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Reunión de Ácidos Nucleicos y Nucleósidos

(Meeting of Nucleic Acids and Nucleosides)

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IX RANN

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Universidad de Oviedo
19 - 21 June
Mieres (Asturias)

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IX RANN Asturias 2013

IX Reunión de Ácidos Nucleicos y Nucleósidos

IX Meeting of Nucleic Acids and Nucleosides

19–21 June 2013

Universidad de Oviedo

Mieres (Asturias)

SPAIN

Organization:

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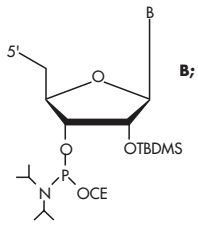
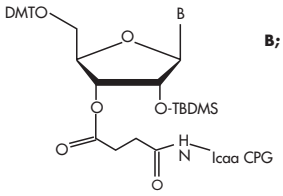
CONVENTIONAL (3′→ 5′ OLIGONUCLEOTIDE SYNTHESIS)



		Catalog #
B;	Adenosine (n-bz)	ANP - 5671
	Cytidine (n-bz)	ANP - 5672
	Guanosine (n-ibu)	ANP - 5673
	Uridine	ANP - 5674
	Cytidine (n-ac)	ANP - 6676

5′-DMT-2′-TBDMS-3′-Amidites



		Catalog #
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	Cytidine (n-bz)	N - 6102
	Guanosine (n-ibu)	N - 6103
	Uridine	N - 6104
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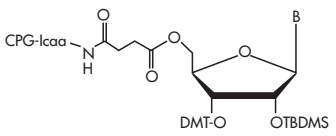
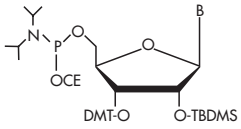


B; A(n-bz)
C(n-bz)
C(n-ac)
G(n-ibu)
U

3′-DMT-2′-TBDMS-5′-CPG



3′-DMT-2′-TBDMS-5′-Amidites

B	Protection	Catalog #
A	n-bz	**ANP-3401 (Amidite)** **N-6201 (CPG)**
C	n-bz	**ANP-3402 (amidite)** **N-6202 (CPG)**
G	n-ibu	**ANP-3403 (amidite)** **N-6203 (CPG)**
U	n/a	**ANP-3404 (amidite)** **N-6204 (CPG)**
C	n-ac	**ANP-3405 (amidite)** **N-6204 (CPG)**

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Welcome

On behalf of the Scientific and Organizing Committees, it is a pleasure to welcome all the participants to the IX RANN that will be held at the Auditorium of the Research Building of Universidad de Oviedo in Mieres.

IX RANN congress will take place from 19 to 21 June 2013 hosted by Universidad de Oviedo. This Congress will involve the participation of young researchers and experienced experts in different areas of nucleic acids and nucleosides to create a multidisciplinary atmosphere stimulating the exchange of knowledge between participants. The aims of the Congress will be to have an update on recent achievements and future tendencies in the field of nucleic acids and nucleosides.

This meeting will continue a series of symposia that had been successfully held every two years since 1997 in Stiges. Later conferences held in El Escorial (1999), Santiago de Compostela (2001), Granada (2003), Alcalá de Henares (2005), Valencia (2007), Jaca (2009) and Sevilla (2011) have made possible that RANN conferences are considered as a prestigious meeting for the scientific community, especially in the areas of Chemistry, Biochemistry and Biology of nucleic acids and nucleosides in Spain.

It will be a pleasure to share with you a few days in Asturias talking about the science that we enjoy in our labs.

We wish all the participants a successful scientific meeting and also an enjoyable stage in Asturias where you will take part in different social events that have been programmed for your entertainment.

Greetings from Organizing Committee

Miguel Ferrero Fuertes

Susana Fernández González

Chairpersons of IX RANN Asturias 2013

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Sponsorship and Acknowledgements

Celebration of this conference will not be able to occur without the generous support from different private companies and governmental institutions. The Organizing Committee wants to express a sincere gratitude to all of them for their efforts.

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General Information

The IX RANN will be held in Mieres, a small town, which is located 15 minutes from Oviedo (17 km), the capital of Asturias. There are regular buses that connect with Oviedo. There are crowded wine areas and as extra incentive, the *fiestas de San Juan* in Mieres, which are very famous in Asturias, held during the week of the congress, which makes it an added attraction.

The rural area is very close to and for who likes hiking there are numerous highly recommended routes. Thus, about 6 km from Mieres, is what the mierenses consider their 'green lung': the valley of Cuna and Cenera. Here you can enjoy a native landscape: chestnut trees, hazel trees, oaks (known as "Carbayones" in Asturian); asturian cuisine in the numerous mesones and sidrerias: mesón "El Molin", mesón "La Panoya", restaurante-sidrería "Palacio de Arriba"...; as well as traditional architecture: hórreos, paneras and rural mansions that are located in the area such as "El Valletu", popular name with the Palace of the Prada Vazquez is known, or the Palace of Viade, 17th-century Manor House. You can also visit the popular hermitage of the martyrs of Valdecuna, which dominates the Valley. For more information, check the following websites:

http://www.ilusionati.com/en_mieres_cuna_cenera/mieres_cuna_cenera.html

http://perso.wanadoo.es/losmartires/Santuario.htm

On the other hand, if you decide to visit other areas of Asturias, you can check the website:

http://www.asturias.es/portal/site/infoAsturias/

About the capital of Asturias

Oviedo is the capital of the Principado de Asturias, situated in the North West of Spain, 450 km from Madrid and represents one of the most important Spanish cultural tourism centre, crossroads the Camino de Santiago (Route to Santiago) and the “Ruta de la Plata”. It possesses great monumental heritage and significant offer in museums that totally display the historic-artistic heritage of Asturias. Today, Oviedo is an open and modern city with a great international projection thanks to the *Prince of Asturias Awards* and the International Opera Festival.

Among its tourist attractions we can mention its Old Quarters, with the Cathedral and its Holy Chamber, and the Campoamor Theatre, venue of many cultural events. Of the outstanding monumental heritage, we can mention Santa María del Naranco**,** San Miguel de Lillo and San Julián de los Prados declared monuments of World Heritage.

For more information, see: http://www.turismoviedo.es

To obtain city maps in pdf format you can visit the website: www.oviedo.es.

Conference Venue

The venue of the conference will be the Auditorium of the Research Building that Universidad de Oviedo has in Mieres.

Research Building (place to celebrate RANN)

Mieres Campus of Universidad de Oviedo

Registration and Lodging

An information desk will be located in the entrance of Auditorium of the Research Building. All questions related to scientific, organizational and financial matters will be initially addressed there. The lodging will be next to auditorium in Students Residence Hall. Breakfast and lunch will be in the cafeteria of the residence.

Students Resident Hall
Mieres Campus of Universidad de Oviedo

Scientific Sessions

The outline of previous RANN conferences will be maintained. Thus, scientific program will consist of oral communications (20-25 min) and there will be no poster sessions. The Opening Lecture will be given by Prof. Masad J. Damha from McGill University (Montreal, Canada).

The congress will be from Wednesday evening 19 June 2013 to Friday night 21 June 2013, and all the scientific sessions will take place in a modern auditorium. Coffee breaks and Stands will be held in the comfortable corridors of the conference venue.

Sports Facilities

Campus of Universidad de Oviedo in Mieres boasts a covered sports hall with a multisport court as well as a multipurpose room, sauna, fitness-gym and administrative offices. There are also four paddle courts, two tennis courts, a synthetic grass seven-a-side football pitch and a cinder running track.

