canonical small RNA produced by a yet unknown biogenesis pathway.

P49

Synthesis, Photo Physical and Biochemical Evaluations of 5- Ethynyl Tropolonyl-2'-deoxyuridine Nucleoside triphosphate (Tr-dUTP)

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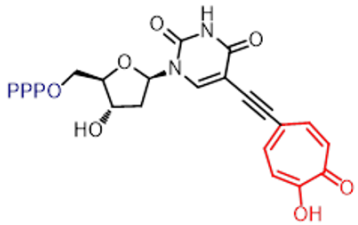
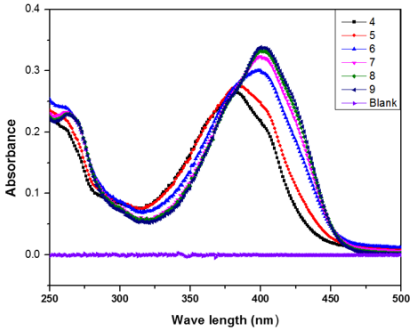
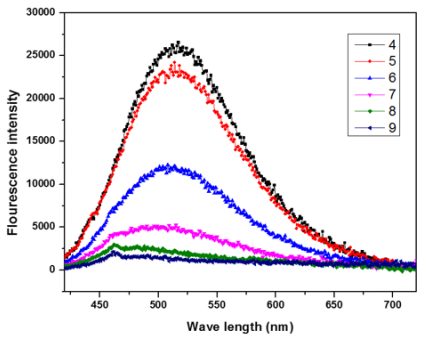
Abstract

Deoxyribonucleic acid (DNA) being a genetic material forms its central role in chemical evolution through replication and amplification. Hence, extensive chemical modifications have been conducted for studying structure and dynamics of nucleic acids. Fluorescent nucleosides have become powerful tool to achieve this purpose along with the conventional applications related to genomics such as gene deletion, single nucleotide polymorphism (SNP) typing and fluorescence imaging. In addition metallated DNA are of great interest exhibiting higher thermal stability and inducing other high-order structures than natural one. These features could lead to construction of not only DNA based nanomaterials, DNAzymes and DNA machines but also potential therapeutic drugs.

Considering the above scenario we have designed DNA containing tropolone moiety, where tropolone is a non-benzenoid seven membered aromatic compound having weak fluorescence but excellent metal coordinating properties. Tropolone shows pH dependent changes in UV and fluorescent properties. Herein we report the synthesis, photo physical and biochemical evaluations of new fluorescent molecule, 5-(ethynyltropolonyl)-2'-deoxyuridine and its triphosphate analogue (Tr-dUTP). We have successfully incorporated it into DNA by primer extension reactions method with many DNA polymerases. Also we have studied the UV and fluorescent properties in different solvents of varying dielectric constant, viscosity and in different pH .This modified nucleoside analogue is sensitive to change in environmental conditions. Hence Tr-dUTP analogues are the potential building blocks to synthesize fluorescent DNA which can sense the changes in environmental conditions.







P50

A modular platform for rapidly optimizing the structure of bispecific antibodies using DNA Nanotechnology

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Abstract

Abstract: Acute lymphoblastic leukemia, ALL, is a disease of the bone marrow that is the most common type of leukemia in children. The clinical success seen in ALL is mostly restricted to children. Most of the adult population will relapse from their disease, while cured patients can have long-term side effects due to chemotherapy. In response to this need, blinatumomab (a bi-specific antibody, bsAb, able to simultaneously bind leukemic B-cells (which display CD3) and normal T-cells (displaying CD19)) was approved by the US FDA in December 2014 for the treatment of ALL. However, a major challenge to the field of bsAb comes from the largely unknown way cells 'display' their antigens. As such, it is presently unknown what the 'optimal structure' of a bsAb should be for productive T-cell engagement, in terms of relative distance of targeting areas or even the flexibility of the entire construct. This project will develop a modular bsAb platform using 'DNA nanotechnology' that would enable us to systematically control the distance between the antigen-binding domains of blinatumomab as well as the overall flexibility of the bsAb structure in order to maximize the ability of the construct to dimerize B- and T-cells.

P51

Modified 7-deazaadenosine ribonucleotides as antiviral agents

Nemanja Milisavljevic[1,2], Eva Konkolová[1], Jaroslav Kozák[1], Jan Hodek[1], Lucia Veselovská[1], Veronika Sýkorová[1], Karel Čížek[1], Radek Pohl[1], Luděk Eyer[3], Pavel Svoboda[3], Daniel Růžek[3], Jan Weber[1], Radim Nencka[1], Evžen Bouřa[1], Michal Hocek[1]

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Abstract

We prepared a library of 7-substituted 7- deazadadenosine triphosphates and corresponding nucleosides and nucleosides monophosphate prodrugs (phosphoramidate and *S*-acylthioethanol esters). Triphosphates were tested for *in vitro* inhibitory activity against selected RNA-dependent RNA polymerases, while antiviral activity of nucleosides and prodrugs was determined in cellular assays (Figure 1).

Modified triphosphates inhibit viral polymerases in micromolar concentrations, while nucleosides show (sub)micromolar activities towards selected viruses. Nucleosides bearing ethynyl and smaller hetaryl groups were more active, but also more cytotoxic, than bulkier heteroaromatic ones. Monophosphate prodrugs were less active than parent nucleoside despite the efficient delivery of bis(*S*-acylthioethanol) prodrug and monophosphate release in the cell.[1]

Funding: European Regional Development Fund, OP RDE (No.CZ.02.1.01/0.0/0.0/16_019/0000729) the Czech Academy of Sciences (*Praemium Academiae* award to M.H.), the Czech Science Foundation (206-12-G151)

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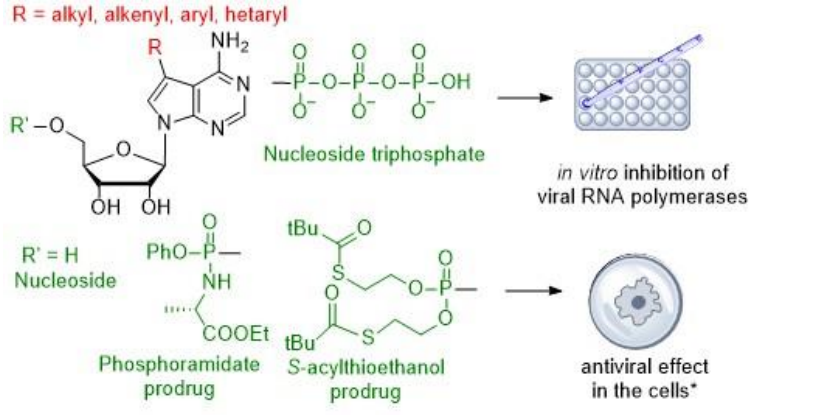

P52

Development of the small molecular binder for the r(CGG) repeat

Hirotaka Murase Ph.D.[1], Fumi Nagatsugi Ph.D.[2], Shigeki Sasaki Ph.D.[1]

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[2]Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, Sendai, Miyagi, Japan

Abstract

The repetitive sequences, whether in translated or untranslated regions, exist in the normal genes, and their abnormal expansions can cause the fatal genetic disorders called repeat disease. A number of disease-associated repeats have been identified in various locations in various genes, such as CGG trinucleotide repeat on 5'- UTR of *FMR1* and G4C2 hexanucleotide repeat on the intron of *C9orf72*, etc. In particular, there has been much interest in developing small molecular ligands that target aberrantly extended DNA and RNA repeats that form the non-canonical structures such as hairpin and G-quartet, etc.

We have previously reported the small molecular binders for the guanines that do not form the hydrogen bonding in the RNA loop structure and for the guanines of the G-G mismatch[1]. Our binding molecule is the dimer-type ligand consisting of the two G-clamp (cytosine analog) units, and selectively binds to neighboring two guanines based on a 1 to 1 binding mode. In this study, we investigated the binding properties of the G-clamp-dimer to the r(CGG) repeat, because r(CGG) repeat associated with fragile X-associated tremor/ataxia syndrome (FXTAS) may form a metastable hairpin structure containing the unpaired guanines in the loop region and the G-G mismatch guanines in the stem region separated by (GC/CG) base pairs.

The UV spectra of G-clamp-dimer showed a red shift and a hypochromic effect in the presence of r(CGG) repeat, clearly indicating the complexation between the ligand and r(CGG) repeat. In the native PAGE analysis, the band mobility of the r(CGG)n hairpin structure was gradually retarded with increasing concentrations of the G-clamp-dimer, suggesting the binding of the ligands at the multiple sites of the r(CGG)n. The CD spectra of the ligands-r(CGG)n complex displayed the similar characteristics to that of A-form RNA, indicating that the complex was formed without any major structural changes. The binding of the ligand to the G-G mismatch region significantly enhanced the thermal stability of the complex. The dissociation constant of the ligand to the r(CGG) repeat was measured by the fluorescence titration experiments and was found to be 20 to 30 nM per single binding site. These results confirm that the G-clamp-dimer has excellent properties as a ligand for r(CGG) repeats.

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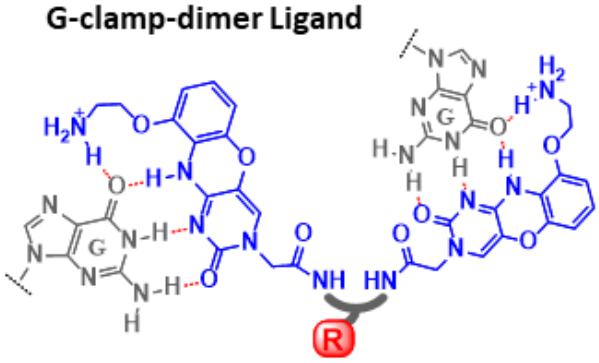
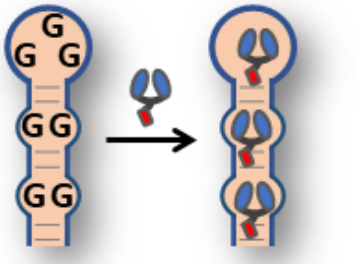
G-clamp-dimer Ligand



r(CGG) repeat



P53

1,2,3-Triazoles as leaving groups: S_NAr reactions of 2,6-bistriazolylpurines with O-, C-, P- and Se-nucleophiles

Dace Cīrule Mg.sc.ing., Kārlis-Ēriks Kriķis B.sc.ing., Agnija Ritere, Andris Jeminejs Mg.sc.ing., Ērika Bizdēna Dr.chem., Irina Novosjolova Dr.chem., Māris Turks Dr.chem.

Institute of Technology of Organic Chemistry, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Riga, Latvia

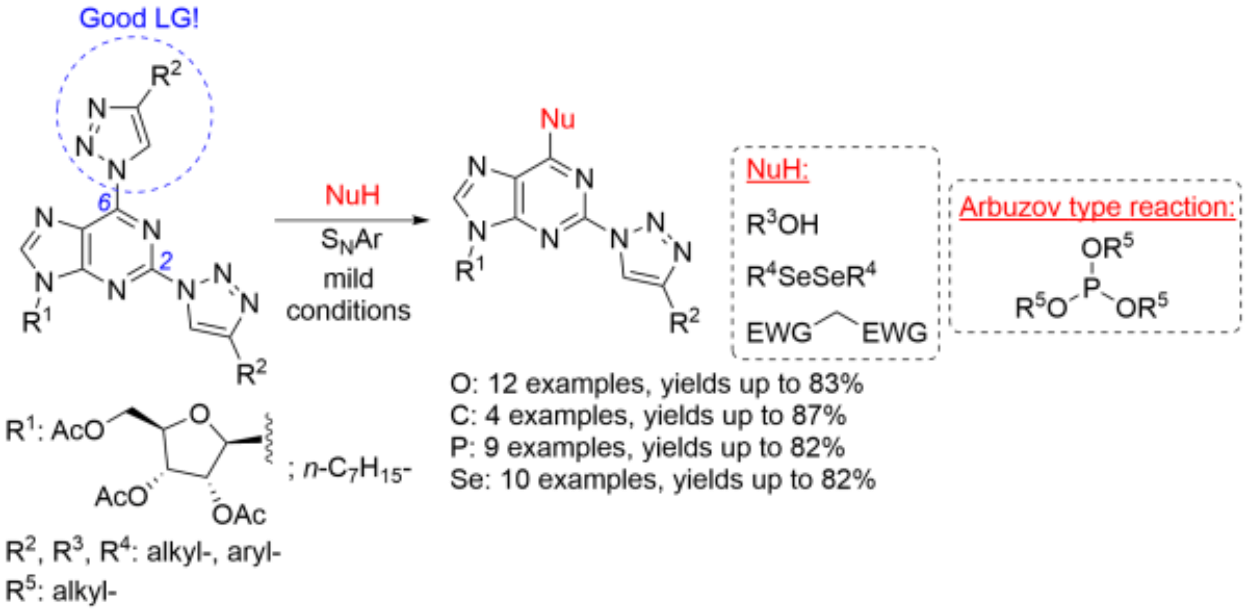
Abstract

Many azolylpurine derivatives possess a wide spectra of biological activities and useful photophysical properties. In 2013, we have introduced 2,6-bistriazolylpurine derivative as a useful intermediate for S_NAr reactions with various N- and S-nucleophiles.

