RESEARCH CONFERENCES

Self-Assembly of Guanosine Derivatives: From Biological Systems to Nanotechnological Applications

Universitätszentrum Obergurgl (Ötz Valley, near Innsbruck) • Austria
20-25 June 2009

Chair: **Gian Piero Spada,** University of Bologna, IT
Co-Chairs: **Irena Drevensek-Olenik**, University of Ljubljana, SI;
Lea Spindler, University of Maribor, SI

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IL-01

GUANOSINE 5'-MONOPHOSPHATE SELF-ASSEMBLE: 100 YEARS LATER

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Almost a century ago, a Norwegian biochemist, Ivar Christian Bang, discovered that one of the simplest nucleotides, guanosine 5'-monophosphate (5'-GMP), gelatinizes under a slight acidic condition but its neutral solution exhibits normal viscosity.[1] Because gel formation was often associated with thymus nucleic acid at that time, Bang used this unusual property of 5'-GMP to argue, incorrectly, against the view held by Kossel and others that guanylic acid is a simple nucleotide similar to inosinic acid. Two years later, Levene and Jacobs[2] reported that the related guanine nucleoside, guanosine, can also gelatinize in the presence of mineral impurity. These authors used this observation to contradict Bang's conclusion about the chemical nature of guanylic acid and, furthermore, their careful elemental analysis showed unequivocally that guanylic acid is indeed a mononucleotide. However, it was not until 50 years later that the structural basis of such gel formation was examined. In 1962, David Davies and his colleagues proposed that 5'-GMP gel formation is a result of nucleotide self-assembly into a helix utilizing a guanine tetramer (now known as the G-quartet) as the building block. They used X-ray fiber diffraction data to show that different GMP isomers form different helical structures. For 3'-GMP, the helical structure is formed by successive stacking of planar G-quartets on top of each other. For 5'-GMP gel formed at pH 5, however, the helix may consist of continuously hydrogen-bonded guanine bases. In 1972, Miles and Frazier[4] reported infrared (IR) spectroscopic evidence that 5'-GMP also self-associates into ordered, perhaps also helical structures, even in neutral solution. Despite consider effort made since the 1970s in pursuing the structure of 5'-GMP self-assembly in neutral solution,[5-13] the answer seems illusive. It is quite remarkable that, although G-quartet structures have now been found in many DNA and RNA sequences, the exact structure of 5'-GMP self-assembled helix, the first class of G-quartet structures, has remained a mystery for so long.

Recently, we have finally solved the structure of Na^+-directed 5'-GMP self-assembled helix in neutral solution using nuclear magnetic resonance (NMR) techniques.[14] In this talk, I will present some historical perspectives of the 5'-GMP self-assembly story and describe our recent findings regarding 5'-GMP self-assembled structures in neutral solution, the role of alkali metal ion binding, and new results on the structure of 5'-GMP self-assembly at pH 5 (i.e., acidic 5'-GMP gel).

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IL-02

CONSTITUTIONAL G-QUADRUPLEX SELF-ASSEMBLY TOWARD FUNCTIONAL SUPRAMOLECULAR MATERIALS

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The functional self-organization can be readily transcribed into hybrid or polymeric nanostructures by using the sol-gel process. Accordingly, we have reported synthetic routes for preparing self-organized systems which have been "frozen" in a solid state matrix, as a straightforward approach for the design of a novel class of nanomaterials. Nucleobases oligomerization can be an advantageous choice to reinforce the controlled communication between interconnected "*dynamic supramolecular*" and "*fixing polymeric*" systems. Moreover, the different interconverting outputs that nucleobases may form by oligomerization define a dynamic polyfunctional diversity which may be "extracted selectively" by polymerization in solid state, under the intrinsic stability of the different nucleobase-pairing and G-quadruplex-based systems.

For all this reasons this presentation will highlight some very recent accomplishments in the field of self-organized hybrid and polymeric materials, focusing on the evolution of discrete nucleobase derivatives from self-assembled dynamic libraries of different devices exchanging in solution to constitutional functional hybrid solid materials.

The primary purpose of this presentation is to describe our recent work on nucleobase-based self-organized membrane materials. The first part begins by describing some recent advances in the area of hybrid supramolecular materials. Then constitutional dynamic amplification of supramolecular architectures will be examined with particular emphasis focusing on such self-organized nucleobase-type systems, presenting combined features of structural adaptation in a specific hybrid nanoenvironment.

The dynamic assembly behaviour of G-quartet and the transcription of its structural information into a functional unimolecular G-quartet or in reversible polymeric gels have been recently described. Very recently, we have reported a long-range amplification of the G-quadruplex supramolecular chirality into hybrid organic-inorganic twisted nanorods followed by the transcription into inorganic silica microsprings by using the sol-gel process (Figure 1).

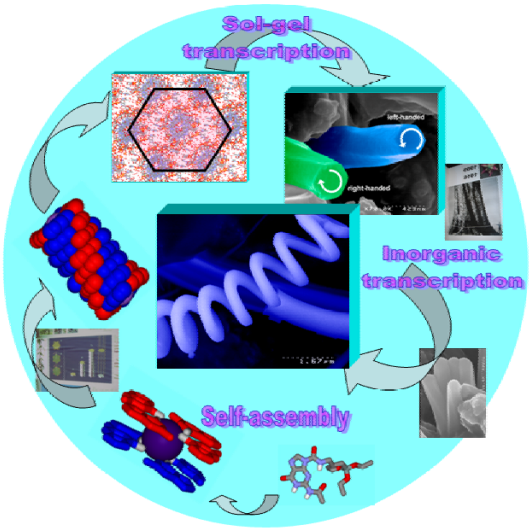

Figure 1: Amplification and transcription of the dynamic supramolecular chirality of the G-quadruplex in hybrid materials

Then the long-range amplification of the G-qudruplex self-organization into macroscopic polymeric functional films will be presented.

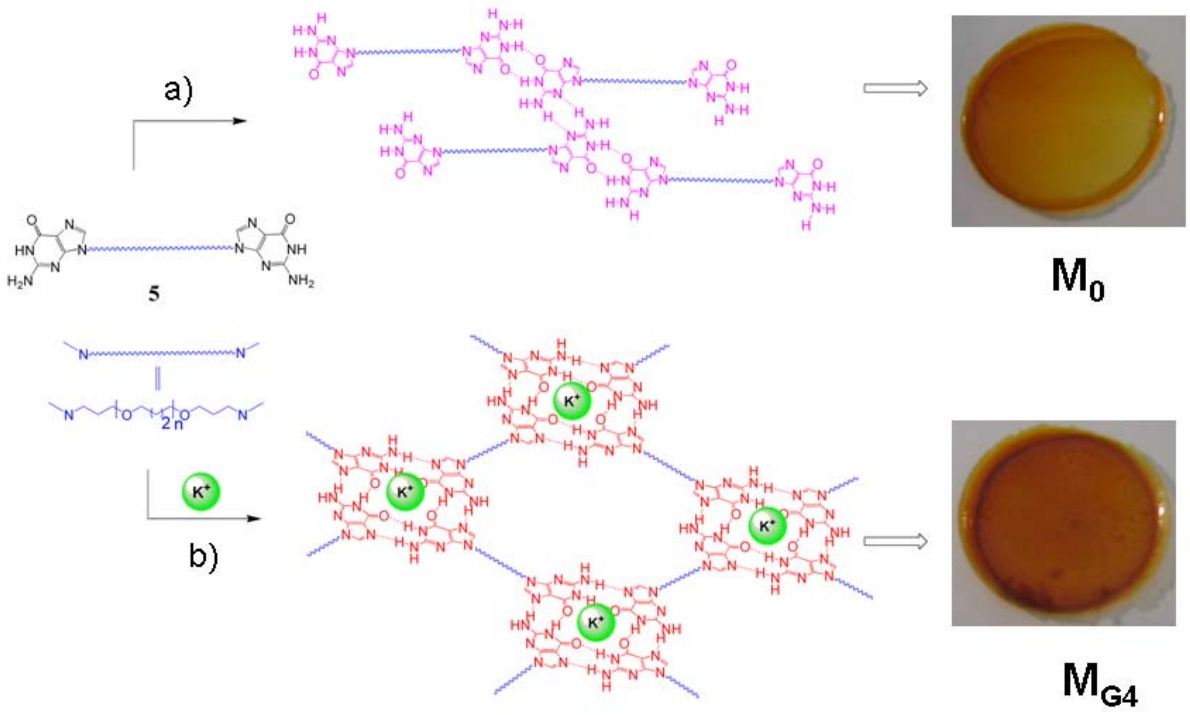

Figure 2. The cation-templated hierarchic self-assembly of bisiminoboronate-guanosine **5** macromonomer gives the G-quartet networks in the solid self-standing polymeric membrane films a) in the absence $\mathbf{M_0}$ and b) in the presence of templating K^+ cation, $\mathbf{M_{G4}}$.