The sport center is open from 9:30 to 18:30 h Monday to Friday. Access is free for IX RANN participants showing the conference credential.

In front of the sport hall there is a municipal heated indoor pool. Admission is free showing the conference credential.

Pool hours:

Monday to Friday, 9:30-22:00 h

Saturday, 10:00-14:00 h and 16:00-20:00 h.

Social Events

Various social events during the IX RANN have been programmed for the enjoyment of all the participants.

Wednesday Evening: Reception

There will be a get-together party at the Students Residence Hall.

Friday Evening: Conference Dinner

On Friday evening we will celebrate all together the conference dinner in Hotel Palacio de Arriba (www.palaciodearriba.es) located 6 km from Mieres, where you will taste delicious traditional dishes from the asturian local area.

Scientific Program

Wednesday 19th June 2013

19:30 − 20:30 Registration

20:30 Welcome Reception

Thursday 20th June 2013

09:30 − 10:00 Registration

10:00 − 10:30 **Opening of the IX RANN**

Session I Chair: **Miguel Ferrero**, Universidad de Oviedo

10:30 − 11:30 **Opening Lecture**

Nucleoside Analogues for Structural and Functional Studies of Nucleic Acids

Masad J. Damha

Department of Chemistry, McGill University, Montreal (Canada)

11:30 − 12:00 Coffee Break (Kindly sponsored by **AME Biosciences**)

Session II Chair: **María José Camarasa**, Instituto de Química Médica-CSIC, Madrid

12:00 – 12:30 Single-molecule manipulation of RNA duplexes and G-quadruplexes

J. Ricardo Arias-González

IMDEA Nanociencia, Madrid

12:30 – 13:00 Molecular Dynamics Simulations of Peptide Nucleic Acids (PNA) based on a Flexible Asymmetric Unit

Carlos T. Nieto

Universidad de Salamanca

13:00 – 13:30 Polypurine Reverse Hoogsteen Hairpins: In vivo Effects, Stability and Immunogenicity

Laura Rodríguez

Universitat de Barcelona

14:00 – 16:00 Lunch at the Residence

Session III Chair: **José Gallego**, Universidad Católica de Valencia

16:00 – 16:30 Rational Design of 3'-Exonuclease Blocking Agents

Montserrat Terrazas

Institut de Química Avançada de Catalunya –CSIC, Barcelona

16:30 – 17:00 Structural Analysis of Hepatitis C Virus 3'X Terminal RNA Domain
Ángel Cantero
Universidad Católica de Valencia

17:00 – 17:30 Developing Methods to Obtain Conformationally Constrained Oligonucleotide Structures
Roger Tresánchez
Universitat de Barcelona

17:30 – 18:00 Coffee Break (Kindly sponsored by **AME Biosciences**)

Session IV Chair: **Ramón Eritja**, Institut de Química Avançada de Catalunya-CSIC, Barcelona

18:00 − 18:30 A Minimal i-Motif Stabilized by Minor Groove G:T:G:T Tetrads
Nuria Escaja
Universitat de Barcelona

18:30 − 19:00 Discovery of new telomeric RNA ligands by ^{19}F-NMR spectroscopy
Miguel Garavís
Instituto de Química-Física Rocasolano-CSIC, Madrid

19:00 − 19:30 Rational drug design for DNA repair mechanism as adjuvant in chemotherapy
Carme Fábrega
IRB Barcelona

Friday 21st June 2013

Session V Chair: **Juan C. Morales**, Instituto de Investigaciones Químicas-CSIC y Universidad de Sevilla

10:00 – 10:30 DNA Stabilized Gold Nanoparticles as Delivery System
Alfonso Latorre
IMDEA Nanociencia, Madrid

10:30 – 11:00 DNA Repair of hAGT Detected over DNA Origami
María Tintoré
Institut de Química Avançada de Catalunya –CSIC, Barcelona

11:00 – 11:30 *SUS1* pre-mRNA Structure and Alternative Splicing
José Gallego
Universidad Católica de Valencia

11:30 – 12:00 Coffee Break (Kindly sponsored by **AME Biosciences**)

Session VI Chair: **Enrique Pedroso**, Universitat de Barcelona

12:00 – 12:30 Modified "Nucleoside" Analogues as Potential Antiviral Inhibitors
Gloria Fernández-Cureses
Instituto de Química Médica-CSIC, Madrid

12:30 – 13:00 Effect of Furanose C2´-Substitution on Structure and Stability: Directing the Folding of the Human Telomeric Quadruplex
Nerea Martín-Pintado
Instituto de Química-Física Rocasolano-CSIC, Madrid

13:00 – 13:30 Functionalization of siRNA Strands with L-Threoninol-Thymine Derivatives
Adele Alagia
Institut de Química Avançada de Catalunya-CSIC, Barcelona

14:00 – 16:00 Lunch at the Residence

Session VII Chair: **Carlos González**, Instituto de Química-Física Rocasolano-CSIC, Madrid

16:00 − 16:30 Carbohydrate Oligonucleotide Conjugates: Tool to Study Fluorosugars-DNA Interactions and Gene Inhibition by Apolar Carbohydrate siRNA
Juan C. Morales
Instituto de Investigaciones Químicas-CSIC – Universidad de Sevilla

16:30 − 17:00 Using Optical Tweezers to Study DNA Replication Dynamics at Single-molecule Level
Borja Ibarra
IMDEA Nanociencia, Madrid

17:00 − 17:30 Threoninol-based Thioctic Acid Derivatives as Suitable Building Blocks to Incorporate DNA Oligonucleotides into Gold Nanoparticles
Santiago Grijalvo
Institut de Química Avançada de Catalunya-CSIC, Barcelona

Session VIII Chair: **Susana Fernández**, Universidad de Oviedo

17:30 – 18:00 DNA Stabilized Silver Nanoclusters
Romina Lorca
IMDEA Nanociencia, Madrid

18:00 – 18:30 **General Discussion and Closing of the IX RANN**

21:30 Conference Dinner

OPENING LECTURE

Nucleoside Analogues for Structural and Functional Studies of Nucleic Acids

Masad J. Damha*[a]

[a]*Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montreal, QC, CANADA H3A 0B8. Tel: 1-514-3987552; E-mail: masad.damha@mcgill.ca*

Chemically modified oligonucleotides have found remarkable utility in an exceptionally diverse range of applications. Modified nucleic acids can now be found in clinically approved drugs, and chemically modified nucleotide analogues have provided valuable tools for studying the structure of oligonucleotides and their interactions with enzymes. Fluorinated nucleosides (arabinonucleosides, ribonucleosides, etc), and ring expanded nucleosides are of particular interest in our research.

I will discuss the synthesis and development of new fluorinated furanose nucleosides and 6 and 7-membered ring nucleosides for a) chemical modification of nucleic acids and b) to probe the nucleotide excision processes of HIV Reverse Transcriptase (RT). Furthermore, I will present results investigating the consequences of our nucleotide modifications on duplex and higher order structures, conformation, stability, and nucleic acid interactions with enzymes such as gene silencing enzymes (RISC).

Acknowledgements

This lecture is dedicated to my present and past graduate and postdoctoral students (http://damha-group.mcgill.ca) and the “Spanish Armada” **Carlos González** (CG), **Modesto Orozco** and their research groups at the CSIC (Madrid) and the University of Barcelona. They have made my work and academic life so much enjoyable. Funding was received from the Canadian Institutes for Health Research (CIHR), The National Science and Engineering Council of Canada (NSERC), and CSIC I-LINK-0216 (grant to CG and MJD).