Herein, we describe an extension of developed method using 1,2,3-triazole at C6 position of purine as a leaving group in S_NAr reactions between 2,6-bistriazolylpurine derivatives and O-, C-, P- and Se-nucleophiles. As O-nucleophiles primary and secondary alcohols were used for nucleophilic substitutions, giving products up to 83% yield. For the C-C bond formation at C6 position of purine malonitrile, dimedone, ethyl cyanoacetate and diethyl malonate were used as C-nucleophiles in the presence of NaH. S_NAr-Arbuzov reaction between 2,6-bistriazolylpurine derivatives and alkyl phosphites was applied for the C-P bond formation and C6-phosphonated 2-triazolylpurine derivatives were obtained up to 82% yield. Selenium containing purine derivatives were synthesized in S_NAr reactions between bistriazolylpurines and alkyl/aryl diselenides in the presence of reducing agent. 10 examples of 6-selanyl-2-triazolylpurine nucleosides were obtained in yields up to 82%. For last 20 years the interest in organoselenium compounds has increased due to their various biological properties and potential application in materials chemistry.

The synthetic routes towards C6-substituted 2-triazolylpurine nucleosides will be discussed.

Acknowledgements: This work was supported by the Latvian Council of Science grant No LZP-2020/1-0348.



O: 12 examples, yields up to 83%
C: 4 examples, yields up to 87%
P: 9 examples, yields up to 82%
Se: 10 examples, yields up to 82%

P54

C-nucleosides derivatives for antiviral activities

Abdelhakim Ouarti PhD tudent

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Abstract

Ribavirin (**RBV**) is a well-known broad-spectrum antiviral nucleoside therapeutic agent [1] and in a repositioning process of active compounds for the drug discovery, RBV is in clinical development for anti-tumor activities.[2]

Moreover, **RBV** still inspires new families of analogues because its broad-spectrum property despite the side effects related to its relative efficiency. From our part, we were interested in developing *C*-ribosyl triazoles, such as SRO-91, as stable compounds against enzymatic processes.[3] Indeed, we have recently shown that these compounds exhibit very interesting antitumoral *in vitro* activities against ovarian cancer and/or glioblastoma.[4]

In parallel to this work, we are interested in studying antiviral activities of prodrugs, especially against the Zika virus and against liver viral infections (Hepatitis C, B….).

Very often, biological activities are limited by the distribution of the drug in the body, thus forcing doses to be increased, resulting in augmented toxicity. In order to address this problem, we present here the synthesis of galactosylated derivatives of *C*-nucleosides to target lectins such as the asialoglycoprotein receptor (ASGPR), for liver injuries. Therefore, modification of the *C*-nucleoside by incorporation of galactoside residues would allow specific targeting of the liver resulting in higher concentration of the *C*-nucleoside and increased endocytosis ability. The synthesis of the prodrug *O*-galactosyl- and the non-hydrolyzable *C*-galactosyl-*C*-nucleoside conjugates are shown as well as their antiviral activities.

We are also interested in the synthesis of *C*-nucleotides analogues for their antiviral activities. Phosphorylation of *C*-nucleosides is often a rate-limiting step that has been overcome by the prodrug strategy and in particular the "ProTide®" developed by Chris McGuigan.[5] We have synthesized a series of phosphoramidates consisting of phenyl or naphthyl as the aryl group and Ala or Val ethyl, isopropyl or benzyl esters for the amino acid moiety, in order to study and compare the antiviral activity of these nucleotides with SRO-91.

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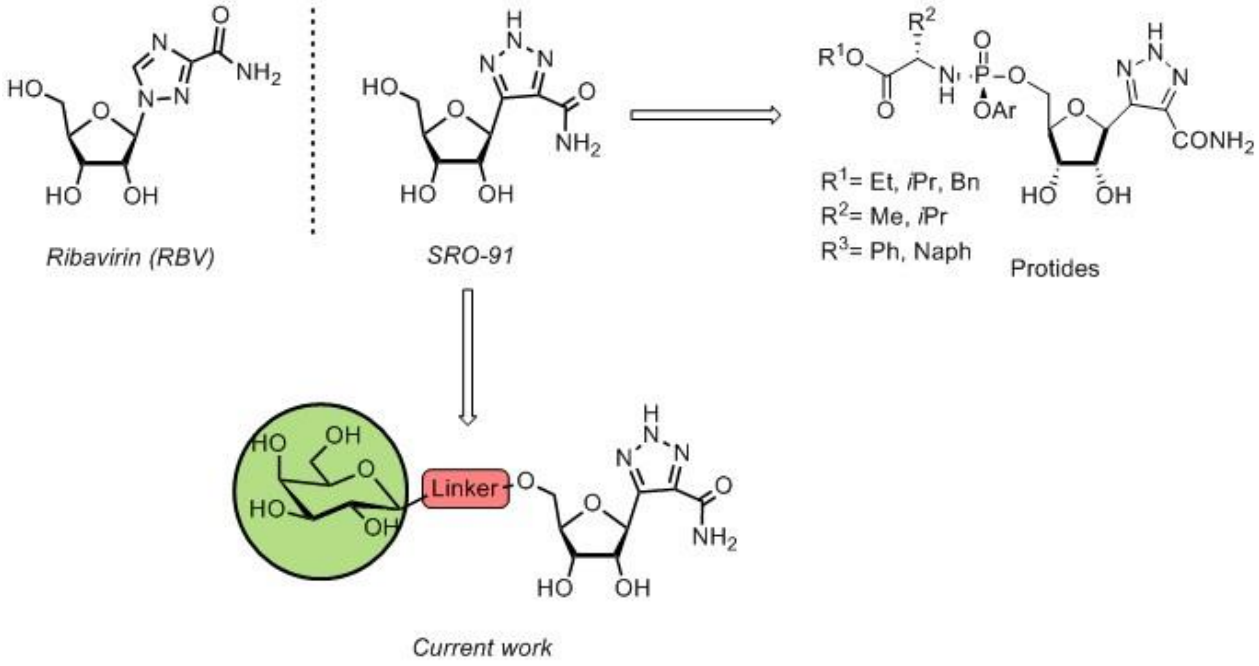
[5] Y. Mechello



P55

Fluorescent DNA: Synthesis and Photophysical Studies of Aminotroponyl deoxyuridinyl DNA Analogues

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Abstract

Fluorescent DNAs are being used as probes for evaluating/detecting the DNA based biochemical processes. Their fluorescent characters are accomplished by introducing the fluorophores at nucleobase or sugar sites.[1] The synthesis of fluorescent nucleobases are emerging tools for making the fluorescent DNA by the chemical modifications at nucleobases of nucleosides, mainly at non-hydrogen bonding sites.[2] The extension of nucleobase conjugations, by benzenoid aromatic/allylic chemical moieties, generate the push-pull type of electronic structure at their purine/pyrimidine rings which may turn nucleobases into fluorescent molecules. It is desired to see the role of non-benzenoid aromatic scaffolds in extension of pyrimidine/purines ring conjugations. Tropolone and related molecules are well known non-benzenoid scaffolds. [3, 4] Recently troponyl conjugated DNA has been synthesized that exhibit fluorescence characters. Herein, the aminotroponyl scaffold has introduced at C-5 position of deoxyuridine and incorporated into DNA. The conjugation of aminotroponyl scaffold at nucleobase uridine is accomplished through ethyne or furan ring fusion. Interestingly, furan ring of deoxyuridine analogues is transformed into pyrrole ring system during the chemical synthesis of DNA. The furan ring fused aminotroponyl deoxyuridine analogue shows better fluorescence character than ethyne linked analogue in organic solvents ACN/DCM. Importantly, the furan fused analogue is transformed into pyrrole fused ring uridine analogue during the DNA synthesis, and remarkably enhanced the fluorescence after the formation of duplex structure.

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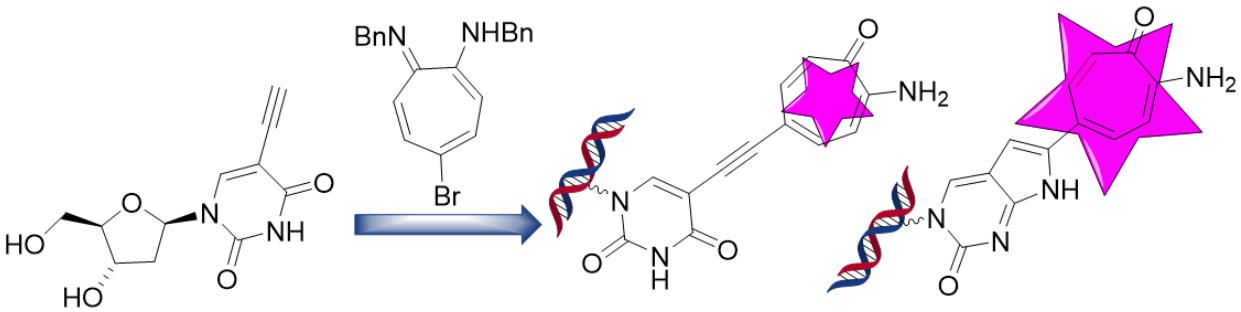

Modified Fluorescent DNA

P56

The minimal in vitro model of G4 DNA in a chromatin context

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Abstract

G-quadruplexes (G4s) are gaining increasing attention as possible regulators of chromatin packaging. In this regard, we aimed to obtain a simple model for assessing their impact on nucleosome occupancy. We designed a dsDNA construct composed of the canonical 165-bp nucleosome positioning sequence and a 50-bp flanking sequence that imitates internucleosomal (linker) DNA. The 50-bp flank harbored a motif predicted to form a stable G4 structure. Mutant dsDNA with a non-G4 50-bp flank was prepared in parallel as a control construct. Using optical methods, electrophoresis, and atomic force microscopy, we confirmed G4 folding within the linker part of the construct. Interestingly, an additional G-rich motif predicted to form a semi-stable G4 was found within the nucleosome positioning sequence. Both constructs were recognized by G4-specific antibodies, but not in the presence of the excess of histone octamers. Both constructs enabled nucleosome assembly, but it was less efficient in the case of the G4 flank. G4 ligands disrupted the nucleosomes at high concentrations, and G4/nucleosome-binding chromatin remodelers induced the formation of multicomponent aggregates. Our results argue that G4 motifs do not prevent nucleosome assembly, while stabilized or intrinsically stable G4s may exclude nucleosomes. This work was supported by Russian Foundation for basic research [19-04-00050] (model design) and Russian Science Foundation [19-15-00128] (studies of G4-ligand/protein interactions).