The proposed strategy for stabilizing the G-quadruplexes in the *double dynamers* systems is novel. It involves the double dynamic connexion between a dynamic supramolecular system (G-quartet/G-quadruplex superstructures) and a rigid polymeric network (Figure 2). This contributes to make functional G-quadruplexes by correlating the reversible supramolecular (G-quartets) with the polymeric self-assembly via the reversible molecular connexions (iminoboronate G-macromonomer bonds) between the components. The fixed ("*frozen*") G-quadruplexes self-correlate with a directional order as proved by XPRD and transport experiments, to generate anisotropic mesophases interconnected via condensed macromonomeric hydrophobic bridges. The G-quadruplex ordered membrane films contribute to the fast electron/proton transfer by the formation of directional conduction pathways. Mixed cationic Na^+/K^+ or selective K^+ transport enabled us to better understand the diffusional ion exchanges along "fixed" G-quadruplex polymeric pathways.

These G-quadruplex-type materials unlock the door to the new self-organized materials world paralleling that of biology.

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IL-03

HOW TO DESIGN HYDROGEN BOND-DIRECTED SUPRAMOLECULAR MATERIALS OF GUANOSINE DERIVATIVES? AN INDIRECT APPROACH TOWARD SUPRAMOLECULAR VESICLES

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Organic supramolecular materials have well-defined organized molecular assemblies, and can exhibit characteristic high functionality based not only on their molecular functionality but also on their organized structures. In order to fabricate the macroscale organized structures by self-assembly, rational molecular design to control intermolecular interactions is essential. The hydrogen bond is an efficient and useful atractive intermolecular interaction for fabrication of supramolecular assemblies because of its relatively strong and directional nature. However, molecular design based only on the hydrogen bond interaction is not sufficient to achieve successful self-assembly. Since molecules are densely packed in the solid state, both atractive and repulsive intermolecular interactions have to be taken in account. In this context, Kitaigorodskii's close packing principles[1)] and Etter's rules for hydrogen bond formation[2)] offer useful concepts to understand the molecular packing in the solid state. Though "all good proton donors and acceptors are used in hydrogen bonding" in crystals, the comment made by Gavezzotti and Filippini[3)] that "close packing is obeyed even for the vast majority of organic molecules that make hydrogen bonds in the solid state" clearly indicates the difficulty in designing hydrogen bond-directed supramolecular assemblies. Since organic molecules have intricate and diverse molecular shapes, it is difficult to predict the close packing state from their chemical structures. To circumvent this problem, we adopted a novel design principle to use a nonpolar adjustable unit to assist and control hierarchical fabrication of the hydrogen bond-directed supramolecular materials. Though multiple intermolecular hydrogen bonds contribute to formation of stable molecular units, they also greatly increase stiffness of the molecular units. We anticipated that the adjustable soft segment might assist hierarchical assemblage of the hydrogen-bonded molecular units into macro-scale supramolecular materials without disruption of the hydrogen bond motifs within the molecular units (Fig. 1).

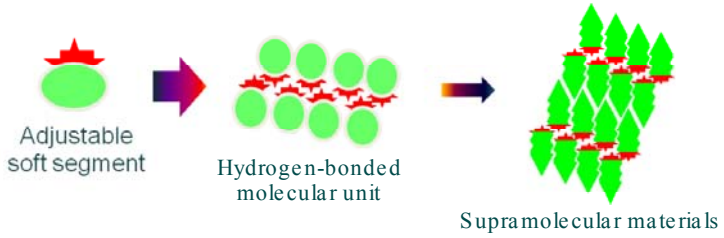

Fig. 1 Role of adjustable soft segements in hierarchical self-assembly

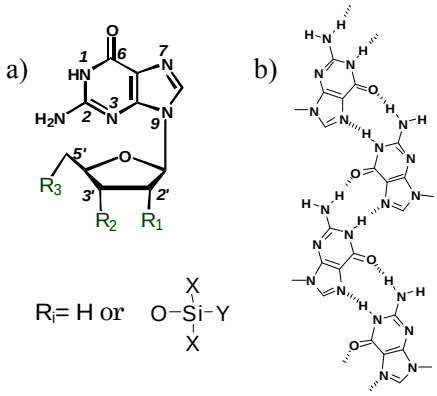

Fig. 2 Structures of a) alkylsilylated guanosine or deoxyguanosine and b) their hydrogen-bonded guanine tape motif

Guanine is one of the key components of nucleic acids, and has multiple hydrogen-bond donor and acceptor sites within the molecular structures. When we used alkylsilylated ribose or deoxyribose as the adjustable soft segment and prepared variety of alkylsilyl derivatives of guanosine crystals, formation of the guanine tape by the N^1-H--N^7 and 2-

NH_2--O=C[6] double inter-base hydrogen bonds was noticed in their crystals irrespective of the shape of the soft segments (Fig. 2) .[3] Detailed analysis confirmed that the close packing was achieved without disruption of the hydrogen-bonded guanine tape due to the adjustable nature of the soft segment, and the packing mode of the guanine tape was explained by the relative volume of the adjustable unit without taking into account their molecular shape. Thus, design and fabrication of the supramolecular materials based on the guanine tape motif can be achieved indirectly by controlling the size of the soft segment. For example, use of the bulky soft segment allows fabrication of the supramolecular fiber by encircling the soft segment around the one-dimensional guanine tape.[5] When the size of the soft segment is small, additional double 2-NH_2--N[3] inter-tape hydrogen bonds are formed between the adjacent tapes, leading to formation of the two-dimensional (2-D) hydrogen bond network (Fig. 3).[6] Both sides of the 2-D hydrogen bond motif is sandwiched by the nonpolar soft segments, and becomes a 2-D sheet-like assembly. In nonpolar alkane solutions, gelation is effectively induced by incorporating the solvents in between the 2-D sheet-like assemblies. Further, introduction of the polar oxyethylene end groups, which located at the surface of the 2-D sheet-like assemblies, enhance the inter-sheet interaction to form the three-dimensionally stacked lamellar-like film structure. The resultant supramolecular film of alkylsilylated deoxyguanosine **1a** is sufficiently flexible and transparent. It is to be noted that the design and fabrication of these hydrogen bond-directed supramolecular materials is achieved not by modification of the hydrogen bonding sites but by that of the soft segments. Thus, the indirect approach to design and control the soft segments that do not participate in the hydrogen bonding interaction is shown to be effective to fabricate hydrogen bond-directed supramolecular materials (Fig. 4).

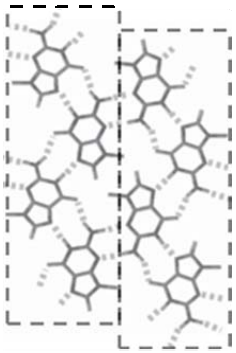

Fig. 3 The 2-D hydrogen bond network of guanine

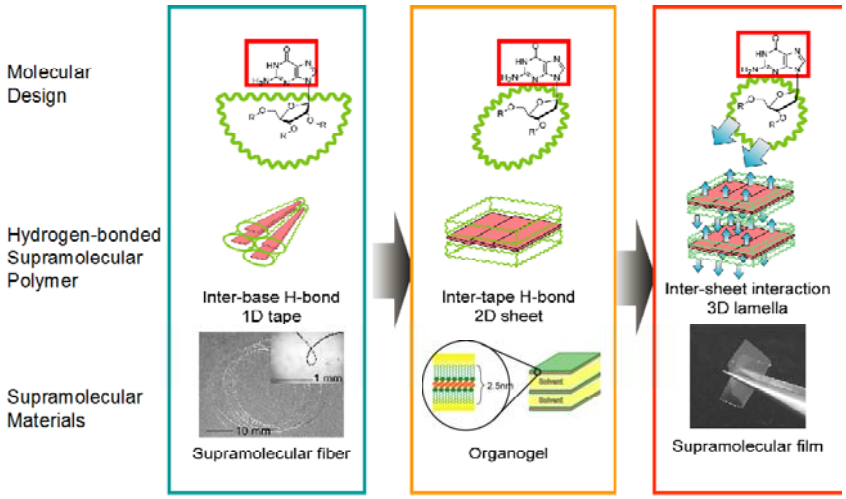

Fig. 4 Fabrication of hydrogen bond-directed supramolecular materials by the indirect method to design the soft segment.

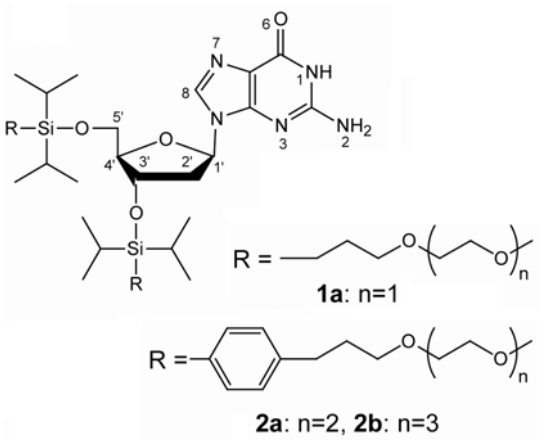

Fig. 5 Molecular structures of alkylsilylated deoxyguanosine derivatives

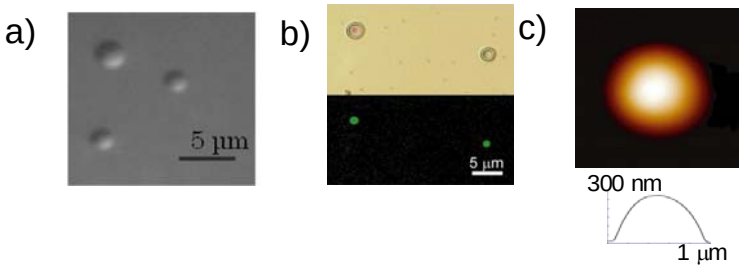

Fig. 6 Hydrogen bond-directed giant vesicles. a) Optical microscope view of **2b** vesicle in water, b) optical and fluorescence microscope images of **2a** vesicles in water, and c) AFM image of **2b** vesicle on a silicon substrate.