ORAL COMMUNICATIONS

Single-molecule manipulation of RNA duplexes and G-quadruplexes

Elías Herrero-Galan,[a,b] María E. Fuentes-Pérez,[c] Carolina Carrasco,[c] José M. Valpuesta,[c] José L. Carrascosa,[c] Fernando Moreno-Herrero,[c] and J. Ricardo Arias-Gonzalez*[a,b,c]

***and* Miguel Garavís,[d,e] Rebeca Bocanegra,[b] E. Herrero-Galán,[a,b] Carlos González,[d] Alfredo Villasante,[e] and J. Ricardo Arias-Gonzalez*[a,b,c]**

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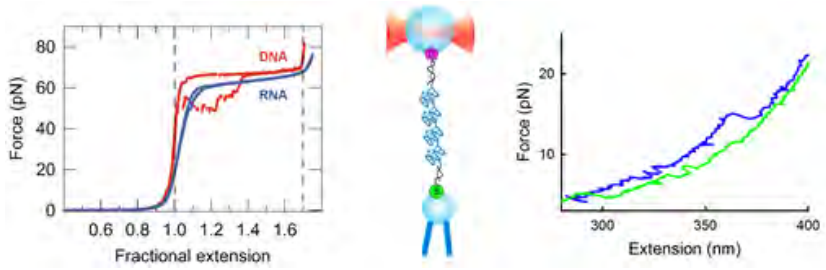
[b]*Centro Nacional de Biotecnología (CNB-CSIC), Calle Darwin 3, Cantoblanco, 28049 Madrid, Spain.*
[c]*Unidad Asociada de Nanobiotecnología CNB-CSIC-IMDEA Nanociencia.*
[d]*Instituto de Química Física Rocasolano, CSIC, C/Serrano 119, 28006 Madrid, Spain.*
[e]*Centro de Biología Molecular "Severo Ochoa" CSIC-UAM, Universidad Autónoma de Madrid, Nicolás Cabrera 1, 28049 Madrid, Spain.*
E-mail: ricardo.arias@imdea.org

RNA are ubiquituous macromolecular platforms that develop multiple biological roles in the cell. Here, we present single-molecule investigations on double-stranded (ds) RNA and on RNA G-quadruplex forming sequences.

DsRNA is the genetic material of a variety of viruses and has been recently recognized as a relevant molecule in cells for its regulatory role. Here, we have engineered long dsRNA molecules for their individual characterization contrasting information with dsDNA molecules of the same sequence.[1] It is known that dsRNA is an A-form molecule unlike dsDNA, which exhibits B-form in physiological conditions.[2-4] These structural types are distinguished at the single-molecule level with atomic force microscopy and are the basis to understand their different elastic response. Force-extension curves of dsRNA with optical and magnetic tweezers manifest two main regimes of elasticity, an entropic regime whose end is marked by the A-form contour-length and an intrinsic regime that ends in a low-cooperative overstretching transition in which the molecule extends to 1.7 times its A-form contour-length. DsRNA does not switch between the A and B conformations in the presence of force. Finally, dsRNA presents both a lower stretch modulus and overstretching transition force than dsDNA, whereas the electrostatic and intrinsic contributions to the persistence length are larger.



Left, force-extension curves of dsRNA and dsDNA, normalized in the extension axis to the A and B forms, respectively. Right, Force-induced unfolding of RNA constructions of GGGUUA repeats by optical tweezers.

G-quadruplexes are nucleic acid sequences that are rich in guanine and are capable of forming a four-stranded conformation. Here, we have studied long human telomeric RNA (TERRA). These non-coding RNA molecules contain subtelomere-derived sequences and an average of 34 GGGUUA repeats at their 3' end.[5] By using optical-tweezers and other biophysical techniques, we have found that long RNA constructions of up to 25 GGGUUA repeats form higher order structures comprised of single parallel G-quadruplex blocks, which unfold at lower forces than their DNA counterparts.[6]

References

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Molecular dynamics simulations of peptide nucleic acids (PNA) based on a flexible asymmetric unit

Carlos T. Nieto*[a], Narciso M. Garrido[a], David Diez[a] and Birgit Schiøtt[b]

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Peptide nucleic acids[1] are synthetic, non-natural DNA homologues where the phosphate-ribose backbone is replaced by a peptide motif. This replacement enhances the binding properties of the hybrids and endows a higher enzymatic resistance. From their beginnings, PNA's have attracted attention as result of their potential properties at the nanoscale: therapeutics (antisense and antigene), biosensing and information tagging.[2] Despite of the different types of peptide monomers elaborated, the applications are only focused on the original aminoethylglicine PNA (Figure 1a).

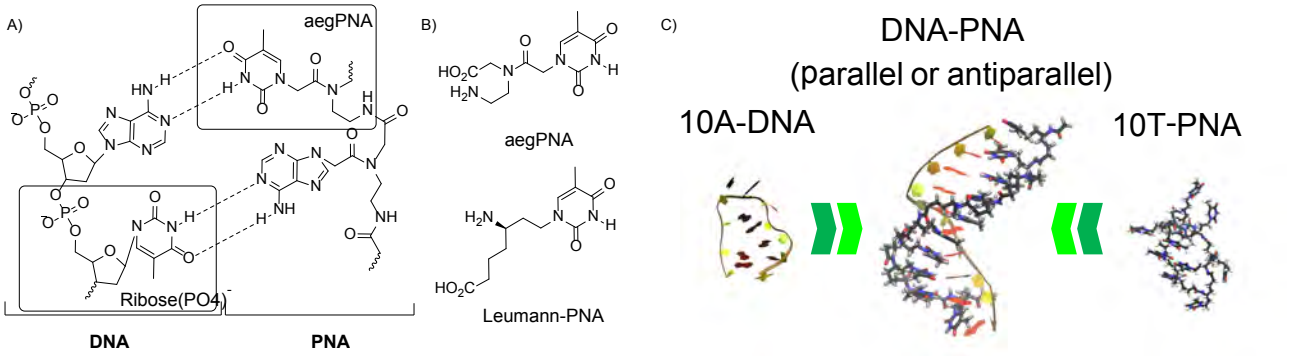

Figure 1: A, structural comparison between natural DNA and aminoethylglicine type PNA (aegPNA). B: comparison between aegPNA monomer and Leumann type. C: oligomeric systems involved in the present study at their final conformations.

A novel thymine-PNA monomer, originally designed by Leumann[3], which possess a flexible backbone is being synthesized in our group in an asymmetric fashion (Figure 1b). Although the properties of the prime aminoethylglicine PNA monomer have been described extensively in literature, novel units require similar studies[4], advancing the knowledge about how interact these systems and the way we could take advantage of them.

We present here one of these studies, a theoretical work about the binding properties of a Leumann type PNA oligomer with a complementary natural DNA strand. We use a molecular dynamics protocol to explore the conformational behavior of the hybrid systems as well as the separated oligomers in water. Binding energies were extracted from the last frames of the simulation by means of MM-PBSA calculations.

The results show a mimetic behavior of the PNA strands compared with natural DNA (Figure 2), presenting helical folding with two different orientations: parallel or antiparallel. Binding energy decomposition indicates a higher binding enthalpy compared with the combination of two anticomplementary DNA natural strands, but a lower solvation free energy, due to removing the negative charge in the PNA scaffold. DNA-PNA systems have higher binding affinities, being more favorable than the original DNA-DNA duplexes.