P57

The effect of nucleotide modifications on specific binding, degradation resistance and inhibitory potential of short poly(A) tail analogs

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Abstract

Poly(A) tail is a regulatory and protective structure located at a 3' end of mature cellular mRNA, composed of multiple adenosine monophosphates. It plays an important role in mRNA stabilization and degradation, as well as translation regulation. Translation, a cellular mechanism of protein synthesis, is preceded by closing an mRNA molecule in a loop formation. This loop closing only occurs after the 3' poly(A) tail is specifically recognized and bound by poly(A) binding protein (PABP). By interacting with a protein complex formed at the 5'end of mRNA, PABP facilitates bringing both mRNA ends together [1].

As mentioned before, poly(A) tail not only plays a regulatory but also a protective role. This long polyadenylated chain located at the 3' end protects the main body of mRNA, coding the protein sequence, from premature degradation by exoribonucleases – specific enzymes that cleave nucleotide subunits from a polynucleotide chain. When necessary, full mRNA degradation begins with deadenylation, which is the removal of the poly(A) tail [2].

Here, we describe the synthesis of short oligonucleotide analogs of the poly(A) tail. Each analog contains at least one or more common nucleotide modification in various positions within the oligonucleotide chain. Among the introduced modifications were: 2'O-Me and 2'F ribose substitutions, thiophosphate modification, m^6A base methylation and guanosine (G) for adenosine (A) base substitution. Multiple 12-mer poly(A) analogs were obtained via solid-state synthesis.

Using a novel and highly effective method of microscale thermophoresis (MST) we were able to determine indirect association constants (K_{app}) of all poly(A) tail analogs binding with PABP. As a result, we also determined the effect modifications and their positioning had on binding affinity with PABP. By performing enzymatic experiments with specific deadenylating enzyme CNOT7 we established different levels of resistance against degradation provided to oligonucleotide chain by aforementioned modifications. Lastly, based on the previously acquired information, we designed three different poly(A) analogs and studied their potential as translation inhibitors in a rabbit reticulocyte lysate (RRL) system.

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P58

A dual prodrug approach for the simultaneous delivery of two bioactive nucleos(t)ides

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Abstract

A novel series of phosphoramidate pronucleotides, including a *S*-pivaloyl-2-thioethyl (*t*BuSATE) moiety as biolabile phosphate protecting group, with the potential to deliver two different anti-HIV nucleoside analogues will be reported. Such constructs were obtained using different phosphorus chemistries and their comparative anti-HIV evaluation shows that such dual prodrugs are able to allow the efficient intracellular combination release of a 5'-mononucleotide as well as another nucleoside analogue.[i] In human T4-lymphoblastoid cells, the pronucleotide incorporating both ddC and AZT exhibits remarkable antiviral activity with an EC_{50} in the nanomolar range and without additional cytotoxicity. Furthermore, these first models exhibit higher selectivity index than the equimolar mixture of their constitutive nucleoside analogues opening the way to further studies with regard to the current use of drug combinations.

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P59

Synthesis and Characterization of O4-Methyl Adducts of C5-Modified-2'-deoxyuridines and Evaluation of Repair by O6-Alkylguanine DNA Alkyltransferases

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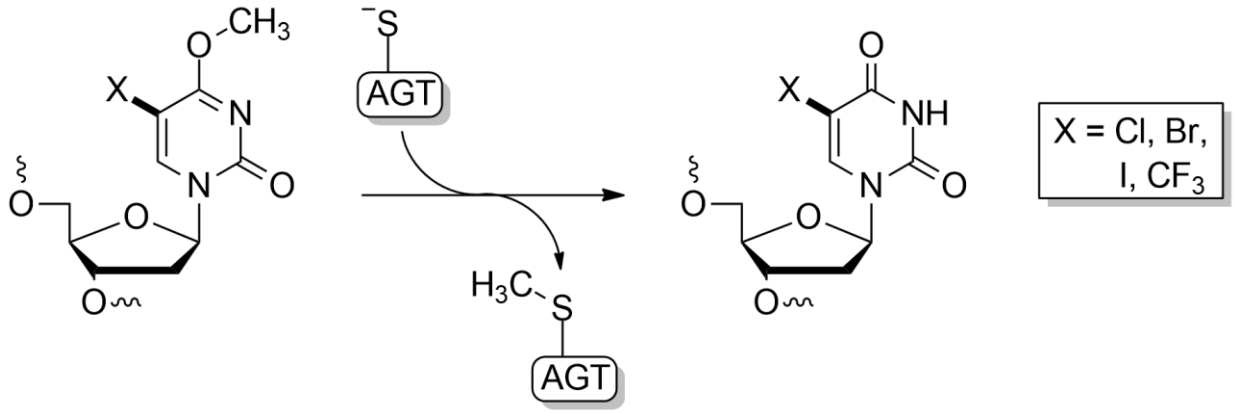
Abstract

DNA is susceptible to the formation of alkyl lesions at the O^6- and O^4- position of 2'-deoxyguanosine and thymidine resulting in GC to AT and TA to CG transition mutations. The DNA repair protein O^6-alkylguanine-DNA alkyltransferase (AGT) can remove a variety of alkyl lesions at these positions via a cysteine mediated alkyl transfer yielding an irreversibly alkylated AGT protein. While AGT is found in numerous organisms, AGT variants demonstrate different repair efficiencies towards these lesions. To better understand the catalytic removal of alkyl adducts by AGT, the influence of C5 nucleobase substitution on O^4-methyl removal by four AGT variants was studied. O^4-methyl-2'-deoxyuridine nucleoside analogs containing C5-chloro-, bromo-, iodo-, and trifluoromethyl modifications were chemically synthesized and incorporated into oligonucleotides. DNA duplexes containing these modifications were studied by UV thermal denaturation which revealed a stability reduction of 11°C for each duplex containing a C5-halogenated O^4-methyl analog relative to their unmodified controls. The ability for DNA repair by hAGT, *E.coli* OGT, Ada-C in addition to a hAGT/OGT chimera (hOGT), was studied using a repair dependant BclI restriction enzyme assay. In all cases, each AGT was capable of removal of the methyl group on the halogenated analogs with varying repair efficiencies. Data reveals that the smaller C5-chloro analog was efficiently repaired by all AGTs while in general, with increasing size of the C5 substituent, repair was reduced. Interestingly, this trend is not observed with the C5-iodo analog which showed increased total repair by all AGTs relative to the C5-bromo and C5-trifluoromethyl substrates. These results suggest that repair efficiency of C5-modified-O^4-alkyl lesions by AGT is dependant on electronic contributions and favorable interactions of the C5-halogens within the AGT active site. Potential favorable interactions with active site residues are discussed. These results provide useful insight on the active site requirements for efficient dealkylation of O^4-alkyl adducts by AGTs.



P60

Synthesis and molecular modeling of purine ribonucleotides as potential ligands of the human G protein-coupled receptor 17 (GPR17)

Marco Rabuffetti[1], Teodora Bavaro[2], Enrica Calleri[2], Stefania Ceruti[3], Chiara Parravicini[3], Daniela Ubiali[2], Giovanna Speranza[1]

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Abstract

GPCRs (G Protein-Coupled Receptors) are important drug targets in medicinal chemistry [1]. The GPR17 receptor, phylogenetically related to both purinergic P2Y and CysLT receptors, is usually over-expressed in the damaged brain tissue and is involved in various disorders characterized by demyelination, such as multiple sclerosis and stroke. Experimental data have shown that it is responsive to both agonists (*e.g.* nucleotides and their adducts) and antagonists (*e.g.* Cangrelor and Montelukast) [2]. Therefore, the human GPR17 receptor is a promising therapeutic target for treatment of neurodegenerative diseases [3].

This evidence prompted us to perform docking studies aided by molecular modeling on a homology model (based on $P2Y_1$ receptors). Among the selected molecules, 8-methylaminoinosinic acid (**1**, **Figure 1**) and three N^2-alkyl/acyl derivatives of guanylic acid (**2**-**4**, **Figure 1**) emerged as the best potential ligands.

As a result, their synthesis was carried out. Compound **1** was obtained by direct phosphorylation of 8-methylaminoinosine, previously prepared by amination of 8-bromoinosine. In the case of **2**, position N^2 of the purine ring was activated as a bromo derivative and subjected to displacement with *n*-octylamine. As for **3** and **4**, N^2-acylations were performed by treatment with a proper acyl chloride or anhydride through a transient protection strategy. Compounds **2**, **3** and **4** were obtained as 2',3'-*O*-isopropylidene adducts of the corresponding nucleotides.

Binding assays will be carried out by Surface Plasmon Resonance (SPR) [4], which has been demonstrated as a reliable technique for the systematic identification of agonists and antagonists of GPCRs, including GPR17 as recently demonstrated by our group [5].

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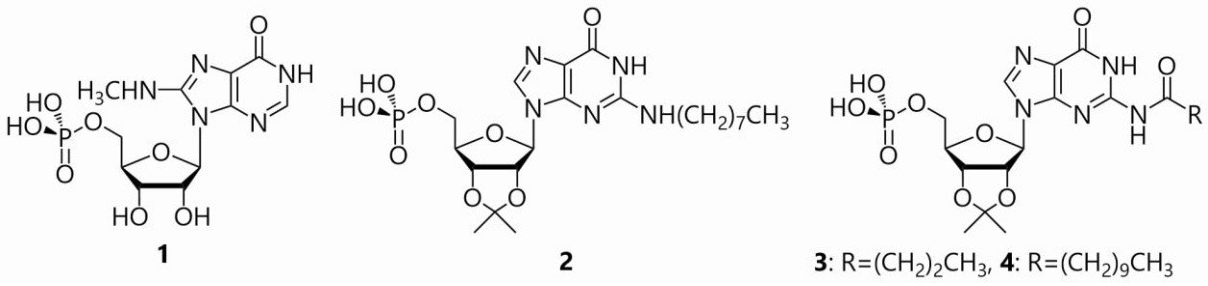

P61

Syntheses and Properties of Stereo-Defined PS-Chiral STOPS™ Molecules for the treatment of chronic hepatitis B

Vivek K. Rajwanshi, David B. Smith, Rajendra Pandey, John K. Cortez, Yuchun Nie, Jin Hong, Julian A. Symons, Lawrence M. Blatt, Leonid N. Beigelman

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Abstract

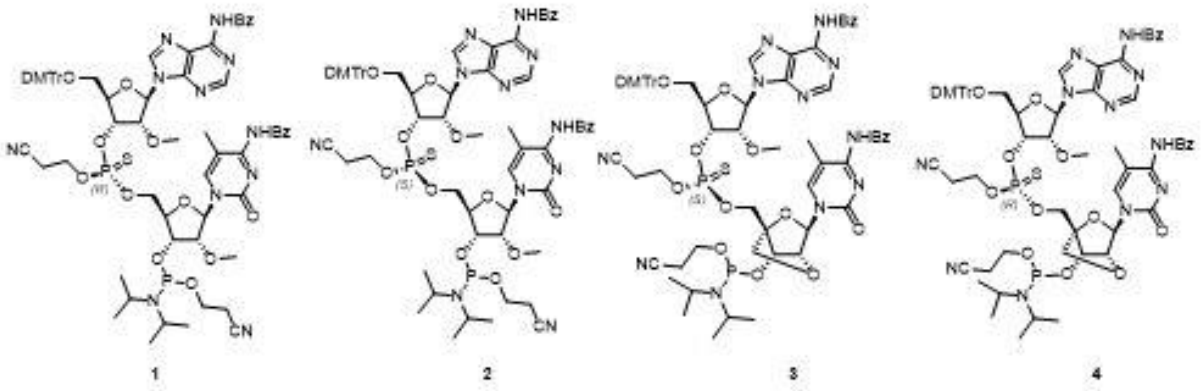
INTRODUCTION:

Chronic Hepatitis B (CHB) is a global public health problem, affecting 300 million people. Current approved treatment options, nucleos(t)ide analogs and / or pegylated interferon, have proven insufficient in attaining meaningful rates of functional cure. Recently, we have reported clinical advancement of ALG-010133, an S-antigen Transport-inhibiting Oligonucleotide Polymers (STOPS) molecule for the treatment of CHB. STOPS molecules are poly-AC oligonucleotides that possess novel chemical features providing for enhanced potency in several HBV cell lines relative to similar agents such as nucleic acid polymers (NAPS). In order to further explore the SAR of STOPS molecules, we have introduced single or multiple predetermined fixed chirality at PS-stereogenic centers which might further influence properties such as potency, stability and target specificity.