For preparation of stable giant vesicles on a micrometer scale, use of the 2-D sheet-like assembly formed by relatively strong and directional hydrogen bonds seems to be a promising approach because of its high flexibility and stability. However, hydrogen-bonding interaction does not generally work effectively in highly polar aqueous media and **1a** was

not sufficiently hydrophilic. Therefore, we refined the structure of the soft segment further in order to increase the hydrophilicity and the shielding effect of the nonpolar part (Fig. 5).[8] The newly designed alkylsilylated derivatives of deoxyguanosine **2a** and **2b** have a rigid phenyl unit within the alkyl chain with the extended oxyethylene units, and the molecular mechanics simulation indicated no apparent steric hindrance of the formation of the 2-D sheet assembly. Macro-scale flexible supramolecular films of **2a** and **2b** were successfully obtained by casting their THF solutions on a Teflon plate. The formation of the 2-D hydrogen-bonded sheet assemblies were confirmed by their IR spectra and the presence of a sharp diffraction peak at 3.80° (2.32 nm) corresponding to the thickness of the sheet assembly in the X-ray diffraction pattern. Micrometer-scale giant vesicles of **2a** were prepared by the thin film method and those of **2b** by injecting the small amount (1 v/v%) of **2b**/THF solution into water. Formation of the vesicles was indicated by TEM, AFM and optical microscopic observation (Fig. 6). Vesicle formation was further confirmed by fluorescence microscope after the incorporation of a water-soluble fluorescent marker (eosin Y) inside the vesicle. The vesicle after replacing the outer aqueous phase with fresh water clearly showed an orange color and green fluorescence due to the trapped eosin Y inside the vesicle.

The vesicle solution of **2b** retained the same turbidity and microscopic image (average diameter 1.24 ± 0.34 μm) after heating at 100 °C for 3 h or kept under ambient conditions for more than a month. To test the stability of the vesicle further, the vesicle on a silicone substrate after removal of water in vacuum was observed by an AC-mode atomic force microscope (AFM) with a silicone tip under ambient conditions. The AFM image showed ellipsoidal μm-size vesicles on the substrate (Figure 6c), and practically unaffected by removal of water. The results indicated that the internal water was preserved even under vacuum, demonstrating that the vesicle membrane has high stability and low water permeability across the membrane. The membrane of the vesicles was suggested to be composed of the multi-layered 2-D sheet-like assemblies (thickness 10~30 nm). Details of the stability and properties of the vesicle membranes will be presented.

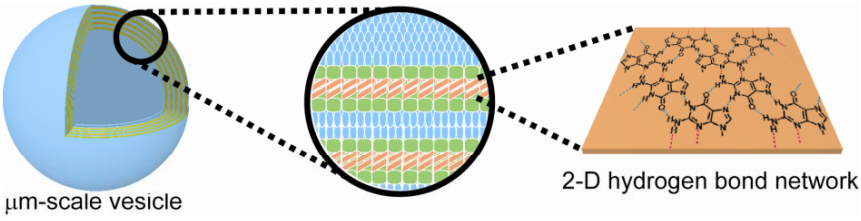

Fig. 7 Structure of the hydrogen bond-directed supramolecular vesicle composed of alkylsilylated deoxyguanosine

Thus, varieties of the hydrogen bond-directed supramolecular materials including the □m-size giant vesicles are conveniently assembled from the alkylsilylated guanosine or deoxyguanosine derivatives, confirming the effectiveness of the strategy to use the adjustable soft segment. The structural hierarchy to form the hydrogen bonded guanine tapes within their structures is observed in these materials, confirming the role of the adjustable soft segment. Further, the mode of the assemblies of the hydrogen-bonded units is effectively controlled by designing the structure of the soft segments that do not participate in the hydrogen bonding interaction. Based on this indirect approach, the hydrogen bond-stabilized giant vesicles are fabricated even in highly polar aqueous media, and the vesicles have high stability. Since the hydrogen bond is the non-covalent bonds, reorganization of the hydrogen bond network can easily be induced. Dynamic properties of the vesicles will also be discussed.

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IL-04

COMPUTER SIMULATIONS ON GUANINE QUADRUPLEXES – FOCUSED ON LOOPS

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G-DNA quadruplexes are multi-stranded molecules characterized by unique structural, dynamical and kinetic properties. One of the amazing features of G-DNA with loops is their enormous structural polymorphism, where a given sequence can adopt multiple folds. Theoretical methods have been also widely applied to investigate various aspects of G-DNA. Among the different theoretical methods for the study of G-DNA atomistic molecular dynamics (MD) with explicit solvent is probably that able to capture with higher accuracy the structure and dynamics of G-DNA in aqueous solution. Unfortunately, when using MD simulations we cannot ignore that they are based on simple empirical force fields which can lead to artifacts in simulations. This means that testing and benchmarking become a crucial step to validate the reliability of any simulation.

Earlier studies indicated very good performance of the simulation technique in studies of G-DNA stems, except of some modest imbalance of the cation positions within the stem. The simulations revealed that the cation-stabilized stem is a uniquely rigid molecular assembly and the ions are necessary for its stabilization. An initially empty stem is capable to attract a bulk ion swiftly, thus, in reality, G-DNA stems should never be left vacant by cations. Alternative topologies of G-DNA stems were found and possibility of such substates was later confirmed by experiments. Simulations were also used to investigate a wide range of double, triple and quadruple stranded species that could occur as intermediates during quadruplex stem formation.

Recently, we have carried out in-depth characterization of the loop topology of the Oxytricha nova d(G4T4G4)2 quadruplex using MD simulations. The main purpose was testing of the capability of the MD simulation technique to describe G-DNA loop topologies, which represent a very challenging task for computational methods. In standard simulations, the diagonal loops were basically stable as taken from the starting experimental structures. However, the ions residing at the stem-loop junction in the X-ray structures were lost. In contrast, Locally Enhanced Sampling (LES) molecular dynamics simulations (aimed at finding the global loop minimum independently of the starting structure) predicted entirely different loop geometry, which was in clear disagreement with the experimental structures. Subsequent free energy computations indicated that the predicted incorrect loop topology is more stable according to the force field than the correct experimental one, further suggesting that the force field is in troubles. These negative results pointed out the difficulties in representing loops by current force fields, which have been always parametrized considering canonical helices or highly compact RNA structures. Nevertheless, existing variants of the AMBER force field were shown to be successful in description of a wide range of noncanonical structures, including many complex RNAs, and also the G-DNA and i-DNA stems.

G-DNA loops represent an excellent benchmark to test the accuracy of current force fields to describe highly irregular nucleic acid structures. Properly tuned force fields, designed to reproduce these complex motifs can provide improved description of many other types of nucleic acids.

IL-05

CONTROL OF QUADRUPLEX SELF-ASSEMBLY

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In order to realize programmed build up of DNA objects, devices, and materials, a systematization of the principles at the basis of control of the assembly process is necessary. Methods are currently being developed in which double helical DNA is utilized as a precisely controllable and programmable scaffold for design and fabrication of objects, devices and materials. Both inclusion of quadruplexes in these macrostructures, or their independent use is based on precepts currently under investigation. Thus far design of topologies has been achieved on known and fairly simple topologies. The discovery of novel topologies has been either serendipitous, or through coincidental emergency. We will demonstrate the design, and control of self-assembly, of novel quadruplex topologies through the use of a rational axiomatic approach based on the two-state disposition of the glycosidic bond angle.

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IL-06

STRUCTURE AND INTERACTION OF G-QUADRUPLEXES

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Guanine-rich sequences are found in many important genomic regions, such as the telomeres and oncogenic promoters. DNA and RNA G-quadruplex structures formed by these sequences represent attractive targets for drug design. I will present our structural study on various G-quadruplex topologies formed by biologically important DNA and RNA sequences. Six different intramolecular G-quadruplex structures observed for human telomeric DNA sequences will be discussed. While G-quadruplex structures formed by human telomeric DNA are diverse, those formed by human telomeric RNA sequences are more conserved. I will discuss the structure of propeller-type parallel-stranded G-quadruplexes formed by human telomeric RNA sequences and present a model for long telomeric sequences based on different stacking interfaces (5'-to-5', 3'-to-3' and 5'-to-3'). The stacking of G-quadruplexes will also be discussed through the structure of a G-rich oligonucleotide, which is an HIV integrase inhibitor. From these structures, we could learn more about different structural elements in G-quadruplexes including base pairing, looping and stacking.