Acknowledgements

The authors are grateful for financial support from the Spanish MICINN (EUI2008-00173), MEC (CTQ2009-11172/BQU), the FSE and Junta de Castilla y León (SA162A12-1) and excellence GR-178. C.T.N. thanks Junta de Castilla y León for a FPI doctoral fellowship.

Notes and References

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Polypurine Reverse Hoogsteen Hairpins: In vivo effects, stability and immunogenicity

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Gene therapy is a new approach highly used both in molecular biology and therapeutic strategies. We recently developed a new gene silencing tool called Polypurine Reverse Hoogsteen Hairpins (PPRHs), which are non-modified DNA molecules formed by two antiparallel polypurine stretches linked by a five-thymidine loop (1). Concretely, there are two types of PPRHs capable of decreasing gene expression, that differ in the location of the target sequence and their mechanism of action: Template-PPRHs, which bind to the template strand of the dsDNA (2), and Coding-PPRHs (3), which bind both to the template strand of the dsDNA and the mRNA.

We decided to further explore important properties of these molecules for their potential therapeutic use such as their stability and immunogenicity. Stability experiments performed in different types of serum (human and murine) and in human prostate cells (PC3) revealed that PPRHs half-life is much longer than that of siRNAs. The activation of the innate immune response was evaluated analyzing the levels of the transcription factor IRF3, the cleavage of the proteolytic enzyme Caspase-1, and the expression levels of several pro-inflammatory cytokines: type-I interferons, TNFα, IL-6, IL-8, IL-1β, IL-18 and IL-33. These determinations indicate that PPRHs do not activate the immune response, unlike siRNAs, and therefore are suitable for in vivo administration.

As we obtained promising results *in vitro* using PPRHs against different genes (*DHFR, telomerase, survivin*), we decided to further explore the *in vitro* and *in vivo* effect of PPRHs in cancer. We chose *survivin* as a target because it is overexpressed in different tumours and plays an important role in the evasion of apoptosis, one of the hallmarks of cancer. We assayed several PPRHs against survivin in different cancer cell lines and chose the most effective one for *in vivo* studies, using a a xenografted model of prostate cancer cells (PC3). The administration of the chosen PPRH, either intratumorally or intravenously, induced a decrease in tumour volume and weight. These findings represent the proof of principle of PPRHs as a new silencing tool for cancer gene therapy.

Acknowledgements

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Rational design of 3'-exonuclease blocking agents

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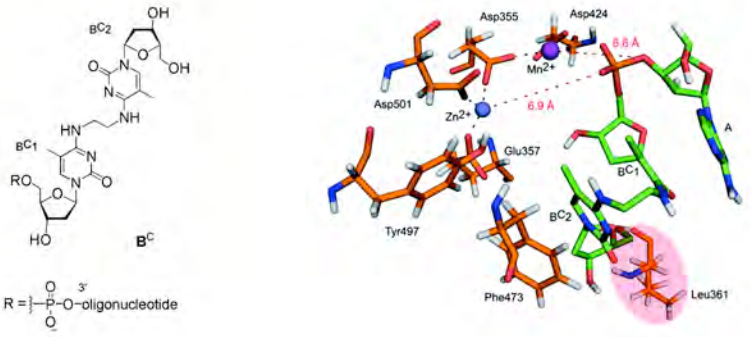
Inhibition of gene expression by synthetic oligonucleotides has been a widely explored field,[1] but the application of oligonucleotides as therapeutic agents *in vivo* faces some key problems. For example, ordinary oligonucleotides are highly vulnerable to serum nucleases. To overcome these limitations, several research groups have been actively studying oligonucleotides with various chemical modifications.[2] Most of the studied modifications increased their biostability, but in some cases modified oligonucleotides were found to have negative effects on activity. In this work, we have created and analyzed a new class of modification aimed at increasing the stability of oligonucleotides against 3'-exonucleases without disrupting their gene-silencing activity.[3] In particular, rational design showed the possibility of blocking the hydrolytic activity of 3'-exonucleases by replacing the two 3'-terminal nucleotides of a natural oligonucleotide strand (linked through a 3'-5' phosphodiester bond) by two nucleotide units linked together by an alkyl chain through the exocyclic amino group of the nucleobase. The resulting dimeric nucleoside (B^C) is connected to the oligonucleotide through a normal 3'-5' phosphodiester bond. Molecular dynamics simulations of a 3'-B^C-modified DNA : 3'-exonuclease (KF) complex suggested that this kind of alteration has negative effects on the correct positioning of the adjacent scissile phosphodiester bond at the active site of the enzyme, due to steric clashes between the alkyl linker and amino acid residues (Leu361). We verified that a 3'-terminal B^C unit completely blocked the hydrolytic activity of 3'-exonucleases. Furthermore, cellular RNAi[4] experiments with 3'-B^C-modified siRNAs showed that this modification was compatible with the RNAi machinery. The studies performed in this work not only provide a deeper insight into the role of nucleobase-protein interaction on 3'-exonuclease function, but can also help to design new potential therapeutic agents.



Acknowledgements

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Structural analysis of hepatitis C virus 3'X terminal RNA domain

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The hepatitis C virus (HCV) is a positive-stranded RNA virus belonging to the *Flaviviridae* family. The HCV genome comprises a single open reading frame (ORF) flanked at either end by untranslated regions (UTRs). The 5'-UTR contains an internal ribosome entry site (IRES) that mediates initiation of protein synthesis, whereas the 3'-UTR is involved in viral replication. The 3'-UTR region consists of a highly variable region immediately downstream of the ORF stop codon, a polypyrimidine tract of variable length, and a highly conserved 98-nt-long domain designated 3'X.

While the three-dimensional structure of the HCV IRES has been extensively studied with nuclear magnetic resonance (NMR), X-ray crystallography and cryo-electron microscopy methods, the structure of the 3'-UTR has only been analyzed by enzymatic and chemical footprinting experiments. These experiments have established that the last 46 nucleotides of the 3'X domain form a stable stem-loop, SL1, but the secondary structure of the remaining 3'X 52 nucleotides is not well defined. Although most reports have concluded that they form two additional stem-loops (SL2 and SL3), alternative folds comprising one or three hairpins have also been proposed. These 52 nt comprise sequences involved in a distal loop-loop interaction with the nearby ORF CRE sequence and in an intermolecular kissing loop leading to genomic RNA dimerization *in vitro*. Since the alternative 3'X structures expose to different degrees these nucleotides, it has been proposed that the 3'X domain may act as a dynamic switch signaling the transition between the replication, translation and possibly packaging processes of the virus[1].

Since the tertiary structure of SL1 is unknown and the secondary structure of the SL2 and SL3 is unclear, we are studying the structure of the HCV 3'X domain using NMR spectroscopy. So far we have separately transcribed SL1, SL2 and SL3 *in vitro* and are currently analyzing these systems. After gathering information on the separate subdomains using natural and $^{13}C/^{15}N$-labeled samples, we will analyze the secondary and tertiary structure of the complete 3'X. Using these data, HCV replicon mutants modulating the structure of 3'X will be designed, and their activity will be evaluated with cellular assays to obtain functional information about this essential HCV RNA region.