RESULTS AND DISCUSSIONS

Here we report an efficient and scalable synthesis of 2'-OMe containing adenine and 5-methyl cytosine stereo defined dimer amidites **1** and **2** for the synthesis of rationally designed stereospecific STOPS molecules. Coupling of activated 2'-OMe-A phosphoramidite with 3'-*O*-levulinyl nucleoside was followed by sulfurization of the resulting phosphite triester to give 5'-*O*-DMT- 3'-O-levulinyl -2'-OMe (5m) C as a stereo random PS dinucleotide. The 3'-O-levulinyl protecting group was removed by treatment with N_2H_4 / pyridine / HOAc. Individual diastereomers were separated using SFC chromatography and characterized by 2D-NMR and ^{31}P NMR as dimer (Rp) **1** and Sp **2**. The procedure describe here is general and was further applied to the syntheses of 2'-OMe-LNA stereo defined dimer amidites **3** and **4.** Subsequent incorporation of these stereo defined dimers **1-4** resulted into stereo-specific STOPS molecules.

The synthesis and characterization of these dimer amidites as well as their incorporation into STOPS molecules along with cell-based biological activity will be presented.



P62

ABN: A fluorescent nucleoside analogue with single-molecule detection capability.

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Abstract

Fluorescent base analogues (FBAs) are widely used as tools for the study of biological systems. Despite recent progress in the field, no FBA with robust single molecule detection (SMD) capability has been made.

Mimicking the structural features of rhodamine, a highly bright conventional fluorophore, while maintaining the Watson–Crick base pair motif led to the design of linked 8-(diethylamino)benzo[*b*][1,8]naphthyridin-2(1*H*)-one nucleoside (ABN). ABN has a similar push-pull motif with extended conjugation system as in rhodamine. This extended conjugation causes ABN to be excited at a longer wavelength with a high extinction coeffecient.

ABN was made in a 14-step synthesis including Vilsmeier, Wittig and Heck reactions in addition to several substitution, protection and deprotection reactions. After the synthesis of ABN nucleoside analogue, phosphoramidite synthesis was performed to synthesize several oligonucleotides using solid phase DNA synthesis. Photophysical measurement studies were performed on ABN free nucleoside and modified oligonucleotides.

ABN shows a high quantum yield of 39% and a high extinction coeffecient of 20,000 M^{-1} cm^{-1} in PBS buffer, making it one of the brightest FNAs ever studied. When incorporated into duplex DNA, ABN shows higher quantum yields (50-53%) when base paired with adenosine, making ABN as the brightest and most red shifted FBA studied in DNA. Due to its high brightness and stability, ABN, as a free nucleoside, is the first FBA to be detected in single-molecule fluorescence upon both one-photon and two-photon excitation.

P63

RE-SELEX: Restriction Enzyme-Based Evolution of Structure-Switching Aptamer Biosensors

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Abstract

Aptamers are widely employed as recognition elements in small molecule biosensors due to their ability to recognize small molecule targets with high affinity and selectivity. One particularly promising class for biosensing applications is structure-switching aptamers because target-induced conformational change can be directly linked to a functional output. However, traditional evolution methods do not select for the significant conformational change needed to create structure-switching biosensors. Modified selection methods have been described to select for structure-switching architectures, but these remain limited by the need for immobilization. We have constructed the first homogenous, structure-switching aptamer selection that directly reports on biosensor capacity for the small molecule target. We used kanamycin A as a model compound to demonstrate the feasibility of our new method. Excitingly, we successfully selected for four structure-switching biosensor sequences that report on kanamycin A concentrations. Further optimization of biosensor conditions afforded facile detection of kanamycin A (90 μM – 10 mM) with high selectivity over three other aminoglycosides. This general approach would be broadly useful for small molecule detection, especially for particularly challenging targets.

P64

Targeted Gene Regulation of GATA-3 in immune cells using Peptide Functionalized Nucleic Acid Nanocapsules as treatment for Asthma

Shraddha S. Sawant, Tyler D. Gavitt, Alyssa K. Hartmann, Arlind B. Mara, Steven M. Szczepanek, Jessica L. Rouge

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Abstract

Asthma is a chronic respiratory condition affecting a major population globally, with no complete cure. Although conventional drugs such as bronchodilators and steroids offer symptomatic relief, treatment of such an intractable disease requires novel targeted therapies. Herein we show the targeted delivery of a GATA-3 specific DNAzyme using a unique oligonucleotide nanocarrier developed by our lab referred to as a nucleic acid nanocapsule (NAN) reduces lung inflammation by efficient knockdown of an asthma associated gene, GATA-3. These enzyme responsive peptide crosslinked NANs are designed to break apart in the presence of specific proteolytic enzymes which are overexpressed during inflammation, thereby releasing DNAzyme-surfactant conjugates. The enzyme specificity was confirmed via a variety of techniques including agarose gel assays and qPCR analysis of a non-cleavable NAN compared with that of a peptide crosslinked NAN. The knockdown efficiency of the GATA-3 NAN was tested *in vitro* in immune cells including human Jurkat T cells in a dose-dependent manner. The inability of mutated (point mutation) DNAzyme NANs to reduce GATA-3 expression in T-cells as compared to active GATA-3 DNAzyme NANs confirms the specificity of our delivery construct. Flow cytometry analysis and confocal imaging confirmed the accumulation of DNAzyme-surfactant conjugates in peripheral blood mononuclear cells, specifically in CD4+ cells without the need of transfection agents or modifications. Moreover, treating house dust mite (HDM) induced asthmatic mice with GATA-3 DNAzyme NANs actively reduced lung inflammation as compared to the untreated control population, as measured based on eosinophil accumulation. This study confirmed the *in-vivo* stability, targeted delivery, and efficiency of NANs as a nucleic acid carrier. The use of GATA-3 DNAzyme NANs may have promising clinical application for the treatment of both acute and chronic symptoms of asthma in humans through transcription-based regulation.

P65

Impact of initiator characteristics on the efficiency of *de novo* DNA synthesis by terminal deoxynucleotidyl transferase

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Abstract

Terminal deoxynucleotidyl transferase (TdT polymerase) has gained prominence due to its template-independent mode of action, extending the 3'-OH termini of single-stranded DNA initiators with dNTPs in random order. The broad substrate range regarding – even modified – nucleoside triphosphates has been investigated in different contexts, including introduction of terminal modifications of DNA oligonucleotides, like fluorescent labels and affinity tags. Thanks to efforts in controlling TdT's activity in a way that allows for addition of single nucleotides, it is also a prominent candidate to facilitate enzymatic *de novo* synthesis of DNA oligonucleotides as an alternative to chemical synthesis, which is linked to accumulation of hazardous waste and features limitations in length of the oligonucleotide products. However, different approaches tackling enzymatic DNA synthesis still rely on chemically synthesized DNA initiator strands of at least 20 nucleotides in length.

One of our recent projects aims at investigating the dependence of strand extension by TdT polymerase on the characteristics of the initiator. Therefore, we used photolithographic *in situ* synthesis to generate permutation libraries of all possible sequence combinations for DNA strands, varying in length from one up to five nucleotides, immobilized to a glass slide. These microarrays allowed us to investigate the impact of initiator length and nucleotide composition on the efficiency of extension by TdT polymerase *via* assessment of its activity on all these initiator variants in a single experiment using a hybridization-based approach for detection. Furthermore, we expanded our investigation beyond the conventional substrate of D-DNA with terminal 3'-OH, and additionally challenged the polymerase with extension of D-DNA (5'-OH), L-DNA (5'-OH), 2'-O-methyl RNA (3'-OH) and even hexaethylene glycol strands.

Although our results clearly show D-DNA with terminal 3'-OH being the preferred substrate for extension, they also reveal the remarkable potential of TdT polymerase to extend other initiators, including strands of hexaethylene glycol and even mirror-image DNA, with deoxynucleotides. Pointing out the impact of length and sequence of such initiating strands on efficiency of extension, important conclusions can be derived for the use of TdT polymerase in enzymatic synthesis of DNA and its applications in synthetic biology.

P66

Synthesis of oligoribonucleotides containing 8-oxoG, m^1G, $m^{2,6}A$, or 8-bromoG and their reactivity with the exoribonuclease, Xrn1

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Abstract

Oxidative damage to RNA and DNA, brought on by reactive oxygen species (ROS) has been correlated to neurodegenerative diseases, mental disorders, and age progression.[1] ROS are a normal biproduct of metabolism, however, aging and external factors, such as ionizing radiation can increase their concentration.[1]Because of the severity of these effects, it is likely that eukaryotes and prokaryotes have developed mechanisms for the removal of oxidatively damaged RNA. Among the four canonical nucleobases, guanine has the lowest oxidation potential, and as a result 8-oxo-7,8-dihydroguanine (8-oxoGua), is observed the most frequently. On the other hand, the 5'-3' exoribonuclease known as Xrn1 has been shown to have a role in the decay of oxidized RNA.[2] Herein we report on the *in vitro* reactivity of Xrn1 with RNA containing 8-oxoG. To this end, we synthesized RNA (20nt long) and incorporated this lesion via solid phase synthesis. Treatment of a 5'-phosphorylated ON with the exonuclease, followed by MALDI-TOF analysis revealed the presence of a fragment that corresponded to enzymatic stalling at 8-oxoG. To corroborate this observation, a bisphosphate uridine nucleotide was prepared to enable data analysis from 3'-end radiolabeling experiments (synthesis and approach described). To gain a better understanding for the nature of this observation, oligoribonucleotides containing m^1G, $m^{2,6}A$, or 8-bromoG were prepared, where the former can be used to probe H-bonding interactions and the latter for conformational changes around the glycosidic bond. Using circular dichroism spectroscopy, we found that all sequences used, lacked structure (a factor known to contribute to exonuclease inhibition), supported by non-characteristic spectral forms and wide thermal denaturation ranges. It was determined that in the presence of sequences containing 8-oxoG, Xrn1 experienced stalling at ~30% when compared to the canonical, m^1G, $m^{2,6}A$, and 8-bromoG sequences. Thus, it can be inferred that the stalling observed is not a result of hydrogen bonding, conformational changes, or secondary structure; but more likely a direct interaction between 8-oxoG-sites and Xrn1.

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P67

Synthesis and photophysical properties of fluorescent purine derivatives

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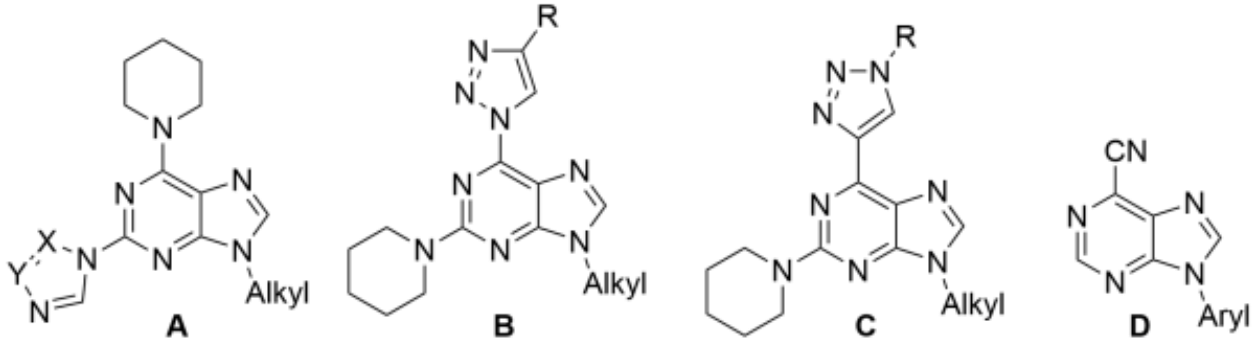
Abstract

In this study we have introduced various substituents at C2 and C6 positions of purine to test their impact on fluorescent properties. Fluorescent purine derivatives have potential for use in materials science, cell imaging and study of DNA/RNA structures. Our initial attempts involved synthesis of compounds of type **A** and **B** which are characterized by push-pull fluorescence. Various azole groups were introduced as electron acceptors, mainly focusing on the use of 1,2,3-triazolyl moieties, while piperidine substituent was used as an electron donating group. Functionalization at N9 was done either with alkyl substituents either with or without bulky triphenylmethyl groups, which provided amorphousing properties for compounds, that are useful in materials science. Compounds of type **A** exhibited visible violet emission with moderate yields, while compounds of type **B** emitted blue light with yields over 90% in DCM solution and over 50% in thin layer film. Compounds of type **C** were synthesized to determine the influence of C-C connection between triazole and purine on stability and emissive properties in comparison with C-N analogues **B**. Compounds of type **C** were synthesized using Sonogashira coupling and subsequent CuAAC with different alkyl and aryl azides. However, differences in stability and emission between compounds **B** and **C** were negligible. Our latest research focuses on 6-cyanopurine derivatives **D**. Such compounds exhibit thermally activated delayed fluorescence and can form emissive exciplexes with carbazole derivatives. These compounds exhibit emission only in solid state and we are investigating effects of steric hindrance and substituent positioning on their photophysical properties.