IL-07

THE NANOCHEMISTRY OF SURFACES AND INTERFACES: MASTERING THE SUPRAMOLECULAR APPROACH

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The nanopatterning, manipulation and quantitative study of the physico-chemical properties of multifunctional materials across multiple length scales are crucial for technological applications in organic electronics.[1] Supramolecular recognition between pre-programmed building blocks incorporating electrically and optically active units can be successfully used to generate multifunctional bi- and multi-component nanopatterns with controlled geometries. [2]

Lipophilic guanosines are very versatile building blocks: depending on the experimental conditions they can undergo different self-assembly pathways, leading to the formation of either H-bonded ribbons or quartet-based columnar structures. Given the possibility to functionalize the guanosines in the side-chains they appear as ideal building blocks for the fabrication of complex architectures with a controlled high rigidity, thus paving the way towards their future use for scaffolding, i.e. to locate functional units in preprogrammed positions. In my lecture I will review results on the 2D self-assembly of various guanosine derivatives we obtained in the last few years,[3] and I will show how ordered guanosines 1D H-bonded architectures can be used to control the 2D patterning of oligo-thiophenes at surfaces. [4]

Another highly directional non-covalent interaction that can be employed for 2D patterning of surfaces is metallo-ligand interaction; I will show how metal-ligand among molecular tectons embedding anthracene moieties can be employed to obtain 2D architectures with controlled geometries.[5] Furthermore we demonstrated for the first time that by applying a supramolecular approach to the development of chemisorbed bi-component SAMs on Au(111), it is possible to form multicomponent crystalline domains, thereby achieving sub-nm control over the molecular patterning of a surface. This represents the first, yet fundamental, step towards the controlled spatial confinement of single molecules or functional groups on a surface. This supramolecular multicomponent array allows potential recognition of target functional groups and could therefore lead to the detection of single-molecule properties. [6]

External stimuli can be used to manipulate single molecules and aggregates embedded in a supramolecular ensemble. Prototypes of light-powered mechano-chemical switches can be developed: Significantly, the photochemical isomerization of a new terminally thiolated azobiphenyl rigid rod, forming a single component and tightly packed SAM on metallic surfaces, was found by STM to be highly cooperative and to be complete over a molecular 2D crystal.[7a] Such an azobenzene SAM has been successfully used to modulate the current through metal-organic-metal junctions. By incorporating the azobenzene SAM between a Au(111) support and a metal coated AFM tip, we could detect a 30-fold difference in the current through the junction, providing the first example of conducting AFM measurement on a bi-stable system. [7b] When incorporated into a macroscopic Hg drop based junction the SAM could also operate as a current photoswitch; interestingly the light induced vertical displacement of the Hg drop revealed that our SAMs acts as light-powered cargo lifter generating a force per unit area as high as $1x10^5$ $N/m^{2.}$.[7b] This result unambiguously demonstrates that our azobenzene SAM represents a prototype of a molecular machine able to transport mass, and in particular to act as a cargo lifter. [7c] Noteworthy, our SAMs undergoing *cis-trans* isomerization represents the first molecular switch/motor operating on a surface according to a cooperative process. Our responsive system can be used to gate optical signals, and therefore has potential for implementing logic operations on arrays of switching elements, and ultimately for high density data storage based on artificial molecular systems.

Change in pH can also be employed to trigger conformational transition in 2,6-bis(1-aryl-1,2,3-triazol-4-yl)pyridine (BTP) molecules physisorbed on surfaces, as observed with a sub-nm resolution by STM at the solid-liquid interface. Upon addition

of trifluoroacetic acid two different BTP molecules, each forming a highly ordered physisorbed monolayer, underwent significant conformational changes from their “rosette” to their “tetragon” forms, as reflected in dramatically altered 2D self-assembly over large areas extending over hundreds of nanometers.[8]

Beyond imaging,[9] Scanning Probe Microscopies make it possible to study quantitatively various physico-chemical properties of architectures based on organic molecules. We have focused our attention to exploration of the electronic properties of mono-[10] and multi-component functional nanostructures by Kelvin Probe Force Microscopy (KPFM).[11]. By KPFM the photovoltaic activity in electron acceptor/donor blends was quantitatively studied both on the hundreds of nanometers[12] and, for the first time, on the few nanometers scale.[13] These results are of paramount for both achieving a full understanding over the fundamental processes of exciton split and electron/hole diffusion in blends for photovoltaics, but also for the optimization of organic solar cells.

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IL-08

G-QUADRUPLEXES: SEQUENCE EFFECTS AND RECOGNITION BY SMALL LIGANDS

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Little data is available to predict the stability of a quadruplex based on primary sequence only. Therefore, it is important to understand the rules that govern the formation of these intramolecular structures and determine their stabilities. Hundreds of different sequences have now been compared in sodium and potassium buffer (1-2). We demonstrate that the stability cannot simply be deduced from total loop length. These observations allow us to propose consensus sequences for high- or low-stability quadruplexes.

We recently demonstratred that the absence of a helicase (Pif1) promotes genetic instability of alleles of the G-rich human minisatellite CEB1 inserted in the Saccharomyces cerevisiae genome, but not of other tandem repeats (3). Efforts are now being made to understand the stability of RNA quadruplexes, as a number of biologically relevant G-rich RNA sequences may also adopt this structure, such as the telomeric transcribed RNA (TERRA)(4) or the 5' end of the RNA component of telomerase, hTERC (5).

To address the issue of the *in vivo* relevance of G-quadruplexes, chemical, biophysical, and biochemical tools that will detect G-quadruplex structures in cells must be developed. A few examples of fluorescent G-quadruplex ligands have been reported to date ; we will present a new series of fluorescent compounds based on Bisquinolinium and thiazole orange moeties (6). Besides their uses as probes, G4 ligands may have interesting antiproliferative effects. Rather than « simple » telomerase inhibitors (7-8), these compounds should be considered as telomere ligands: a number of biological effects are indicative of an induced telomeric dysfunction by such compounds. We will report our current progress on the design of selective ligands, that exhibit preferential binding to some quadruplex topologies.

Finally, quadruplexes may also be interesting for nanotech applications, and recent developments will be briefly described (9-10).

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IL-09

SIMULATION OF G4-DNA QUADRUPLEXES

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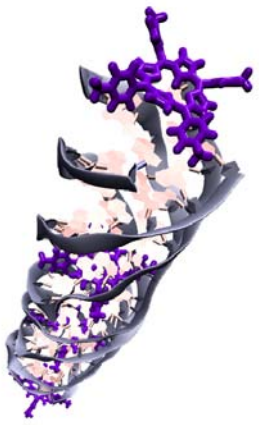
The G-quartet is a planar macrocycle formed by the assembly of four guanines held together by eight hydrogen bonds, four internal and four external. It is the fundamental building block of the supramolecular structures formed: (i) by oligoguanylates and G-rich oligonucleotides in water and (ii) by lipophilic derivatives of guanosine in organic solvents. All such supramolecular objects are characterized by the presence of a helical stack of the basic tetrameric unit and of a row of cations filling the central cavity. Depending on their size, the cations are coplanar with G-quartets or sandwiched between them.

The ability of guanosine derivatives to self-associate was first reported in 1910 and the G-quartet structure was identified in 1962 [1]. In 1988, it was found that synthetic oligonucleotides, containing short G-rich sequences present in the immunoglobulin switch regions, self-associate at physiological salt concentration to give four stranded structures. Since stretches of adjacent guanosines are present in several chromosomal locations, G4-quadruplexes were proposed to play a role in several biological processes, in particular those governing telomere function. By now, the interest for the structures formed by the self-assembly of a variety of guanosine derivatives has become very wide, going from molecular biology to nanotechnology, as described in the excellent review by Davis [2]. Applications in the field of nanotechnology are more strongly motivated by superior behavior of G4-DNA relative to double-stranded DNA for what concerns resistance to substrate deposition and response to applied electric field [3].

In this presentation I tackle issues related to the electronic structure and the conformational stability of G4-quadruplexes. The electronic structure is computed by means of quantum simulations in the framework of Density Functional Theory with the PWscf software (www.pwscf.org) [4]. The atomic structure in the presence of an external water environment and of an intercalated porphyrin species is described by means of classical molecular dynamics with the NAMD software (http://www.ks.uiuc.edu/Research/namd) [5,6]. I will put a special emphasis on the rationale for exploring the use of guanosine self-assembled structures in nanoelectronics.

By means of quantum simulations, it is found that G4-quadruplexes filled with K^+ ions, under suitable conditions, are expected to exhibit an effective behavior of wide-band-gap semiconductors [4]. This feature, along with the possibility of forming extended stacked wires at the nanoscale length, makes them appealing for the development of biomolecular electronics.

By means of classical simulations, it is found that different cation species assist the quadruplex stability to different extents [5]. It is also found that the TMPyP porphyrin is a viable stacking intercalator in long quadruplexes, which may be exploited for nanotechnology applications [6].