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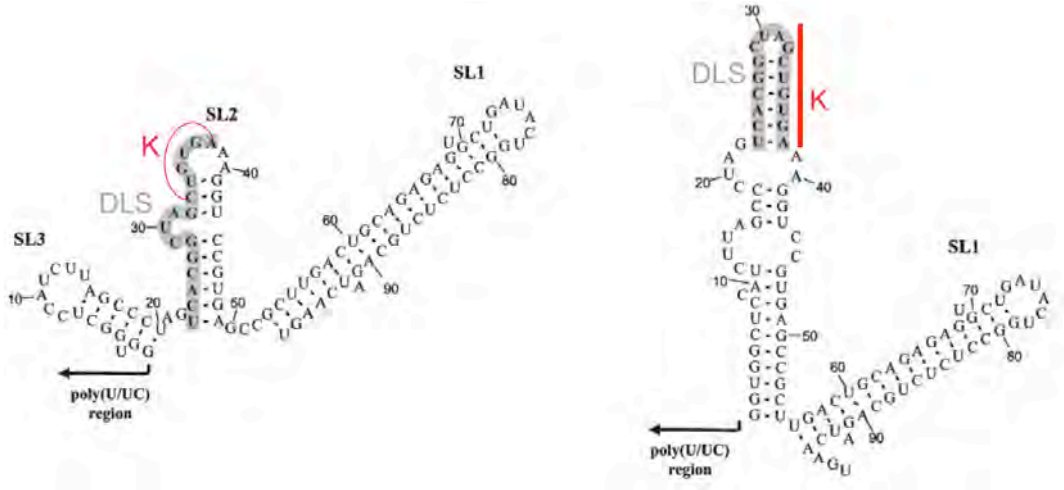

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Developing Methods to Obtain Conformationally Constrained Oligonucleotide Structures

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In addition to its classical depiction as a B-type double-stranded helix, DNA, and RNA in particular, can fold into a large number of structures. Z-DNA, G-quadruplexes, i-motifs, A-motifs, hairpins, triplexes and cruciforms are some examples of non-B DNA conformations.[1]

These alternative conformations affect key genetic events, such as replication, transcription, recombination and repair, causing genome instability, and are responsible for different human illnesses[2] such as the neurological diseases caused by triplet repeat expansion.[3,4]

In this context, understanding the conformations of nucleic acids is crucial. However, the use of short oligonucleotides as models of large DNAs and RNAs is hampered by their low structural and thermal stability compared to the native nucleic acids. In this regard, introducing conformational constraints into the oligonucleotide constructs may facilitate their structural study.

The incorporation of an interstrand cross-link (ICL) is one of the best and well-established approaches to constrain nucleic acids conformations. These modifications can naturally take place as a result of environmental agents or be caused by therapeutic drugs.[5] Furthermore, synthetic cross-linked oligonucleotides can be used to study the effects of such linkages on nucleic acid structure as well as to get insight into the repair mechanisms.[6]

We will report on a novel method to introduce an ICL into nucleic acids. The diethylsulfone linkage between nucleobases (**Figure 1**) can be easily introduced and, besides increasing notably the duplex thermal stability, does not significantly perturb B-DNA conformation. Moreover, the reversibility of the diethylsulfone junction fulfils one of the desirable properties of an ideal cross-link.

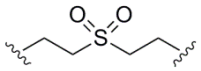

Figure 1. Structure of the diethylsulfone linkage.

On the other hand, cyclization is another common way of constraining oligonucleotide conformations, which is especially useful in thermodynamic studies because cyclic oligonucleotides denaturate in a monomolecular fashion. We will also describe some results of our atempts to synthesize circular oligonucleotides containing repetitive sequences by template-assisted ligation, which has proved to be a challenging task.[7]

Acknowledgements

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A minimal i-motif stabilized by minor groove G:T:G:T tetrads

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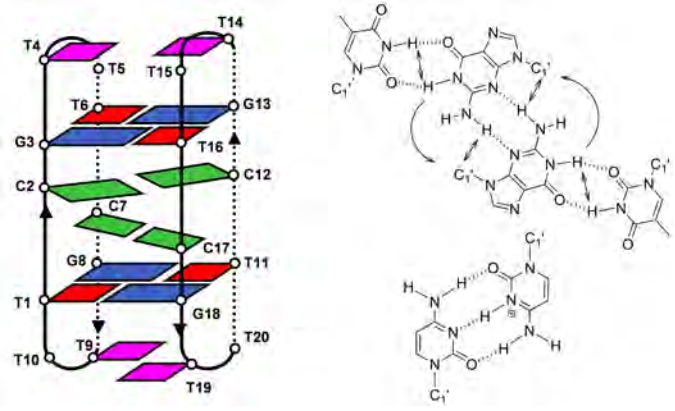
Non-canonical nucleic acids secondary structures are commonly associated with specific repetitive patterns that, interestingly, are found in functionally important genomic regions such as telomeres, centromeres and gene promoters.[1] The tendency to form such structures may be determinant to the biological role of these sequences and converts them in promising therapeutic targets.[2] The most extensively studied non-canonical structures are quadruplexes formed by G-rich sequences (G-quadruplexes) but increasing interest is being adressed to the complementary C-rich sequences that form intercalated quadruplex structures[3] (i-motifs).

Although a diversity of quadruplex folding patterns have been observed, the coexistence of different quadruplex-forming elements in the same structure remains less understood. Here we report the structural study of the oligonucleotide d(TCGTTTCGT) and its cyclic analog d<pTCGTTTCGTT>.[4] Both sequences form dimeric quadruplex structures consisting of a minimal i-motif of two hemiprotonated C:C$^+$ base pairs capped, at both ends, by slipped G:T:G:T minor groove-aligned tetrads. These mini i-motifs, which do not exhibit the characteristic CD spectra of other i-motif structures, can be observed at neutral pH, although they are more stable at acidic conditions. This finding is particularly relevant since these oligonucleotide sequences do not contain contigous cytosines. Importantly, these structures resemble the loop moiety adopted by an 11-nucleotide fragment of the conserved centromeric protein B (CENP-B) box motif,[5] which is the binding site for the CENP-B.

Interestingly, the complementary sequences form a distinct non-canonical structure at acidic conditions, which is stabilized by homo base pairs. The structural characterization of this motif and its equlibrium with the duplex form will be discused.



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Discovery of new telomeric RNA ligands by ^{19}F-NMR spectroscopy

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G-quadruplex forming sequences are located preferentially in gene promoters and telomeres.[1] It is believed that these structures play a role in gene expression and telomere regulation. Human telomeres are formed by tandem repetition of the hexanucleotide G-rich sequence d(TTAGGG), ending in a single strand 3' overhang able to self-associate into G-quadruplex structures. Maintenance of telomeres is reached by the enzyme telomerase, a reverse transcriptase that synthesizes additional d(TTAGGG) repeats.

DNA G-quadruplexes are good anticancer targets because there are small molecules that, through specific quadruplex-stabilization, can suppress oncogene transcription or inhibit telomere extension in cancer cells.[1]

The recent discovery of a telomeric-repeat containing RNA (TERRA) and the suggestion of its possible role in triggering the alternative mechanism (ALT) for uncontrolled proliferation of cancer cells[2], as well as the ability of 5'UTR G-quadruplexes to inhibit mRNA translation, have converted RNA G-quadruplexes in a primary research topic.

In the last decade, many efforts have been devoted to rationally design and evaluate drugs showing optimal binding to telomeric G-quadruplex structures. However, screening of large number of compounds has not been exploited in this field. The possibility of testing many kind of compounds opens up a way to find molecules without the relatively common features shared by G-quadruplex ligands found so far, that will serve as new starting points for the development of finest drugs.