The synthetic routes, structure diversity of modified purine derivatives and their photophysical properties will be discussed.

Acknowledgements: This work was supported by the Latvian Council of Science grant No LZP-2020/1-0348.



P68

A carbazole-derived nitroxide that is an analogue of cytidine – a rigid spin label for DNA and RNA

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Abstract

To understand the function and behavior of biomolecules, such as nucleic acids, information about their structures and dynamics is required. Electron paramagnetic resonance (EPR) spectroscopy is a valuable technique to access such information. For this method, a paramagnetic center is required. Nitroxides, commonly used as spin labels, are small organic molecules that possess an unpaired electron. Such radicals are persistent and can be readily synthesized. With dipolar EPR spectroscopy, such as Pulsed Electron-Electron Double Resonance (PELDOR), it is possible to obtain information about structure through measurement of distances between two spin labels attached to a biomolecule. The rigid spin labels **Ç**[1] for DNA and **Çm**[2] for RNA (**Figure 1A**), are ideal reporters for EPR. Not only do they yield accurate distances but can also give information about relative orientations of spin labels inside nucleic acids.[3-4] Although the crystal structure of **Ç** in DNA shows that the base is fully planar, a crystal structure of the nucleobase showed a non-planar geometry, with a ca. 20° bend at the oxazine linkage, presumably due to crystal packing.[5] Bending around the oxazine linkage may be due to the 16 π electrons **Ç** and **Çm** have, which makes them formally antiaromatic. Here we describe the design and synthesis of new rigid spin labels, **Ċ** and **Ċm** (**Figure 1B**), for nucleic acids that are aromatic and their crystal structures indicate that they are more rigid than **Ç** and **Çm**.

Acknowledgment: This work was supported by a grant from the Icelandic Research Fund (173727-051).

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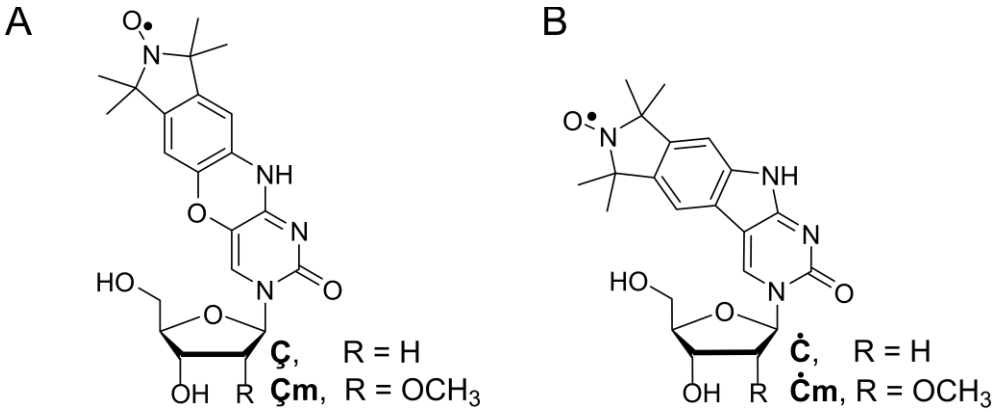

Figure 1. A. Rigid nucleosides **Ç** and **Çm**. B. New rigid nucleosides **Ċ** and **Ċm**.

P69

Synthesis of 2´ -O-carbamoylethyl nucleic acids incorporating various alkyl chains on the carbamoyl nitrogen to evaluate their hybridization properties, resistance toward 3´ -exonuclease, and their lipophilicity.

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Abstract

2´-O-modified oligonucleotides have been widely studied for the development of nucleic acid-based technologies, such as nucleic acid drugs. For these purposes it is important to expand the chemical space of oligonucleotides in terms of their physicochemical properties. Recently, we have reported the synthesis and properties of various 2´-O-carbamoylethyl-modified oligonucleotides bearing methyl or aminoalkyl groups on the carbamoyl nitrogen. These carbamoylethyl oligonucleotides showed increased stabilities toward nuclease digestion, affinity comparable to 2´ -O-methyl derivative toward complementary RNAs, and applicability as antisense oligonucleotides.

In this study we report 2'-O-carbamoylethyl nucleic acids incorporating ethyl, n-propyl, n-butyl, n-pentyl, n-octyl and some arylalkyl groups on their carbamoyl nitrogen. The corresponding thymine nucleosides were synthesized using 2´-O-benzyloxycarbonylethylthymidine after being converted to the carboxylic acid through hydrogeneration. Then, the carboxylic acid derivative was condensed with the appropriate amines to give the desired nucleosides, and their phosphoramidites. Using the phosphoramidites, we synthesized oligonucleotides incorporating them, and studied the effect of the 2´-O-alkylcarbamoylethyl modifications on the thermal stability of the duplexes, tolerance toward a 3'-exonuclease, and lipophilicity of the oligonucleotides.

P70

The structure of i-motif/duplex junctions at neutral pH

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Abstract

The i-motif is a four-stranded intercalated structure stabilized by the formation of hemiprotonated C:C$^+$ base pairs, and therefore usually stable at acidic pH. This structure is attracting an extraordinary attention since its recent observation in human cells. Several studies *in vitro* and *in vivo* have found i-motifs in many biologically relevant C-rich sequences. It is believed that i-motifs are formed transiently *in vivo*. Since DNA is mainly a double helix in the cell, local i-motif formation entails the formation of interfaces (junctions) with canonical B-form DNA regions. G-quadruplex/duplex junctions are being extensively studied, and found to be an interesting target for selective recognition.[1] However, very little is known about the structure of i-motif/duplex junctions (IDJs).

In this communication, we present the first three-dimensional structure of an i-motif/duplex junction (IDJ) obtained by NMR methods. One of the difficulties of this kind of studies is that the optimal experimental conditions for i-motif and duplex formation are different. To avoid this problem, we have taken profit of the recent observation that minor groove tetrads (MGTs) permits the stabilization of i-motif structures at physiological pH.[2] Thus, several constructs were designed including a MGT at one end of a monomeric i-motif and a stem/loop hairpin at the other side, exploring different interfaces. The most stable construct includes a T:T base pair in the interface following the intercalation arrangement of the i-motif.

The structural determination of this construct confirms that these two secondary structures coexist in the same molecule at physiological pH. Moreover, it opens the door to study possible IDJ formed by sequences with biological relevance, such as KRAS or NMYC oncogene promotors. Furthermore, the IDJs are an ideal model to screening ligands, allowing to study the selectivity of the ligands for different DNA topologies in one experiment or even recognizing IDJ interface, a possible unique binding mode of DNA.[3]

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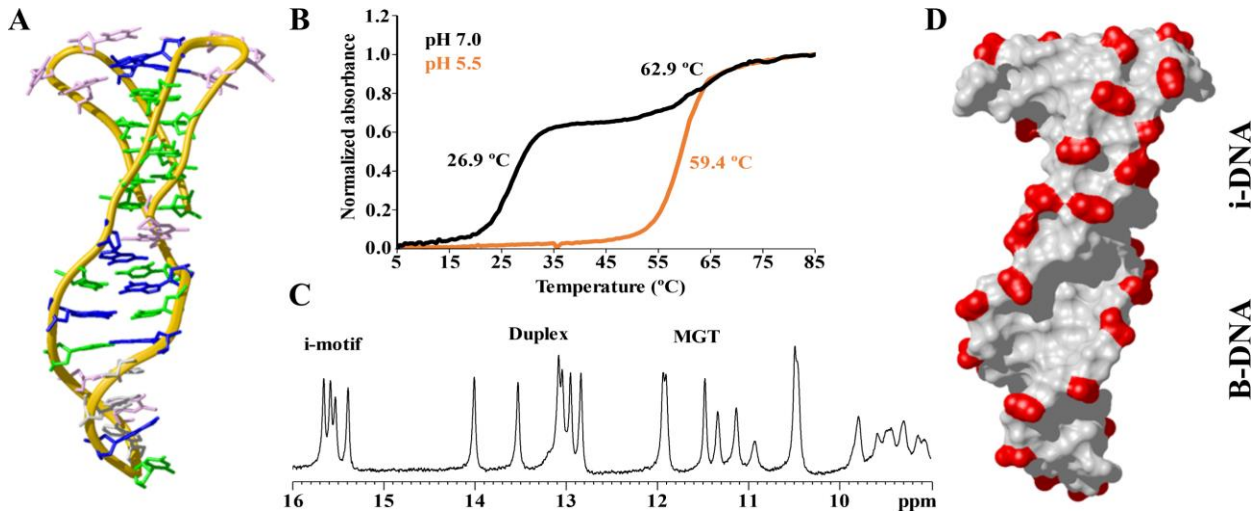
A
B
pH 7.0
pH 5.5
62.9 °C
26.9 °C
59.4 °C
Normalized absorbance
Temperature (°C)
C
i-motif
Duplex
MGT
ppm
D
i-DNA
B-DNA

P71

Fluorescent DNA Analogues: Synthesis and Photophysical Studies of Troponyl DNA/RNA Analogues

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Abstract

The native DNA and RNA are non-fluorescent biomacromolecules, which are genetic materials of living systems and regulate most biological processes. The fluorescent DNA/RNA are outstanding probes to recognize the nucleic acid-dependent biological process for understanding and finding their specific role in real-time cellular environments.[1-3] The conjugation of fluorescent dyes either at sugar ring or nucleobases are commonly used to prepare the fluorescent DNA/RNA. The non-fluorescent nucleobases may turn fluorescent by the structural modification of pyrimidine/purine rings. The native nucleobases participate in Watson-Crick (W-C) type hydrogen bond base-pairing in the duplex structure of DNA. The structurally modified fluorescent nucleobases have chemically prepared by either preserving or non-preserving the W-C hydrogen base pairing. The non-benzenoid aromatic compounds also occur in nature as *Troponoides* natural products containing *Tropolone, Aminotropone*, and *Aminotropimine*.[4] Tropolone and related compounds exhibit the broad range of bioactivities such as antimicrobial, antiviral, antifungal, antibiotic and anticancer. Unlike benzene, Tropolone is a seven-membered ring aromatic molecule comprising unique photophysical behaviours, intramolecular hydrogen bonding, and excellent metal-chelating ability with transition metal ions suc as Cu^{2+}, Zn^{2+} and Ni^{2+} (Figure).[5] However the conjugation of non-benzenoid structural moiety to the biomacromolecules are under utilized as compared to the benzenoids moiety. The extension of pyrimidine/purine ring conjugation could be extended with non-benzenoids scaffolds for making the fluorescent nucleobases. So far there is no report about the conjugation of tropolonoids moiety with DNA/RNA analogues, except our previous report. Recently, we have appended non-benzenoid aromatic scaffold *Tropolone* at the C5-position of the deoxyuridine ring, through an ethyne linker, and it's DNA analogues that exhibit pH-dependent fluorescence characters, inversely proportional to pH, though their quantum yield are low (Figure 1a).[6] Herein, we have attempted to introduce a 2-aminotropone unit at C5-position of deoxyuridine and its DNA through ethyne linker and fused ring (furanyl & pyrrole ring). This report describes the synthesis, biophysical and photophysical studies of aminotroponyl-DNA analogues.