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IL-10

MODELLING MULTIMERIC G-QUADRUPLEX-DRUG INTERACTIONS

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Most cancer cells prevent shortening of telomeric DNA by utilizing the reverse transcriptase activity of the telomerase enzyme complex. Telomerase expression is a key marker of cellular immortalization and has been observed in 80-85% of cancer cells. The extreme 150-250 nucleotides at the 3' end of telomeric DNA are single-stranded and appropriate small-molecule ligands can induce the formation of higher-order folded DNA structures. These can inhibit telomerase activity since elongation and catalysis require a single-stranded DNA substrate for effective hybridization by the RNA sub-unit of the telomerase complex, leading to telomere shortening and cell death in neoplastic cells. In order to form higher-order DNA, the small molecules compete with the single-strand binding protein POT1, and with the end-capping function of telomerase. Their displacement rapidly leads a DNA damage response signal, which can be detected, for example by γ-H2AX. This in turn activates apoptotic pathways. This approach is currently of interest as a selective therapeutic strategy in human cancer.

Human telomeric DNA comprises tandem repeats of the sequence d(TTAGGG). The formation of higher-order structures in the single-stranded telomeric DNA overhang is a consequence of the ability of a guanine base to form hydrogen bonding interactions on both its Watson-Crick and Hoogsteen faces. This enables guanines to readily self-associate to form a highly stable structural motif involving four guanines held together via eight Hoogsteen hydrogen bonds in a coplanar array, termed a G-quartet. Several G-quartets can stack on top of another, to form a four-stranded G-quadruplex arrangement, with the G-quartets held together by nucleotides from the sequences that occur between each G-tract.

G-quadruplexes can be highly polymorphic, adopting a variety of folds, especially when the loops are 3-4 nt. Several distinct structural topologies of G-quadruplexes have been identified using NMR and X-ray crystallography including those from human telomeric DNA and Oxytricha nova telomeric DNA. However, no structural information is currently available on tandem repeats of quadruplexes (multimers) such as may be formed along the length of the single-stranded telomeric DNA overhang, although several models have been proposed. There is only restricted structural data available to data on quadruplex-ligand complexes. Two categories of topologies have been found: (i) the bimolecular diagonal loop crossover topology from Oxytricha nova telomeric DNA in complexes with disubstituted acridines and (ii) the parallel-stranded propeller-loop topology from human telomeric DNA, observed in three bimolecular and one unimolecular complexes, with three very different types of ligand. This suggests that a parallel topology is an appropriate starting-point for models of higher-order quadruplex multimers with bound ligand, which may also provide insight into the structural requirements of the DNA damage response. No NMR or crystallographic structures relevant to the human four-repeat unimolecular quadruplex have been observed to date with (3+1) structural features.

In this study, we report a molecular model for drug binding to tandem repeats of human telomeric G-quadruplexes by using the 2.5 Å resolution G-quadruplex crystal structure complex with the experimental anticancer drug BRACO19, a 3,6,9-trisubstituted acridine. The 5' to 3' continuity of the two quadruplexes in the biological unit enables the ready construction of a higher-order model based on the scaffold observed in the crystal structure. This has enabled a unimolecular model of the complex to be built whilst retaining all the features present in the original crystal structure, using explicitly-solvated all-atom molecular dynamics (MD) simulation methods to obtain low-energy structures and relative free energies estimated using molecular mechanics and the Poisson-Boltzmann surface area approximation

(MM-PBSA). The model adopts a parallel-stranded, propeller-loop topology with a drug binding site at the interface between them. The pattern of external TTA loops and their dynamics suggest that they can act as recognition features and play a role in the quadruplex recognition of telomere-associated proteins such as poly(ADP-ribose)polymerase and in the DNA damage response to the loss of telomere capping proteins.

IL-11

DYNAMIC G4 DNA IN THE GENOME

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G-rich DNA can form structures stabilized by interactions between guanines, referred to as G4 DNA. Whether such structures form or have specialized functions in vivo has been a subject of considerable controversy. Our laboratory has shown how and where these structures may form, and identified the enzymes that recognize and remove them, to maintain genomic stability or – paradoxically – promote instability.

Essentially all genomic DNA is duplex, and guanines within a G-rich region in duplex DNA are prevented from forming G4 DNA by canonical G/C pairing. However, the duplex undergoes transient denaturation during transcription, replication, and recombination. We have demonstrated that transcription of a G-rich region results in formation of novel structures, which we refer to as G-loops. G-loops contain a stable cotranscriptional hybrid on the template strand and G4 DNA interspersed with single-stranded regions on the G-rich nontemplate strand.

G-loops and G4 DNA are recognized by factors that can both stabilize and destabilize genomic structure. The RecQ family helicases are essential for maintaining genomic stability and preventing cancer and aging. RecQ family helicases actively unwind G4 DNA, using a conserved domain that binds G4 DNA with very high affinity to bind to their DNA substrates. RecQ family enzymes are especially important at G-rich regions of the genome, such as the telomeres. RecQ helicases work together with another G4 DNA helicase to unwind G4 DNA that forms during normal DNA replication. Another conserved factor, MutSα, normally promotes repair of base mismatches and small loops, but may have a more paradoxical role at G-rich regions. MutSα binds tightly to G4 DNA and can synapse structures containing G-loops. MutSα may thereby promote both stability and instability of G-rich regions. The structure-specific nuclease, Exonuclease 1, similarly has a dual role, eliminating G-rich DNA to relieve the genome of potentially toxic structures.

Does G4 DNA have specific functions in the human genome? To address this question, we have mapped regions with potential to form G4 DNA (G4P), and asked if G-richness reflects or confers special properties. Three chromosomal domains with specific functions are notably high in potential to form G4 DNA: the telomeres, the ribosomal DNA, and the immunoglobulin class switch regions. Strikingly, high and low G4P also correlate with gene function: proto-oncogenes, which are activated or overexpressed in tumors, are characterized by very high G4P; while tumor suppressor genes, which maintain genomic stability to prevent tumor development, are characterized by very low G4P. Thus there is selection for and against G4P in human genes. Near transcription start sites, human genes exhibit a characteristic profile of G-richness. Much of the G-richness may reflect well-characterized regulatory features; nonetheless, even with such motifs masked, G-rich elements stand out. One of these is at the very 5'-end of the first intron, where nearly half of all human genes contain a region with considerable potential to form G4 DNA. These G-rich intron 1 (GrIn1) elements are in the nontemplate DNA strand, and may contribute to regulation at the level of transcription or RNA processing.

IL-12

G-QUADRUPLEX DNA: A NEW TARGET FOR GROOVE BINDING AGENTS

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Recognition is a very important phenomenon in biology and chemistry, and the molecular basis of the interaction between small ligands and biopolymers is the subject of numerous investigations aimed at the rational design of molecules with specific biological activities. In particular, during past years, a great number of manuscripts have been appeared in literature dealing with the development of low molecular weight sequence-selective agents interacting with DNA.

Most of the investigations were focused on targeting duplex DNA. Only recently, upon the identification of G-quadruplex motif as biologically crucial structure, several studies about ligand/DNA-quadruplex interactions have been reported. G-quadruplexes are four stranded helical structures, comprising stacks of G-tetrads, which are the planar association of four guanines in a cyclic Hoogsteen hydrogen-bonding arrangement.[1] This structural motifs seems to play a very important role in a number of biological processes and they are widespread in genomic regions like gene promoters and telomeres[2]. Telomeres are ensemble of proteins and specialized non-coding DNA sequences. Telomere protects the ends of the chromosome from damage and recombination and its shortening has been implicated in cellular senescence. Telomeric DNA consists of tandem repeats of simple short sequences, rich in guanine residues, which, therefore can form G-quadruplexes.[2] Telomerase, the enzyme which elongates the G-rich strand of telomeric DNA, is active in about 85% of tumors, leading the cancer cells to infinite lifetime. The inhibition of telomerase has become an attractive strategy for the anticancer therapy and, because telomerase requires a single-stranded telomeric primer, the formation of G-quadruplex complexes by telomeric DNA inhibits the telomerase activity. Interestingly, it has been found that small molecules that stabilize G-quadruplex structures are effective telomerase inhibitors.[3]

Most of the reported G-quadruplex binding agents bind to DNA by interacting with the wide π-stacking surface of the G-tetrads at the edges of the quadruplex. Generally, intercalation (or more generally end-stacking interactions) and groove-binding modes are characterized by a very different specificity. Groove-binding recognition process generally offers an higher extent of selectivity and a deep understanding of factors which determine the complex formation and stabilization is important for the drug design. For this reason, we have investigated the interaction of distamycin A, a classical minor groove binder of duplex DNA, with the quadruplex $[d(TGGGGT)]_4$.[4] The interaction between distamycin A and the parallel DNA quadruplex $[d(TGGGGT)]_4$ has been studied by ^1H NMR spectroscopy and isothermal titration calorimetry (ITC). In order to unambiguously assert that distamycin A interacts with the grooves of the quadruplex $[d(TGGGGT)]_4$, we have analyzed the NMR titration profile of a modified quadruplex, namely $[d(TGG^{Me}GGT)]_4$, and we have applied the recently developed Differential-Frequency Saturation Transfer Difference (DF-STD) method, for assessing the ligand–DNA binding mode. The three-dimensional structure of the 4:1 distamycin A / $[d(TGGGGT)]_4$ complex has been determined by an in-depth NMR study followed by dynamics and mechanics calculations. All results unequivocally indicate that distamycin molecules interact with $[d(TGGGGT)]_4$ in a 4:1 binding mode, with two antiparallel distamycin dimers that bind simultaneously two opposite grooves of the quadruplex. The affinity between Dist-A and $[d(TGGGGT)]_4$ enhances (~10-fold) when the ratio of distamycin A to the quadruplex is increased.