We present here the application of ^{19}F NMR spectroscopy to the screening of libraries composed by small fluorinated compounds, and the discovery of new binders able to interact with long human telomeric RNA. A total of 20 hits out of 237-compound library have been found. Biophysical data and a variety of NMR methods were used to confirm the interaction. The selectivity of the hits by RNA G-quadruplex was also evaluated indicating that some of the binders do not interact with other RNA and DNA structures.

Acknowledgements

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Rational drug design for DNA repair mechanism as adjuvant in chemotherapy

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The endonucleolytic activity of human apurinic/apyrimidinic endonuclease (AP endo, Ape1) is a major factor in the maintenance of the integrity of the genome(1). On the other side, as an undesired effect, Ape1 overexpression has been linked to resistance to radio- and chemo-therapy treatments in several human tumors(2). Inhibition of Ape1 using siRNA or the expression of a dominant-negative form of the protein have been shown to sensitize cells to DNA-damaging agents, including various chemotherapeutic agents(3). Therefore, inhibition of the enzymatic activity of Ape1 might result in a potent antitumor therapy. Small molecules have been described as Ape1 inhibitors; yet, those compounds are in an early stage of development(4). Here we report for the first time the identification of new compounds as potential Ape1 inhibitors by using docking-based virtual screening technique. Some of these identified compounds are shown to be active in vitro with activities in the low to the medium micromolar range. Interaction of these compounds with Ape1 protein was observed by mass spectrometry. These molecules also potentiate the cytotoxicity of the chemotherapeutic agent methylmethane sulfonate in fribrosarcoma cells. This study demonstrate the power of the docking and virtual screening techniques as a first step for potential drugs design and opens the door to the development of a new generation of Ape1 inhibitors.

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DNA STABILIZED GOLD NANOPARTICLES AS DELIVERY SYSTEM

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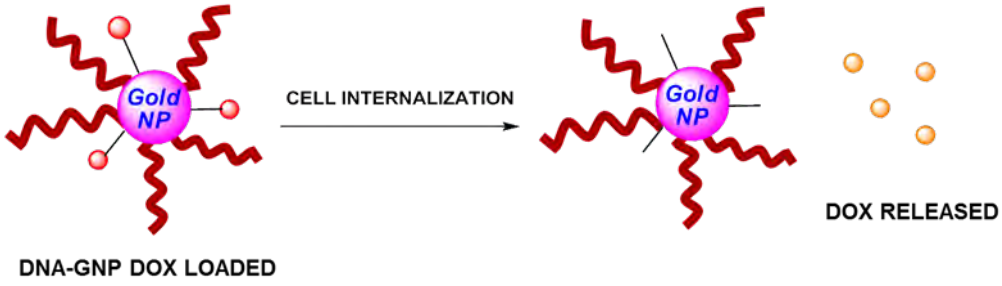
Multidrug resistance (MDR)[1] has been one of the major impediments to the success of cancer chemotherapy. A great interest is growing in the development of drug delivery systems using nanotechnology to overcome this problem. Gold nanoparticles (GNP)[2] have emerged as attractive nanomaterials for biological and biomedical applications because of their physical and chemical properties, including their biocompatibility, low toxicity and ability to internalize in cells.

Our group is focused on the development of a delivery system of chemotherapeutic drugs, such as doxorubicin (DOX), and oligonucleotides, using DNA stabilized gold nanoparticles (Scheme 1). Particularly, DOX is tethered onto GNP surface using a thiolated linker designed to release the drug inside the cell upon an internal stimuli. Different single DNA strands are being evaluated to stabilize the nanostructure and get the best drug release efficiency. Furthermore, we are exploring the use of aptamers to develop a targeted therapy against MCF-7 breast cancer cells.



Scheme 1

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DNA Repair of hAGT Detected over DNA Origami

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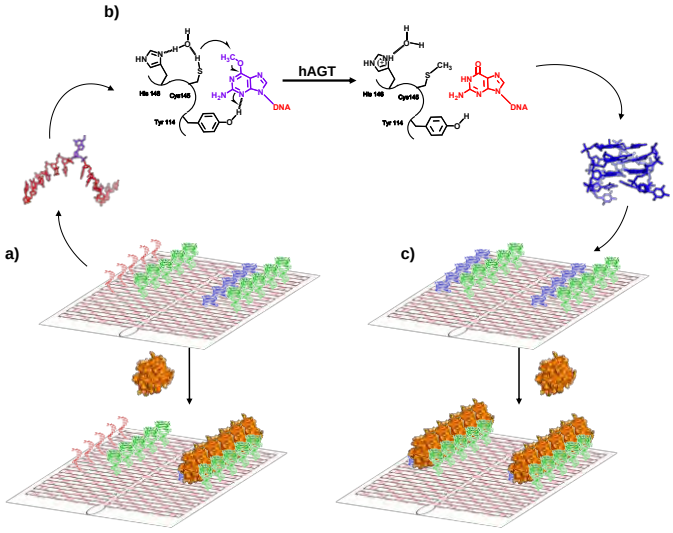
The self-assembly of DNA molecules provides an attractive route towards the formation of complex structures at the nanoscale. Specifically, the direct folding Origami DNA allows the assembly of 2D and 3D structures using single stranded 7kB long viral DNA held together by a few hundred synthetic "staple" DNA strands in a rational and desired shape[1]. This methodology is an excellent platform to create nanosensor for biomedical applications at the single molecule level using atomic force microscopy. In the other hand, the identification of key mechanisms involved in the DNA repair of the adducted induced by chemotherapeutic drugs (Alkylating agents). As an example, human O^6-alkylguanine-DNA alkyltransferase is a DNA-binding protein responsible for the repair of the alkytated adducts produced in the O^6 positions of the guanine, contributing to the resistance to chemotherapeutic agents[2]. Intense research efforts have been devoted to the identification of small molecules capable of inhibiting hAGT activity and enhancing the cytotoxic effect of the alkylating agents in tumour cells[3]. In this work, we describe the use of the DNA origami as a nanosensor to analyze the enzymatic DNA repair activity of hAGT via conformational changes of a DNA G-quadruplex that condition α-thrombin interaction with DNA aptamers. These findings illustrate the potential use of the DNA origami as a protein recognition biosensor.



Scheme 1. a). Tridimensional view of the asymmetric binding of α-thrombin to TBA aptamers of methylated DNA origami. b). MethylTBA repair by hAGT, allowing the G-quadruplex formation. c). Tridimensional view of the symmetric binding of α-thrombin to the repaired DNA origami. quadruplexes.

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SUS1 pre-mRNA structure and alternative splicing

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The structures formed by pre-mRNA molecules modulate splicing through a variety of mechanisms that need further characterization. Sus1 (ENY2 in mammals) is an evolutionary conserved protein involved in chromatin remodelling and mRNA biogenesis. Unlike most yeast genes, the *SUS1* pre-mRNA of *Saccharomyces cerevisiae* contains two introns and is alternatively spliced[1,2], retaining one or both introns in response to changes in environmental conditions. *SUS1* splicing may allow the cell to control the expression of its mRNA export machinery, but the mechanisms that regulate this process remain unknown.