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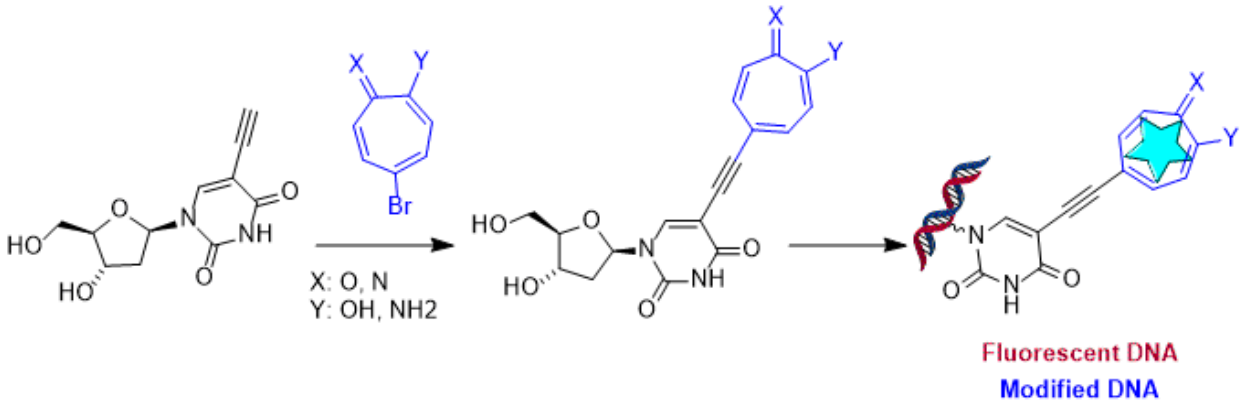

P72

Development of 2-amino-7-deaza-6-vinylpurine-deoxyriboside as an efficient cross-linking nucleoside for anti-miRNA therapy

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Abstract

MicroRNAs (miRNA) play a critical role in regulating gene expression. One of the general strategies to inhibit miRNA function is to use synthetic anti-miRNA oligonucleotides (AMOs), and several chemically modified AMOs have been employed to improve the inhibitory activity. We have been focusing on the reactive nucleosides and developed 2-amino-6-vinylpurine (AVP) nucleosides (Fig. 1a). AVP has a vinyl group which functions as a Michael acceptor to form a covalent bond (or cross-link) with the counter uridine in RNA.[1] Such cross-link formation would increase the thermal stability of the duplex structure and is expected to induce stronger inhibition of the miRNA function.

In our previous report, we have designed 2'-OMe-RNA strand incorporating 2-amino-7-deaza-7-propynyl-6-vinylpurine-riboside (ADpVP).[2] The propynyl group on the 7-position was shown to enhance the kinetics of the cross-link formation with the target RNA strand by orienting the 6-vinyl group into the s-*cis* conformation. However, the synthesis of ADpVP proved to be an arduous process. To overcome this hurdle, we hypothesized that substituting the sugar moiety of ADpVP with deoxyribose while keeping the rest of the strand as 2'-OMe-RNA will achieve a comparable effect while allowing a simpler synthesis. To assess our hypothesis, we designed a 2'-deoxy analogue, 2-amino-7-deaza-7-propynyl-6-vinylpurine 2'-deoxyriboside (dADpVP; Fig. 1a). The phosphoramidite of dADpVP was synthesized in 10 steps and incorporated into CFO composed of 2'-OMe-RNA using an automated DNA synthesizer. The cross-linking reaction was conducted using the FAM-labeled complementary RNA strand, and the reactions were analyzed by denaturing polyacrylamide gel electrophoresis. We found that dADpVP exhibits the uridine selective cross-link formation with a comparable reaction rate to the previously reported ADpVP (Fig. 1b). The slight decrease in the reaction rate is presumably due to the local conformational difference between the deoxyribose and 2'-OMe-ribose structures. Nevertheless, the reaction rate of dADpVP is considerably faster than the parental AVP, indicating its potential utility for anti-miRNA methodology. The details of the synthesis and cross-link reactions will be reported in the presentation.

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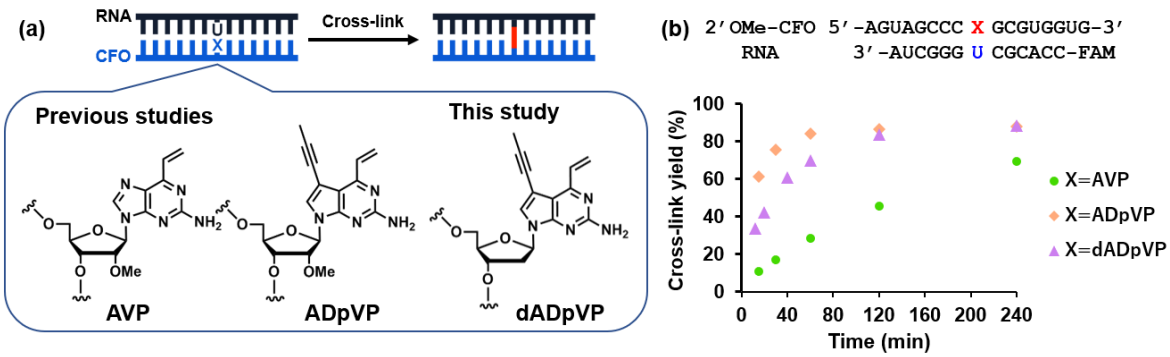

Fig. 1 (a) Structures of cross-linking AVP derivatives. (b) The cross-link properties of each AVP derivative.

P73

Microscale thermophoresis-based assay for the evaluation of interaction of IFIT1 with RNA cap analogs and capped RNAs

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Abstract

During viral infection, higher organisms, including humans, activate a number of immune-related defenses aimed at inhibiting the pathogen. One of the main signaling factors secreted in the cell in such a situation is interferon, which in turn affects the expression levels of many proteins including those from the IFIT family. IFIT1 protein (Interferon-induced protein with tetratricopeptide repeats 1) is localized in the cytoplasm where it plays a role of RNA sensor. IFIT1 specifically recognizes cellular mRNAs carrying cap 0 structures, whereas it has significantly lower affinity towards mRNAs carrying cap 1. Once mRNA is recognized by IFIT, it cannot be accessed by cellular translation machinery. The preferential binding of IFIT1 to cap 0 RNA has been confirmed in several previous experiments and is thought to contribute to translational shutdown of viral mRNAs. mRNAs exogenously delivered into cells as therapeutics can also be potentially recognized by IFIT1. In our study, we sought to examine how various natural and synthetic modifications of mRNA 5' cap affect the affinity of RNA for IFIT1 protein. To this end, we expressed HisTag-modified IFIT1 protein in a prokaryotic E.coli-based expression system and purified the protein using affinity chromatography, ion exchange chromatography, and gel filtration methods. MicroScale Thermophoresis assay (MST) was used to examine the affinity of fluorescently labelled IFIT1 with ligands (cap analogs and capped RNAs) by determining the dissociation constants (KD) between each ligand and IFIT1. First, conditions optimization and protein stability measurements were performed using a Tycho NT.6 instrument. Then, a series of KD measurements were performed for IFIT1 and different caps ligands, including: m7GpppApG (cap 0), m7GpppAmpG (cap1), m7GpppUpG, m7GpppGpG, m7GpppCpG, TMGcap, NAD-cap, FAD-cap. Finally, a series of KD measurements of the IFIT1 protein were performed with the 35 bp and 70 bp long RNA ligands capped with the same cap analogs, which allowed us to determine differences in affinity and find the strongest and weakest interacting ligands relative to the cap 0 and cap 1 references. The project is financially supported from Development of experimental anti-cancer immunotherapy based on therapeutic mRNA modified with innovative next generation cap analogs POIR.01.01.01-00-0920/19

P74

Large Stokes shift fluorescence activation in an RNA aptamer by intermolecular proton transfer to guanine

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Abstract

Fluorogenic RNA aptamers are of growing interest for RNA imaging and as small-molecule sensors. Chili is an engineered 52-nt RNA aptamer that binds to derivatives of 3,5-dimethoxy-4-hydroxybenzylidene imidazolone (DMHBI) with high specificity and affinity, and activates their otherwise quenched fluorescence. The resulting green or red emission is shifted from the excitation maximum by 140 nm, making Chili–ligand complexes mimics of large Stokes shift (LSS) fluorescent proteins. Particularly high fluorescence quantum yields and low-nanomolar binding affinities were achieved by designing ligands with a permanent positive charge, such as $DMHBI^+$ and $DMHBO^+$ [1].

In this work, we elucidated the structural and mechanistic principles underlying the LSS behavior by solving the co-crystal structures of Chili with two if its cationic ligands and, for the first time, applying femtosecond-resolved optical spectroscopy to this kind of fluorogenic RNAs . Our studies revealed a two-tiered G-quadruplex that serves as a central ligand binding platform and is aided by stacking with an unusual base pair between two guanines. The binding site further contains a canonical G:C base pair involving G15 that is coplanar with the bound ligand. In this arrangement, a K^+ ion is coordinated by the ligand, the coplanar base pair and one of the G-quartets, which helps to establish a short hydrogen bond between the phenolic hydroxy group of the ligand and N7 of G15.

Excited state proton transfer (ESPT) from the neutral ligand to the RNA can proceed along this hydrogen bond and constitutes the basis for LSS emission. Due to the preorganized nature of the hydrogen bond, time-correlated single photon counting (TCSPC) did not resolve the kinetics of the ESPT step, which was therefore investigated by a combination of broadband fluorescence up-conversion (FLUPS) and transient absorption (TA). We found a time constant of 130 fs that is associated with the proton transfer, followed by a continuous geometric relaxation of the ligand on the ps time scale and fluorescence emission with a lifetime of 1.4 ns [2].

While the presence of a G-quadruplex at the binding site is a feature Chili shares with other fluorogenic aptamers [3], its mode of action centered around highly efficient ESPT is a first for fluorogenic aptamers and widens our perspective for the growing functional diversity of structured RNA.

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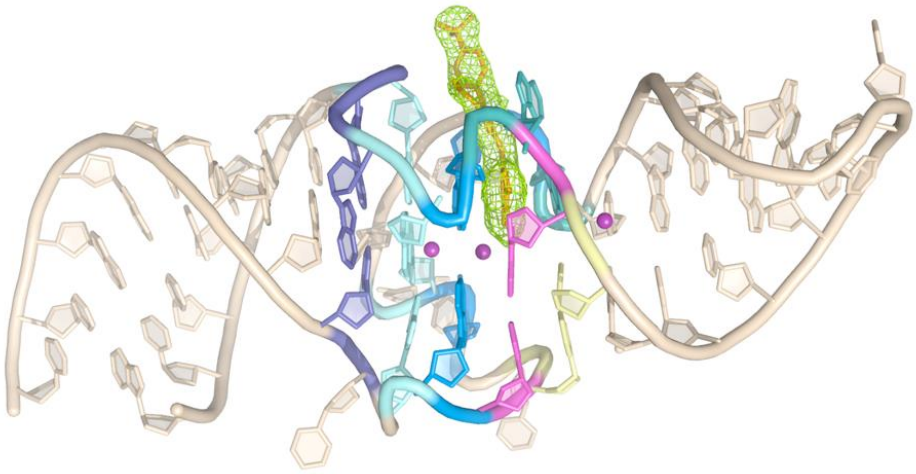

P75

TMG cap conjugates with GFP fluorophore analogs as molecular probes for snurportin

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Abstract

Trimethylguanosine cap structure (TMG cap) is present at the 5 'end of the chain of some small nuclear RNA (snRNA) and is specifically recognized by snurportin, an adaptar protein that initiates the transport of snRNA from the cytoplasm to the nucleus. TMG capped snRNAs are involved in a number of biochemical processes related to RNA transport and pre-mRNA maturation [1]. Fluorescent Molecular Rotors (FMR) are two-segment molecules in which one segment rotates freely relative to the other [2]. FMR fluorescence is based on the phenomenon of intramolecular rotation of one of the marker segments around the σ bond with charge transfer, which results in a non-radiative (OFF) or a radiant (ON) relaxation process. Application of FMRs is based on "switching on" of fluorescence only as a result of interaction with the protein. This properties can make FMR-conjugates useful tools as molecular probes.