All these findings have stimulated other investigations aimed to shed light onto the recognition processes between small organic molecules and quadruplex structures. So we have focused our attention on the interaction of Dist-A and its two derivatives (compounds 1 and 2), with quadruple helices of different sequence and molecularity. Compounds 1 and 2 are two carbamoyl analogues of distamycin A, containing four and five pyrrole units, respectively.

In a first study we demonstrated that Dist-A displayed a very different stoichiometry and titration behaviour with respect to K^+ buffer condition.[5] These results strongly indicate that, as also observed in other study,[6] the nature of the ions present in solution does affect the drug/quadruplex recognition processes.

Therefore, in order to get further insight into the influence of the ions on the binding behaviour of Dist-A and its derivatives, we have also reported an NMR study on the interaction of the quadruplex $[d(TGGGGT)]_4$ with Dist-A in Na^+ buffer, and with compounds 1 and 2 in both Na^+ and K^+ buffers.[7] The study is also completed by the ITC analysis of the binding of 1 to $[d(TGGGGT)]_4$ in K^+ buffer. Finally, we also report ITC data about the interaction of Dist-A, 1 and 2 with the human telomeric sequence $d[AG_3(T_2AG_3)_3]$ in both K^+ and Na^+ buffers.

Experiments reveal that distamycin A and compound 1 bind the investigated quadruplexes in both solution conditions, conversely compound 2 appears to have a poor affinity in any case. Moreover, these studies indicate that the presence of different cations in solution affects the stoichiometry and thermodynamics of the interactions. The interaction of quadruplex structures with other new ligands will also be reported in this communication.

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IL-13

G-QUADRUPLEX OLIGONUCLEOTIDES AS THERAPEUTIC AGENTS

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For several years, we have been investigating the mechanism and therapeutic potential of guanine (G)-rich oligodeoxynucleotides as novel treatments for cancer [1-7]. Our collaborative research efforts have led to the development of AS1411 (formerly AGRO100), an unmodified 26-base oligodeoxynucleotide, which has cancer-selective antiproliferative activity against a wide range of malignant cell types. A Phase I clinical trial of AS1411 indicated an absence of serious side effects and evidence of clinical activity, and the drug is now being tested in Phase II studies for the treatment of renal cancer and acute myeloid leukemia (AML).

Our mechanistic studies indicate that AS1411 acts as an aptamer to nucleolin, a highly multifunctional protein that is expressed at high levels in the nucleolus, nucleoplasm, and cytoplasm of cancer cells, as well as on the surface of cancer cells and on angiogenic endothelial cells. Nucleolin was identified as a candidate target for G-rich oligonucleotides (GROs) based on its characteristics, and then binding of endogenous or recombinant nucleolin to GROs was verified using southwestern blotting, western blotting, UV crosslinking, EMSA, and mass spectrometry techniques. Correlations between the ability of GROs to bind nucleolin and their antiproliferative activities suggested a functional role, which was confirmed by experiments showing specific changes in nucleolin in AS1411-treated cells and inhibition of these changes by nucleolin siRNA. Previous research on AS1411 has revealed that the aptamer can interfere with the interactions between nucleolin and its binding partners leading to a number of biological effects, including: (i) inactivation of constitutive and TNFα-induced NF-κB signaling due to cytoplasmic sequestration of a NEMO/nucleolin complex [5]; (ii) expression of tumor suppressor ST7 due to cytoplasmic sequestration of a complex containing nucleolin and PRMT5, a transcriptional repressor [6]; (iii) reduction in levels of the anti-apoptotic Bcl-2 protein due to inhibition of the stabilizing interaction between nucleolin and Bcl-2 mRNA [8]; and (iv) altered nuclear-cytoplasmic distribution of the methylated form of nucleolin [6] and of numerous nucleolin-associated proteins [unpublished]. Interestingly, AS1411 seems to affect only a small proportion of the nucleolin pool [6], which explains why when total nucleolin protein was examined there was little change due to AS1411 treatment. We conclude from these studies that AS1411 affects the trafficking of a subset of nucleolin-containing complexes.

Research from our group and others have suggested that AS1411 is not the only G-rich oligonucleotide with biological activity. Several other G-rich sequences are currently being evaluated as novel treatments for cancer, infectious diseases, and neurological disorders (reviewed in [7]). In general, G-rich oligodeoxynucleotides have been found to have unusual physical and biological properties compared to non-G-rich sequences, including enhanced nuclease-resistance, cellular uptake and aptameric activity. Recently, we have been investigating the use of combinatorial G-rich oligonucleotide libraries as starting points for SELEX, which is an *in vitro* evolution method used to generate target-specific aptamers. Our hypothesis was that such libraries would be enriched in quadruplex-forming sequences, which may lead to fast evolution of aptamers that have useful properties for *in vivo* studies. During the course of these studies, we examined the baseline characteristics of various oligonucleotide libraries and made the unexpected discovery that some G-rich DNA libraries have strong cancer-selective antiproliferative activity, even before SELEX was applied. This intrinsic activity was associated with quadruplex formation, nuclease resistance, efficient cellular uptake and protein binding. Our results indicate that the ability to inhibit the growth of cancer cells, while sparing non-malignant cells, may be a general property of quadruplex-forming oligonucleotides.

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IL-13

G-QUADRUPLEX OLIGONUCLEOTIDES AS THERAPEUTIC AGENTS

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IL-14

LIPOPHILIC G-QUADRUPLEXES AS TRANSMEMBRANE ION TRANSPORTERS

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Synthetic ion channels and pores not only represent models of natural transmembrane ion channels, but also have potential applications in the areas of drug delivery, biosensors, antimicrobial agents and other molecular devices. In this presentation, lipophilic guanosine derivatives that combine both "molecular recognition" and "membrane soluble" features are utilized for the development of synthetic ion channels.

The potential of lipophilic G-quadruplexes to function as synthetic ion channels has been investigated by first tracing the cation exchange process between free cations and G-quadruplex bound cations. Cation exchange between bulk cations (K^+, NH_4^+) in solution and the bound cations in lipophilic G-quadruplexes such as (G **1**)$_{16}$•4Na^+•4DNP^- was first investigated by electrospray ionization mass spectrometry and by ^1H and ^{15}N NMR spectroscopy. The ESI-MS and ^1H NMR data showed that G-quadruplexes containing "mixed cations" formed through a sequential ion exchange process. The use of NMR-"visible" $^{15}NH_4^+$ cations in the NMR titration experiments allowed the determination of two "mixed-cation" intermediates by ^{15}N-filtered ^1H NMR and selective NOE spectroscopy. A "central insertion" pathway was proposed for the cation exchange process from $(G)_{16}$• 4Na^+• 4DNP^- to $(G)_{16}$• 4NH_4^+• 4DNP^-. In the lipophilic G-quadruplex, the "central" Na^+, bound between the 2 symmetry related G_8-Na^+ octamers, is bound less strongly than are the 2 "outer" Na^+ ions sandwiched within the G_8-octamers. These results demonstrated the dynamic nature of the lipophilic G-quadruplex in solution and suggested the design of a ditopic guanosine-sterol conjugate as an approach toward making synthetic ion channels.

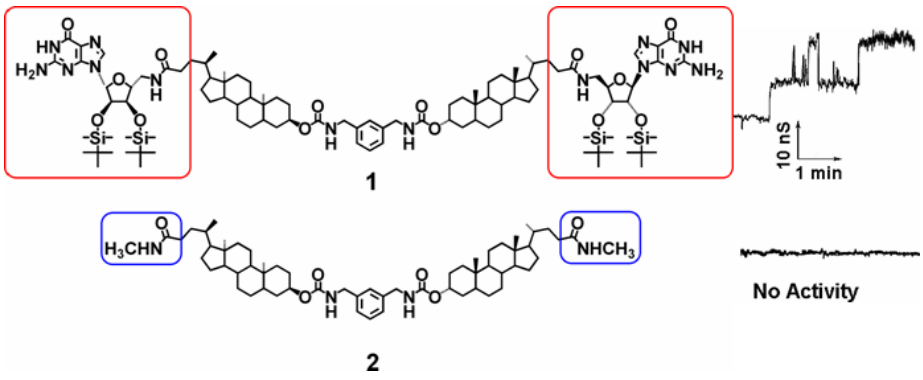

Figure 1. Ditopic G-sterol **1** and control bis-lithocholamide **2**. Typical traces of conductance vs. time, after addition of **1** or **2**.