An *in silico* analysis of *SUS1* pre-mRNA predicted the formation of two presumably stable RNA structures, one contained in the exon (E2) separating the two introns, and the other one in the downstream intron (I2). A cellular assay based on a Cup1 reporter system revealed that *SUS1* mutants containing altered I2 and E2 structures had significantly impaired splicing activity *in vivo*. Semi-quantitative RT-PCR experiments indicate that these mutants accumulate unspliced pre-mRNA. Nuclear magnetic resonance (NMR) spectroscopy and UV thermal denaturation experiments have confirmed that I2 forms a sub-stable 37-nucleotide A:U-rich stem-loop structure containing the branch site near its apical loop and the 3' splice site at the 3' terminus of the stem. E2 may form a more complex structure involving a 79-nucleotide four-helix junction and, up to now, NMR and UV melting experiments have confirmed that one of the E2 stem-loops forms an unusual and stable structure. RNA modification experiments (*SHAPE*) are currently underway to characterize the secondary structure of the entire E2 sequence. Investigating the role of pre-mRNA structure in *SUS1* splicing may allow us to identify new functional RNA motifs and will contribute to elucidate the mechanisms that regulate the splicing of an essential eukaryotic protein.

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MODIFIED "NUCLEOSIDE" ANALOGUES AS POTENTIAL ANTIVIRAL INHIBITORS

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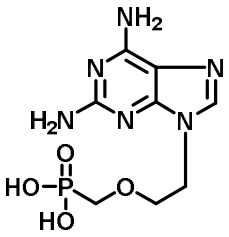
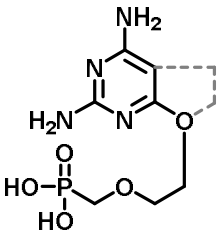
Recently, a new structural subclass of acyclic pyrimidine nucleoside phosphonates (ANP) named as PMEO-DAPym (2) was identified.[1,2] Unlike other ANPs, these derivatives are recognized by HIV-1 RT as a purine nucleoside instead of a pyrimidine nucleoside and incorporated facing thymine (in DNA) or uracil (in RNA). PMEO-DAPym represents a prototype compound of a novel class of pyrimidine acyclic nucleoside phosphonates with antiviral or antimetabolic properties.[3]

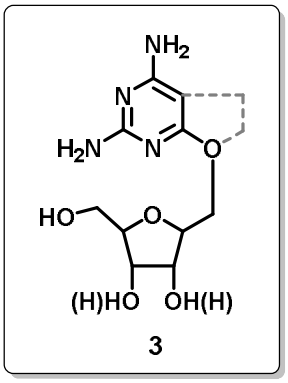
We are currently working on the design and synthesis of new "nucleos(t)ides" analogues of PMEO-DAPym (3) where the acyclic alkyl chain has been replaced either by an intact furanose or a 2',3'-dideoxyfuranose rings. In order to study whether the corresponding "nucleoside" analogues would be converted into triphosphates by cellular kinases and incorporated into DNA as a purine nucleotide. The peculiar structure of these novel nucleosides may represent a potential new family of antivirals.



PME-DAPym (1)



PMEO-DAPym (2)



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Effect of furanose C2´-substitution on structure and stability: Directing the folding of the human telomeric quadruplex.

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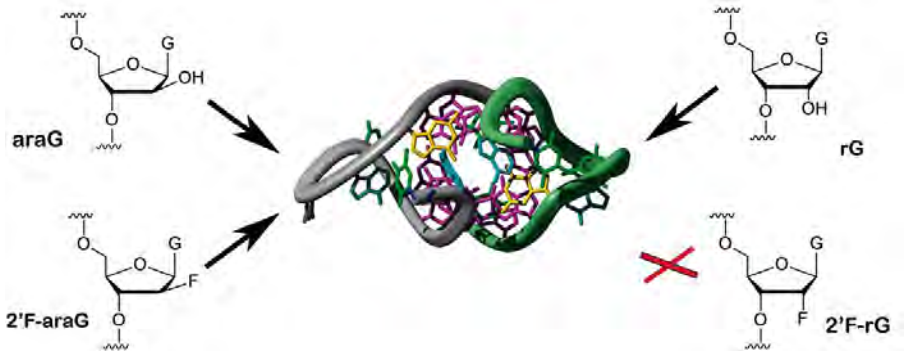
Nucleic acids analogs containing modifications at the C2' sugar position are interesting compounds for their potential applications as gene-targeting drugs applying antigene or antisense strategies. The impact of arabinose (ANA),[1] 2'fluoro-arabino (2'F-ANA)[2] and 2'fluoro-ribose (2'F-RNA)[3] substitutions in duplex and hybrid structures has been extensively studied in the last few years.

In this communication, we present our studies on C2' sugar-modified G-quadruplexes. Human telomeric DNA quadruplexes can adopt different conformations in solution. We have found that ANA, 2′F-ANA, and RNA substitutions stabilize the propeller parallel G-quadruplex form over competing conformers, allowing NMR structural determination of this particularly significant nucleic acid structure. 2′F-ANA substitution provides the greatest stabilization as a result of electrostatic (F-CH---O4′) and pseudo-hydrogen bond (F---H8) stabilizing interactions. In contrast, 2′F-RNA substitution provokes a dramatic destabilization of the quadruplex structure due to unfavorable electrostatic repulsion between the phosphate and the 2′-F.[4]



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Functionalization of siRNA strands with L-threoninol-Thymine derivatives.

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The RNAi pathway is a specific and natural cellular process, triggered by endogenous dsRNA and involved in regulation of gene expression during embryo development, cell proliferation, hematopoiesis and apoptosis (1).
In 2001, it was reported that synthetic siRNAs could induce RNAi in mammalian cells (2), since then a wide number of new synthetic RNA molecules have been evaluated for studying gene function and for approaching new specific gene therapy. But, in spite of these huge applications, their low biological stability hinders an effective *in vivo* use. Over the past years, a wide variety of chemical modifications have been proposed to address this issue. They can be classified into three groups: sugar modifications (2'-OMe; 2'-F; 2'-MOE, LNA), base modifications (5-bromo-uracil; 2-thio-uracil; difluorotoluene) and backbone modifications (phosphorothioate; boranophosphate; PNA) (3).
Recently, a new foldamer has been developed. The foldamer (named as aTNA) carries the natural nucleobases (A, G, C, T) connected to the acyclic L-threoninol backbone (aTNA) and phosphodiester linkages (4).
These ongoing discoveries led us to explore the effects of this class of modification on the biological properties of siRNAs. To carry out such studies, hybrid oligonucleotide molecules containing acyclic L-threoninol backbones in place of natural ribose rings at certain positions of the strand have been synthesized. For example, siRNAs containing two L-threoninol-modified building blocks in place of the natural 3'-dinucleotide overhang. These derivatives have been tested for their effects on the RNA interference process. *In vitro* cellular RNAi assays have demonstrated that this class of modification is well tolerated by the RNAi machinery. In fact, our analogues turned out to be more active than the native (unmodified) siRNA. On the other hand, the effect of the 3'-L-threoninol functionalization on the 3'-exonuclease stability of siRNAs has been evaluated. Interestingly, this class of modification conferred strong 3'-exonuclease resistance. In view of these good results, has been planned: (i) to evaluate the stability of our analogues in human serum; (ii) to apply this pattern of modification to the knockdown of a therapeutically relevant gene: ApoB; (iii) to determine whether the modification might avoid the activation of innate immune response.