Here, we reports the synthesis, spectral, and biochemical properties of conjugates of TMG cap analogs with various GFP-like FMRs. TMG cap conjugates are specific probes for snurportin. The affinity of these conjugates for snurportin was tested using a fluorescence quenching titration (FQT) technique. The introduction of a marker in the form of FMR in some cases significantly increased the affinity to the protein and thereby, conferred attractive properties enabling their use as molecular probes for snurportin. Saturating the ligand with protein at different excitation wavelengths was used to determine the fluorescent properties of the conjugates. Based on these studies, the compounds with highest affinity and highest protein-induced fluorescence enhancement were selected as the most promising fluorescent candidates for in vivo studies with snurportin. The impact of various structural modifications, among others, a) the type of second nucleotide (G/A), the length of the nucleotide sequence, b) modifications in the triphosphate bridge, c) the length of the phosphate bridge, and d) the tag insertion site, were determined.

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P76

Effective sequestering of miRNA-122 by GalNAc-conjugated tiny LNAs.

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Abstract

MicroRNAs (miRNAs) are ~22 nucleotides small non-coding RNAs that control post-transcriptional expression of target genes and play pivotal roles in various biological processes. The function of miRNAs can be specifically inhibited by chemically modified antisense oligonucleotides (anti-miRNA ASOs) for the development of novel therapeutics. One promising configuration of anti-miRNA ASOs termed “tiny LNAs (Locked Nucleic Acid)” is seed-targeting ~8-mer fully LNA oligonucleotides, which enables efficiently to block a liver-specific miRNA-122 [1]. However, the pharmacokinetic control of tiny LNAs has been required. Here, we first report a series of *N*- acetylgalactosamine (GalNAc) conjugated anti-miR-122 tiny LNAs to improve pharmacokinetics and demonstrate the in vivo activity and in vivo/ex vivo imaging studies.

The use of GalNAc for hepatocyte targeting mediated by liver-specific asialoglycoprotein receptor (ASGPR) has been shown to be a promising paradigm for ASO therapeutics. We have previously exhibited the usefulness of an “on-support clustering” approach using a monomer-type hydroxy-L-prolinol-based GalNAc (GalNAc$_{hp}$) phosphoramidite unit [2]. In the present study, we applied a series of GalNAc$_{hp}$-conjugated tiny LNAs and unconjugated-tiny LNAs to mice to evaluate the pharmacological and pharmacokinetic effect [3]. The GalNAc$_{hp}$-conjugated tiny LNAs significantly improved in vivo activity and its median effective dose (ED50) is ~300-500 times more potent than the traditional unconjugated tiny LNAs. Moreover, incorporation of GalNAc$_{hp}$ via a biolabile phophodiester bond into the 5' end is preferable to the 3’ end. Through the in vivo/ex vivo imaging study, we found that the GalNAc$_{hp}$-conjugated tiny LNAs increased the accumulation in the liver more than 5-fold compared to the unconjugated counterparts. We concluded our monomeric GalNAc approach has shown to be successful for this specific class of anti-miRNA ASOs.

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[3] *Pharmaceutics,* **2021**, 13, 817.

P77

Synthesis and Biological Evaluation of Novel Class of Cyclic Dinucleotides for STING Dependent Anti-Tumor Activity

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Abstract

The STimulator of INterferon Genes (STING) protein has been identified as an attractive target for cancer immunotherapy due to its central role in inducing T-cell mediated tumor control. Herein, we describe the discovery of a selected set of highly potent and novel cyclic dinucleotides (CDNs) STING agonists with North (*N*)-methanocarba (bicyclo[3.1.0]hexane) sugar modification (ALG-031048) or isothiazolo[4,3-d]pyrimidine nucleobase modification (ALG-031052 & ALG-031078). Single-step cyclization using 2-cyanoethyl-*N*,*N*,*N*',*N*'-tetra(isopropyl)phosphoramidite served as a key step in building a CDN scaffold. These sugar or nucleobase CDNs have demonstrated improved binding (up to 3.4-fold) to the STING R232 protein in the Differential Scanning Fluorimetry (DSF) assay compared to the clinical stage STING agonist, ADU-S100. These CDNs were 5- to 9-fold more potent than ADU-S100 in the IFN-β induction and IRF HEK 293T STING R232 reporter assays. In vivo anti-tumoral activity of ALG-031048 & ALG-031052 was tested in the syngeneic CT26 mouse colon carcinoma model. Three intra-tumoral (IT) doses of 100 µg (3xq3d) caused complete tumor regression in all animals (Figure 1a), while treatment with 25 µg (IT 3xq3d) resulted in the survival of 60% (ALG-031048) and 90% (ALG-031052) of animals. In contrast, only 10 and 44% of mice showed complete tumor regression when treated IT 3xq3d with 25 or 100 µg ADU-S100, respectively. To study whether induction of a protective immune response was associated with STING agonist treatment, surviving animals from ALG-031048 treated group was re-challenged with a second inoculation of CT26 cells on the contralateral side. All naïve animals formed tumors within 23-30 days. In contrast, 8 out of 9 animals pre-treated with ALG-031048 in the first part of the study were tumor-free for 40 days after re-challenge (Figure 1b). These results with Aligos' STING agonists indicate an immune-based, long-lasting mechanism of action for tumor regression and prevention. In summary, we have performed a structure–activity relationship study with sugar-, nucleobase-modified CDNs and identified several novel CDNs which demonstrated potent and long-lasting tumor suppression in in vivo mouse models. The strong anti-tumoral activity of some of these CDNs warrants further development of potentially best-in-class STING agonists.

1a) 1b)

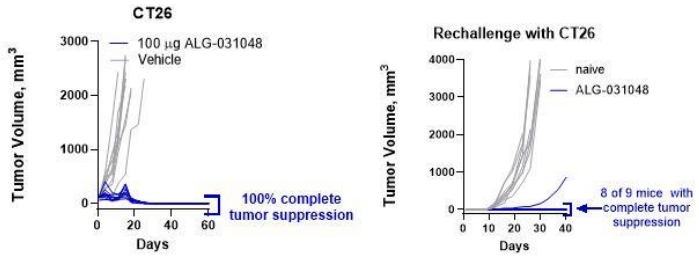

Figure 1: a) Complete tumor regression in CT26 bearing Balb/c mice treated with ALG-031048; b) Re-challenge study with CT26, 8 out of 9 animals pre-treated with ALG-031048 were tumor-free for 40 days.

P78

Scalable Synthesis of DNA and RNA Oligomers Using Mechanochemistry

James D Thorpe BSc, Masad J Damha BSc, PhD

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Abstract

In recent years, oligonucleotide therapeutics have been thrust to the forefront of drug discovery due to their wide-range of potential targets, particularly for those that were previously thought of as undruggable.[1, 2] Demand for synthetic oligonucleotides has reached an all-time high and is expected to increase exponentially in the coming years for both therapeutic and diagnostic purposes. Despite an increased interest of pharmaceutical companies in therapeutic oligonucleotides, large-scale synthesis remains a challenge due to scalability and environmental factors. The use of large amounts of environmentally detrimental and potentially toxic solvents such as acetonitrile, pyridine, and dichloromethane has plagued solid-phase oligonucleotide synthesis for years. The large demand and environmental impact of solid-phase oligonucleotide synthesis has led to the development of alternate synthetic strategies such as solution-phase synthesis[3] and the use of soluble supports.[4]

Previous work in our lab has demonstrated the mechanochemical coupling of nucleotides using both phosphoramidite and H-phosphonate chemistry using stoichiometric amounts of solvent.[5] Coupling and oxidation of internucleotide linkages has been achieved in generally good yields (80-90% per step) using vibration ball milling (VBM) and minimal amounts of solvent. Herein, we report the synthesis of DNA and RNA oligomers using mechanochemistry. By adopting a block coupling approach,[3, 6] we have been able to assemble longer oligomers than previously reported using mechanochemistry. Additionally, this approach provides access to oligonucleotides containing phosphodiester and phosphorothioate backbones based on the choice of reagents. We expect this method to find general utility in the preparation of chemically modified oligonucleotides of therapeutically relevant lengths.

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P79

Remarkable Enhancement of RNaseH Cleavage Activities of RNA Complexed with Chimeric DNA-Peptide Ribonucleic Acid (PRNA)

Nozomu Ishiwata BD[1], Masahito Inagaki PhD[1,2], Masaki Nishijima PhD[1], Hironori Hayashi PhD[1], Yasuyuki Araki PhD[1], Eiichi Kodama PhD[1], Takehiko WADA PhD[1]

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Abstract

We have recently proposed a new strategy and a practical tool for cancer cell-selective oligonucleotide therapeutics artificial nucleic acids, named Peptide Ribonucleic Acids (PRNAs) with active *on-off* control of functional RNA activities corresponding to cancer cell-specific intracellular conditions. The PRNAs can be actively *off* to *on* switching the complexation behavior with target mRNA, miRNA, and siRNA induced by lowered pH of cancer's cytoplasm. This strategy utilizes a new category of artificial nucleic acid that carries a ribonucleoside unit tethered to a peptide backbone as recognition and stimulus-sensitive module. In this artificial nucleic acid called PRNA, the 5'-amino-pyrimidine ribonucleoside unit, which is in the *syn* conformation in normal cellular cytoplasm condition induced by intramolecular cyclic borate ester formation with incorporated phenyl borate moiety, functions as a built-in switching moiety of *syn* to *anti* nucleobase orientation switching function triggered by cancer cellular lowered pH. The results are promising, validating that the original PRNAs with *anti*-oriented nucleobases form stable complexes with the target RNA under cancer's low cytoplasm pH (pH=ca.6.2), which are readily dissociated under normal cellular cytoplasm pH (pH = ca. 7.2). This means that the PRNA strategy can be used as a powerful tool for *on-off* switching the RNA complexation behavior, which is potentially applicable to the cancer cell-selective oligonucleotide therapeutics of the next generation.

Meanwhile, RNase H activities of antisense molecules would be one of the most crucial factors for a practical antisense strategy. Thus, in this study, we have been designed PRNA-DNA chimeras, in which both PRNA and DNA domains work as recognition sites for the complexation with target RNAs and PRNA moieties work as recognition control/switching devices, while DNA-RNA hybrids formed in the DNA domains of the chimera should be substrates of RNase H and then target RNAs cleaved by the enzyme. To improve cleavage efficiency, we focused on the binding mechanism of DNA/RNA duplex to RNaseH's basic binding channel and proposed a chimeric neutral amide backbone of PRNA connected with negatively charged DNA's phosphate-sugar backbone (Fig. 1). In the design, the cleavage site of the target RNA should be restricted to the position of the junction site of the chimera. Fortunately, efficient and very enhanced cleavage of target RNAs compared with those with DNAs was observed for PRNA-DNA chimera/RNA complex by RNaseH. Regulation of protein synthesis by PRNA-DNA chimera was also evaluated by *in vitro* cell-free protein synthesis system, and effective regulation was observed.

P80

Synthesis and Application of a ^{19}F-labeled Fluorescent Nucleoside as a Dual-mode Probe for i-Motif DNA Structures

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Abstract

Emissive isomorphic nucleoside analogues are versatile tools for the investigation of DNA conformations due to their stable orientations within the DNA structure from base-pairing and stacking interactions.[1] Furthermore, in contrast to conventional small molecules or protein dyes, emissive isomorphic nucleosides cause minimal disturbance to native DNA folding and interactions, allowing for a more accurate picture of nucleic acid structures.