Guanosine-sterol conjugate **1** was prepared by coupling 2', 3'-bis-TBDMS, 5'-amino guanosine with a bis-lithocholic acid derivative. Voltage clamp experiments demonstrated a series of stable, single ion channel conductances when compound **1** was incorporated into a planar phospholipid membrane. These channels are large, with nanoSiemens conductance values and the channels have relatively long lifetimes, staying "open" for seconds. This feature distinguishes them from most synthetic channels, which typically conduct in the picosiemens range with millisecond lifetimes. Structure-function studies using the bis-lithocholamides **1** and **2** demonstrated that the guanosine moiety plays an essential role in the self-assembly of the trans-membrane ion channel (see **Figure 1**). The sizes of the most prevalent single channels calculated by Hille's equation are much larger than the diameter of a G-quartet, which suggested that the ion transport proceeded through larger pore(s) that form upon

self-assembly of lipophilic guanosine-lithocholate **1** within the phospholipid membrane. The large transmembrane pore(s) could be due to a supramolecular structure that has hydrophobic walls formed by the bis-lithocholate linker and supporting pillars formed by a cation-filled G-quadruplex.

To test this structural hypothesis we used a bis-urea functionality in the bis-lithocholic acid linker to generate a new guanosine-sterol conjugate **3**. The ion channel activity of **3** was demonstrated by voltage clamp experiments. Large ion channels formed from **3** had even longer life-times than those formed from compound **1**. The extra stabilization of self-assembled ion channels may be attributed to the ability of the bis-urea group to partcipate in hydrogen bonding. The stable large transmembrane ion channels self-assembled by lipophilic guanosine derivatives have potential for delivery of drugs or biomolecules. Progress in this area of using lipophilic guanosine analogs as transmembrane transporters will be discussed.

For some recent references to our studies on transmembrane ion channels formed from guanosine analogs, see:

"A Unimolecular G-Quadruplex that Functions as a Synthetic Transmembrane Na+ Transporter." Mark S. Kaucher, William A. Harrell, Jr. and Jeffery T. Davis, *J. Am. Chem. Soc.* **2006**, *128*, 38-39.

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IL-15

CATION LOCALIZATION AND MOVEMENT STUDIED BY NMR

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The formation of G-quadruplex, B-type duplex or nonB-DNA structures within the cell will depend on their relative stabilities. The basic building block of a G-quadruplex is the G•G•G•G quartet, which is composed of four hydrogen-bonded guanine nucleotides in a horizontal planar arrangement. G-quartets are linked together by eight hydrogen bonds in a Hoogsteen pairing geometry. Cations play a major role in stabilization of G-quartets by reducing repulsions amongst guanine carbonyl oxygen atoms. A list of cations that have been shown to promote G-quadruplex formation includes the monovalent cations K^+ and Na^+ as well as Rb^+, Cs^+, NH_4^+, Tl^+ and the divalent cations Sr^{2+}, Ba^{2+}, Pb^{2+} and Ca^{2+}. In addition, cations contribute to enhanced base-base stacking interactions in a G-quadruplex. The coordination of cations by the closely spaced carbonyl oxygen atoms of a G-quartet was postulated long before the first high-resolution structure of a G-quadruplex was determined. G-quartets interact with dehydrated cations via inner sphere coordination. It is therefore not surprising that formation, stability and structural details of G-quadruplexes are dependent on cation species and cation concentration.

Early studies on guanosine gels established a strong correlation between the melting temperature and the ionic radii of cations which was an indication of site-specific ion binding by G-quartets. The strong interaction between cations and G-quartets originates from electrostatic interactions involving the guanine O6 oxygen atoms. On the other hand, electrostatic repulsions between cations within a G-quadruplex are substantial. Thus, the exact locations and coordination geometries of cations within a given G-quadruplex are the result of a balance between attractive interactions with carbonyl oxygen atoms and mutual cation repulsion. A series of stacked G-quartets produces a regular geometry, and potential cation coordination sites, with four O6 atoms within the plane of a G-quartet, or with eight O6 atoms between two stacked G-quartets. Ions such as K^+ and $^{15}NH_4^+$ (ionic radii 1.33 Å and 1.43 Å, respectively) are too large to coordinate in the plane of a G-quartet, whereas Na^+ (ionic radius 0.95 Å) is small enough to be coordinated within the plane of a G-quartet

Ammonium ion has been shown to stabilize G-quartets to an extent that is similar to that observed for Na^+. Hud and Feigon *et al.*[1] used this fact to exploit $^{15}NH_4^+$ ion as a solution-state probe of monovalent cation coordination sites. Our laboratory has furthered insight into localization of cation binding sites and movement of cations along the central axis of the quadruplex of different stoichiometries and topologies.[2-11] NMR studies utilizing $^{15}NH_4^+$ ions as a probe revealed that $^{15}NH_4^+$ ions move between different coordination sites and bulk solution in a manner that is controlled by

structural and other factors.

Our insights into the possible biological roles of G-quadruplexes and into the origins of their topological sensitivity to cation species and concentration have benefited by recent studies on structure, stability and interactions of cations with constituent G-quartets. However, it is not currently possible to delineate general rules governing the folding of G-rich DNA sequences, and more specifically the role of cations in this process. Different G-rich sequences adopt distinct quadruplex topologies and, in addition, a given sequence can also fold into various different conformations, which can coexist. Human telomere sequences represent one of the best illustrations of the structural diversity and complexity of G-quadruplex structures. The change of metal ion species present can induce structural changes, which can be significant for some DNA sequences. The simple change in cation (salt) concentration can result in large changes in structure and stability of a G-quadruplex.

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IL-16

SYNTHESIS AND PROPERTIES OF NOVEL LONG G4-DNA-BASED NANOWIRES

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The DNA molecule has attracted extensive interest over the past decade as a possible candidate for nanoelectronics. The self-assembling properties of DNA, its accurate synthesis, and specific interaction with proteins, guided by recognition are extremely useful for implementing self-assembly in molecular circuits. One of the main challenges with such molecules, however, is the control of their electrical conductivity. The current viewpoint is that native double stranded DNA is an insulator (poor semiconductor) rather than conductor. This prompted us to develop two types of novel continuous G4-nanowires, namely the wires composed of a single long (thousands of bases) self-folded G-strand [1,2] and the wires composed of four parallel poly(G) strands clustered together by avidin [3].

Production of the former monomolecular G-wires includes of the following steps: 1) Polymerase catalyzed synthesis of double stranded DNA composed of long (Kbases) continuous G-strand and short (15-30 base) poly(C) fragment not covalently connected with each other [2]; 2) Separation the G-strand from the C-fragments by size exclusion HPLC at high pH; and 3) Assembling of G-strands into G4-structures by lowering pH. The G4-structures produced using the above method characterized by a narrow length distribution and do not contain nicks along the polymer. These structures are more rigid compared to double stranded DNA, resistant to heat (up to 100^{o}C) and mechanical forces and insensitive to the DNase treatment. In contrast to short G-rich sequences the G4-wires are stable in the absence of "stabilizing" monovalent cations, K^{+} or Na^{+}. The results of AFM imaging of the molecules show that the average contour length of the G4-wires is approximately equal to one fourth of the parent poly(dG)-poly(dC) proving the monomolecular mechanism of the G-strand folding

In addition to monomolecular, G-strands can be assembled into tetra-molecular G4-structures. These structures however, do not spontaneously form in the G-strands solution. At high concentration, required for the tetra-molecular assembly, long (Kbase) G-strands aggregate into bundles comprising of a large number of strands. To overcome this problem and to promote a tetra-molecular G4-DNA assembly, we developed a novel approach based on the avidin-biotin recognition. By using avidin, a tetramer glycoprotein, it is possible to bring together four G-strands, end-labeled with biotin, thus enabling their integration into a parallel tetra-molecular structure. An overall procedure of the tetra-molecular G-wires production includes the following main steps (see Fig.1): 1) Polymerase catalyzed synthesis of double stranded poly(dG)-poly(dC) labeled at the 5' end of the G-strand with a biotin residue; 2) Preparation and HPLC purification of the avidin-5'biotin-Poly(dG)-Poly(dC) conjugates comprising four poly(dG)-poly(dC) molecules attached to the avidin tetramer (see AFM image of the structures in Fig 2, left panel); 3) Separation of the complex of four G-strands connected to the avidin from (dC)-strands by size exclusion HPLC at high pH and 4) Self-assembly of the four strands connected to the avidin into G4-structures by lowering pH of the solution. Similarly to the monomolecular G4-wires**,** the tetra-molecular G4-structures produced using the above procedure are uniform and characterized by a narrow length distribution (see Fig 2, right panel). They are rigid, resistant to heat and mechanical forces even in the absence of K^{+} or Na^{+} and insensitive to the DNase treatment.

The results of AFM imaging of the molecules presented in Fig. 2 show that an average contour length of the G4-wires is approximately equal to that of a parent poly(dG)-poly(dC) proving the tetra-molecular mechanism of the G-strands folding into the G4-quadruplex.