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Carbohydrate oligonucleotide conjugates: tool to study fluorosugars-DNA interactions and gene inhibition by apolar carbohydrate siRNA

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Carbohydrate-nucleic acid contacts are known to be a fundamental part of some drug-DNA recognition processes. Most of these interactions occur through the minor groove of DNA, such as in the calichaemicin or anthracycline families. Recently, we have used carbohydrate oligonucleotide conjugates (COCs) to show that carbohydrate-DNA interactions are also possible through sugar stacking on a DNA double helix and on a G-quadruplex context. We observed that mono- and disaccharides attached to the 5'-end stabilize both DNA structures with respect to the DNA controls.[1] These results are remarkable since these carbohydrates are highly polar molecules in contrast to typical capping agents used in DNA such as hydrophobic aromatic rings. Carbohydrate-DNA stacking was also confirmed by NMR experiments and molecular dynamics calculations. Moreover apolar sugars such as permethylated carbohydrates were also able to stack onto DNA duplexes and a G-quadruplex stabilizing them much more than the corresponding natural sugars.[2]

Here, we present results on the interaction of carbohydrates with different modifications (carboxylate, methyl, N-acetylamino and fluorine) when stacking onto DNA double helices. We have observed that more hydrophobic carbohydrates and fluorosugars stabilize DNA more than their natural sugar versions.

We have also used COCs as potential tools for gene inhibition. Glucose and galactose modified RNAs were examined with limited success. Here we report on the RNAi activity of permethylated carbohydrate-siRNA conjugates as potential vectors to improve delivery and gene inhibition. All the apolar carbohydrate-siRNA derivatives were compatible with RNA interference machinery if transfected with oligofectamine. In the absence of a transfection agent, some of them exerted some reduction of gene expression. The use of a hydrophobic carbohydrate platform to improve siRNA entrance showed a limited success.

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Using optical tweezers to study DNA replication dynamics at single-molecule level

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It has been over two decades since methods of single molecule manipulation were first introduced in biochemical research. Since then, the application of these methods to an expanding variety of biological problems has grown rapidly, from mechanical properties of nucleic acid to mainly all processes involved in nucleic acid metabolism (replication, transcription, translation, recombination, repair, packaging, etc), protein folding and molecular motion of a great variety of enzymes (such as kinesin, dynein, myosin, etc). The ability to analyze biological systems at single molecule level opens avenues of investigation that are not possible using more 'classical' techniques that measure aggregate properties of molecular populations.

Optical Force Microscopy or optical tweezers forms much of the backbone of the field of research devoted to study (and manipulate) biology at single molecule. This technique offers the possibility to: 1) measure the mechanical properties of proteins and nucleic acids, 2) follow the activity of a single motor protein in real time and 3) measure the mechanical force exerted by the protein during its biochemical cycle and apply external mechanical force on the protein. This information is crucial to quantify the real time kinetics and the mechano-chemical processes of a biological reaction. I will describe the series of optical tweezers-based assays we have employed in our laboratory to study the dynamics of an essential biological reaction, DNA replication.

Threoninol-based Thioctic Acid Derivatives as Suitable Building Blocks to Incorporate DNA Oligonucleotides into Gold Nanoparticles

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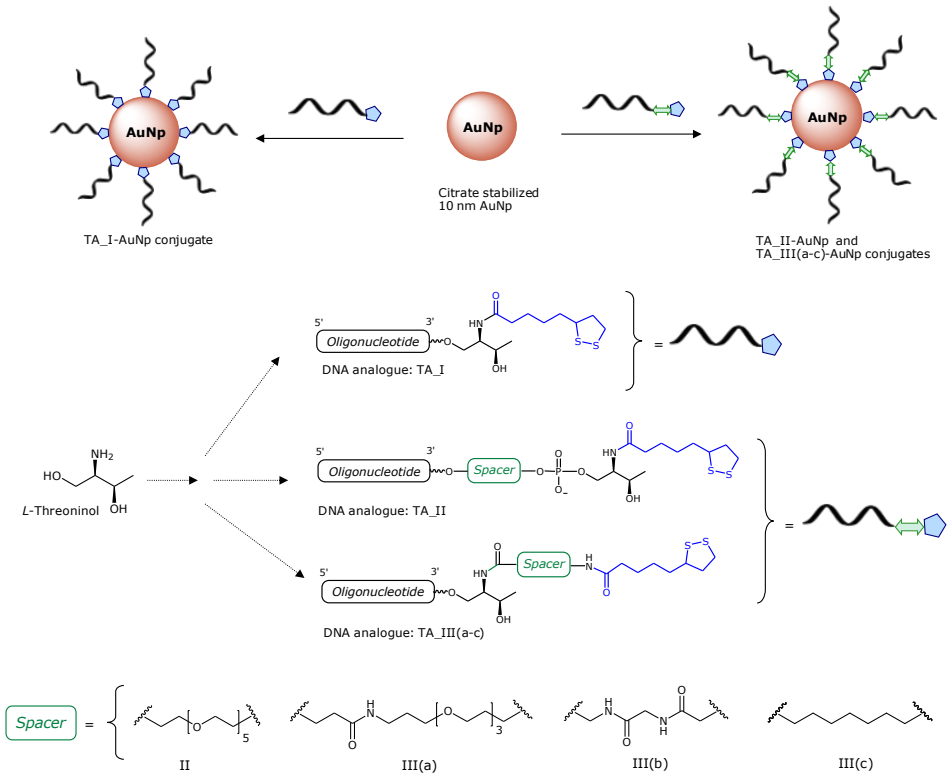
Thiol-chemistry has been widely used to date as the general approach to functionalize gold nanoparticles with nucleic acids. In most cases, thiolated oligonucleotides have been simply the strategy used to incorporate DNA or RNA molecules onto gold surfaces. However, the stability of such nucleic acid–gold nanoparticle conjugates in certain conditions is clearly a limitation so that aggregation process normally occurs. There are some examples reported in which the aforementioned stability of the gold nanoparticle conjugates is improved by modifying the linkage between gold nanoparticles and nucleic acids.[1,2] Here, we describe a straightforward synthesis based on the use of several thioctic acid-*L*-threoninol-based modified oligonucleotides containing five different spacers, which were attached onto gold nanoparticles (Figure 1). The corresponding thioctic acid-*L*-threoninol-based oligonucleotides were fully characterized and stability experiments along with surface coverage were successfully performed.



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DNA Stabilized Silver Nanoclusters

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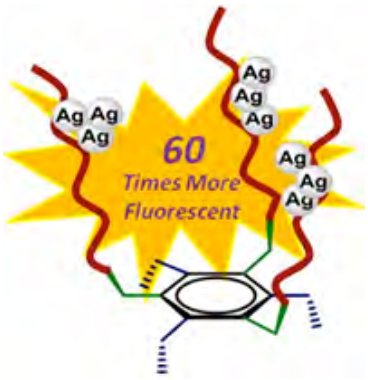
DNA stabilized silver nanoclusters (AgNCs) are promising optical materials, whose fluorescence properties can be tuned by the selection of the DNA sequence employed in their preparation.[1] This phenomenon has allowed the preparation of a wide range pallete of colours by changing the nucleotide sequence. The fluorescence properties of AgNCs depend also on other parameters such as buffer, pH, concentration of reagents, size and oxidation state of the silver clusters. The environment of AgNCs can also affect their fluorescent properties. In this sense, the structural changes promoted by the interaction with a given analyte have been exploited to develop sensors for different entities such as ions, small molecules, DNA, microRNAs, proteins or tumour cells.

We have prepared a family of branched derivatives composed of DNA strands connected through a bezene ring. These derivatives have allowed the preparation of fluorescent silver nanoclusters with improved stability and up to 60 times more fluorescence than structures with unmodified oligonucleotides.[2]



Acknowledgements

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