Herein, we report the synthesis of a fluorine-labeled fluorescent cytosine analogue, ^{FP}dC, and its incorporation into i-motif-forming DNA sequences.[2] DMTr-protected ^{FP}dC phosphoramidite was synthesized in eight steps and successfully utilized in solid-phase synthesis to obtain the desired oligonucleotides. Compared to previously reported fluorescent tricyclic cytosine derivatives, ^{FP}dC monomer presented a four-fold improvement in brightness (12 000) due to its high molar absorptivity (24 000 mol^{-1} dm^3 cm^{-1}) and quantum yield (0.50). When incorporated into oligonucleotides and upon formation of i-motif structures, significant changes in fluorescence intensity and lifetime, as well as ^{19}F NMR chemical shifts were observed. The changes in fluorescence intensity were observed to be highly reversible when the folding and unfolding of an i-motif structure was induced with Ag(I) ion and cysteine.

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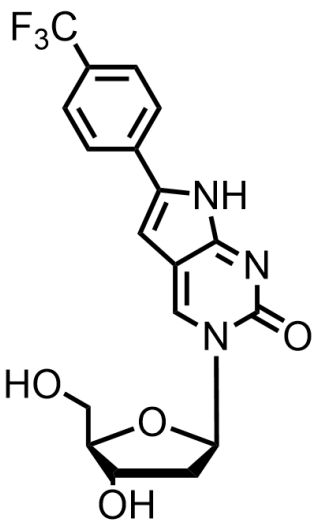
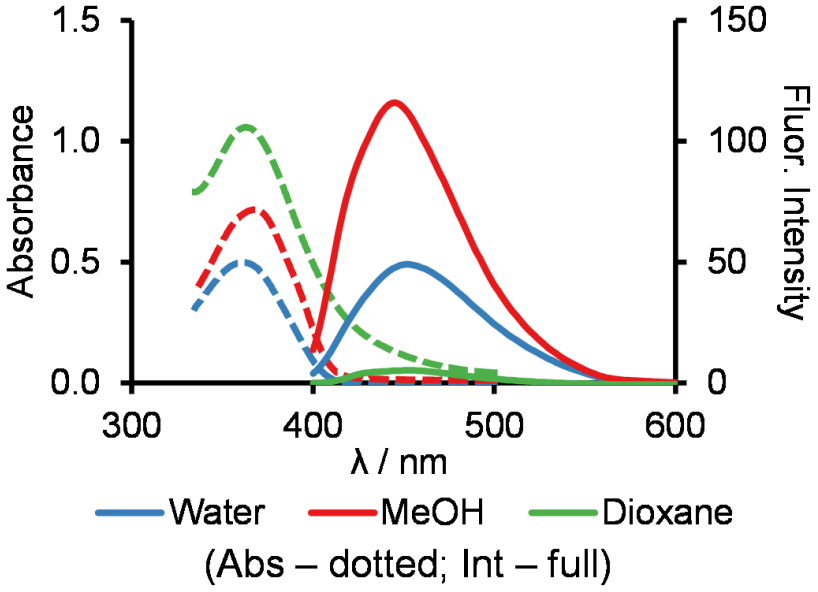

F3C
NH
N
N
O
HO
O
OH

^{FP}dC


1.5
1.0
0.5
0.0
Absorbance
150
100
50
0
Fluor. Intensity
300
400
500
600
λ / nm
Water
MeOH
Dioxane
(Abs – dotted; Int – full)

P81

The Network of Replication, Transcription, and Reverse Transcription of a Synthetic Genetic Cassette

Hui Yang, Elena Eremeeva, Mikhail Abramov, Piet Herdewijn

Rega Institute, Leuven, Belgium

Abstract

The present life employs two informative polymers to encode for hereditary information, DNA and RNA, replication, transcription and reverse transcription are the three central biological processes with which DNA and RNA duplicate themselves or interconvert into each other. To diminish some inherent drawbacks of natural biopolymers, including biological instability and limited chemical and structural diversity, synthetic nucleic acids became the key targets of biomedical research. Here, a comprehensive analysis of PCR amplification, transcription, reverse transcription, and cloning was done to screen for alternative genetic monomers. A small library of six modified nucleobases was selected: the modified 2'-deoxyribonucleoside (dZTPs) and ribonucleoside (rZTPs) triphosphates of 7-deaza-adenine (A1), 5-chlorouracil (T1), 7-deaza-guanine (G1) or hypoxanthine (G2) together with 5- fluorocytosine (C1) or 5-bromocytosine (C2) (Figure a). Our study revealed that information encoded in entirely morphed informational polymers could be successfully propagated by natural enzymes. The fragments composed of one to four modified nucleotides (denoted as DZA) have been successfully obtained in the presence of different set of modified dZTPs using Taq DNA polymerase, then, they were recognized and transcribed to natural or modified RNA (denoted as RZA) by T7 RNA polymerase. In turn, the fully modified RZA fragment could be reverse transcribed by M-MuLV enzyme and then amplified by Taq DNA polymerase in the presence of various dZTPs. Noticeably, modified fragments could function as genetic templates in vivo by encoding the 678 base pair gene of a fluorescent protein in bacteria (Figure b). These results demonstrate the existence of a fully simulated genetic circuit that uses synthetic materials.

P82

Introduction of Amino Acid-Nucleic Acid Hybrids (ANHs) as Novel Building Blocks for DNA Modifications

Ji Hye Yum[1], Soyoung Park PhD[1], Hiroshi Sugiyama Professor[1,2]

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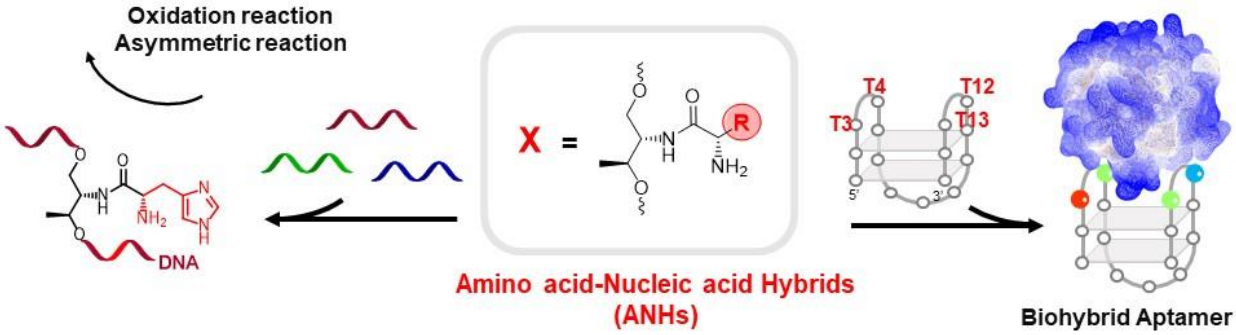
Abstract

The rapid development of innovative biochemical tools has demonstrated that DNA and RNA are more than simple genetic materials and serve diverse and active roles that are relevant to interdisciplinary research. Chemical modifications on innate DNA/RNA can significantly improve their functions. Our group has established a systematic modular strategy to incorporate amino acid residue into DNA oligonucleotides via an acyclic D-threoninol backbone, entitled Amino Acid-Nucleic Acid Hybrid (ANH), for the functionalization of oligonucleotides. We first chose histidine residue, a versatile amino acid residue that plays a critical role in the active sites of many metalloenzymes, for the enantioselective catalysis. Histidine-conjugated DNA oligonucleotides were synthesized by combining DNA alphabets and the natural metal-binding amino acid, and their applications as novel biohybrid materials have been demonstrated. In the presence of various metal ions, His-modified DNAs displayed excellent metal-coordination capability as robust molecular depots. Besides, histidine-conjugated DNA oligonucleotides could be successfully used in asymmetric catalysis (up to 90% conversion and 95% *ee*) as DNA metalloenzymes and ABTS oxidation reactions horseradish-peroxidase (HRP)-mimicking DNAzymes with suitable metal cofactors.

In parallel, we introduced our modular strategy using amino acid-nucleic acid hybrids (ANHs) to modify DNA aptamer to increase its inhibitory activity and binding affinity. Because of the importance of thrombin in the blood-clotting process and cell signaling pathways, we adopted structurally and functionally well-explored TBA sequence (TBA15) as the basis for developing ANH-based TBA sets. Carefully selected seven different ANH building blocks were incorporated instead of thymines on the loop through solid-phase DNA synthesis. We confirmed that all devised TBAs form antiparallel G-quadruplex structures based on spectroscopic studies regardless of incorporated amino acid residue. ANH-aptamers with hydrophobic amino acids (**Phe**, **Met**, and **Trp**) to replace T3 of loop region (T3F) afforded remarkably enhanced thrombin inhibition property, resulting from prothrombin assay. Moreover, surface plasmon resonance assay and molecular modeling results support the significant difference in anticoagulation activity. Our results showed a great potential of our amino acid-modification method for the post-SELEX process. Overall, original methods of synthesizing nature-inspired amino acid conjugates and their successful applications are presenting.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Asymmetric reaction
DNA
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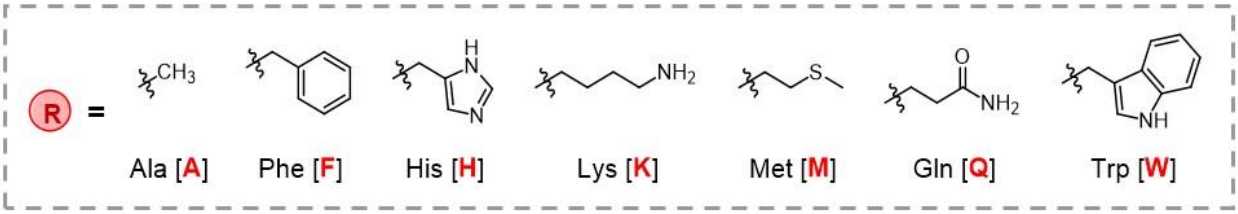
R
NH2
Amino acid-Nucleic acid Hybrids
(ANHs)
T4
T12
T3
T13
5'
3'
Biohybrid Aptamer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CH3
NH2
S
O
NH2
H
N
N
NH
Ala [A]
Phe [F]
His [H]
Lys [K]
Met [M]
Gln [Q]
Trp [W]

P83

DNA mimicry polymers containing adenine and thymine nucleotides

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Abstract

Natural DNA is becoming increasingly important in the biomedical field, however its usage, for example, in gene therapy has been restricted by its high cost and limited shelf life.[1, 2] In this regard, artificial polymers bearing DNA substituents are being developed to tackle these issues. Polymers containing nucleobases,[1] nucleosides[3] and oligonucleotides[4] have been synthesised utilising either bioconjugated monomers or post-polymerisation techniques.[1, 3-4] While these types of synthetic nucleobase-polymers display intriguing properties, the main drawbacks are that they do not contain all three DNA components, i.e., the nucleobase, the deoxyribose and the phosphate group, associated with the main DNA building block, namely the nucleotide. The inclusion of the entire nucleotide cannot be ignored if true DNA mimicry is to be achieved.

Herein, we report on the first synthesis of nucleotide bearing monomers containing either adenine (A) or thymine (T) nucleobases and their polymerisation through pre- or post-functionalisation methods. The synthesis of the monomers relied on the coupling of 2-hydroxypropylmethacrylate to protected commercially available phosphorimidites. A reversible addition fragmentation chain transfer (RAFT) polymerisation was used to achieve higher control over the size and polydispersity of the DNA mimickry polymers. Successful synthesis of the monomers and polymers was demonstrated using mass spectrometry, proton and phosphorus nuclear magnetic resonance spectroscopies and ultraviolet-visible spectroscopy. This study opens up new possibilities to fabricate biomimetic polymers for a broad range of biomedical applications.

TS3NA IRT

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AUGUST 26–27

Phosphates Run the World: Chemical Biology and Applications of Nucleosides, Nucleotides and Nucleic Acids

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