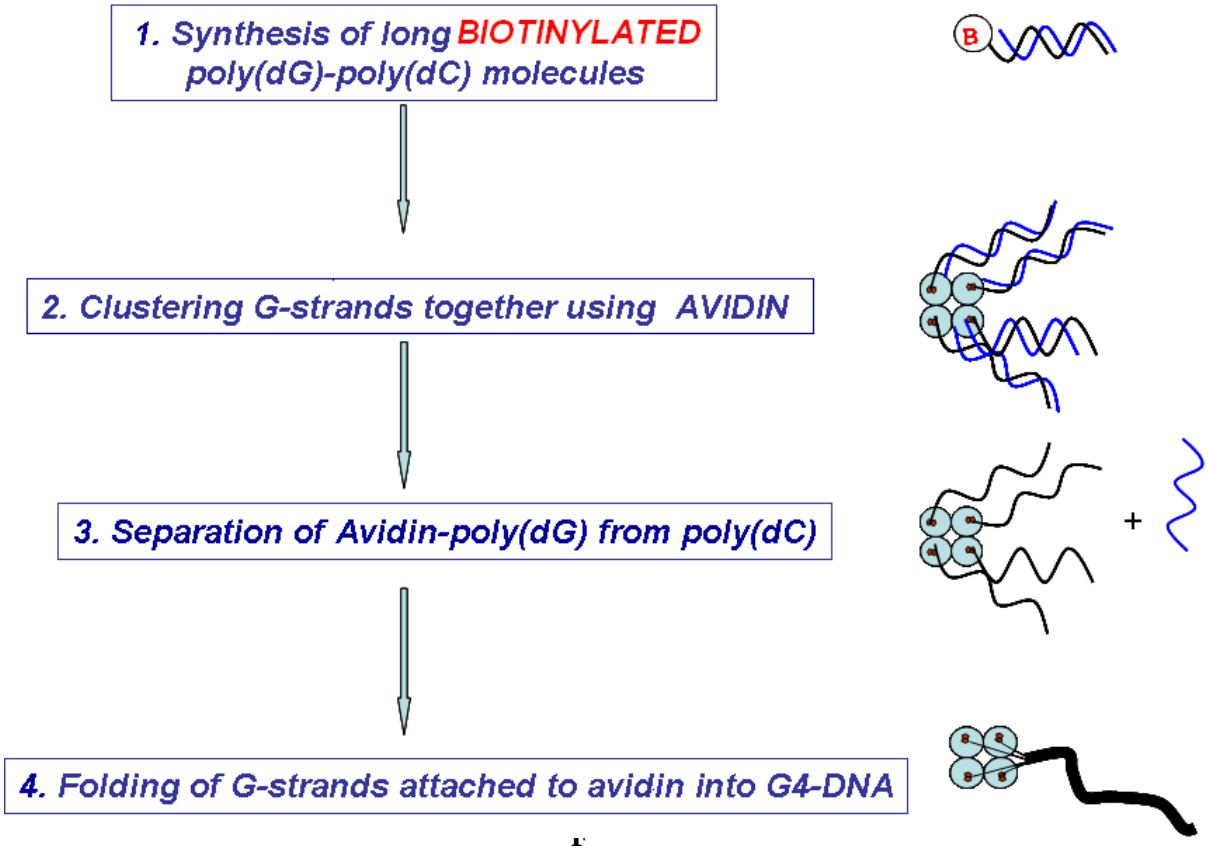

Figure 1. *Schematic representation of a procedure for the preparation of tetra-molecular G4-DNA wires.*

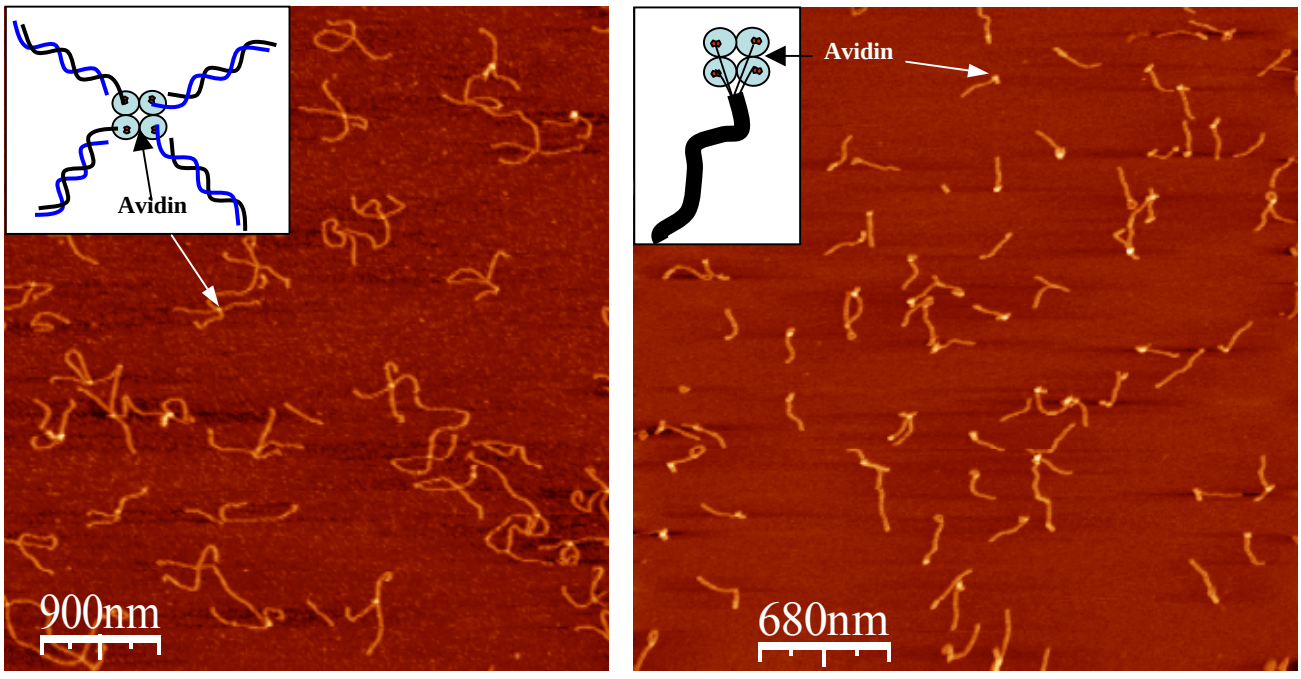

Figure 2. *AFM images of avidin-poly(dG)-poly(dC) (left panel) and avidin-G4-DNA (right panel) complexes. Insets show schematically complexes of corresponding avidin-DNA complexes.*

Both monomolecular and tetra-molecular G4-wires prepared here comprise a large number of stacked guanine tetrads providing better conditions for π overlap compared to base-pairs of the canonical double stranded DNA. A

high content of guanines, which have the lowest ionization potential among DNA bases, also makes charge migration through the DNA highly probable. These properties strongly indicate that the conductivity of G4-DNA is potentially better than that of dsDNA, making G4-DNA a valid alternative to dsDNA to develop DNA-based nano-electronics.

We have also demonstrated that a cationic porphyrin**,** 5,10,15,20-tetra(N-methyl-4-pyridiniumyl)porphyrin **(**TMPyP**)** forms a very stable complex with the wires. DNA [4]. The complex does not significantly dissociate during chromatography and characterized by a high ratio for TMPyP to G4 binding equal to 0.5. We have shown that the mechanism of the porphyrin binding includes intercalation of TMPyP between each pair of successive G-tetrad planes in the G4-DNA. The planar porphyrin molecules are intercalated within G4-DNA (see Fig 3) and stabilize the complex through favorable π-stacked interactions between aromatic residues. A stable complex is also formed between TMPyP and the tetra-molecular G4-wires. The porphyrin to the tetrad ratio in the latter complex is equal to 0.15. This value is much lower than that estimated for the complex of the monomolecular G4-wires with the dye. The π-stacking between the G-tetrads and the porhyrin within the 4G-DNA-porphyrin complexes might induce high conductivity of the wires and thus can provide electrical properties that are suitable for molecular electronics. A metal-free and Zn-porphyrins are photoactive and characterized by long lasting excited triplet state. The triplet state characterized by low redox potential and is capable of abstracting an electron at electrical potentials much lower than the ground state. When complexed with G4-DNA the intercalated porphyrin molecules will carry the current through the polymer at relatively low applied electrical potentials only in the presence of light. This property is essential for development molecular electro-optical devices.

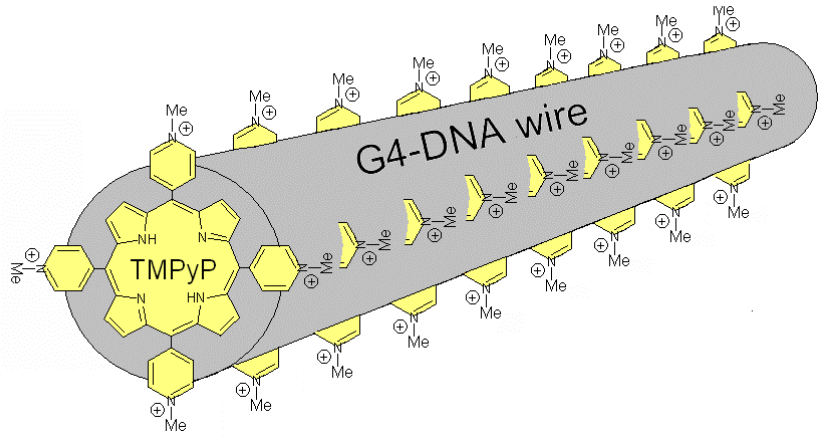

Figure 3. *Schematic presentation of G4-TMPyP nanowire*

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IL-17

GUANOSINE BASED NANODEVICES

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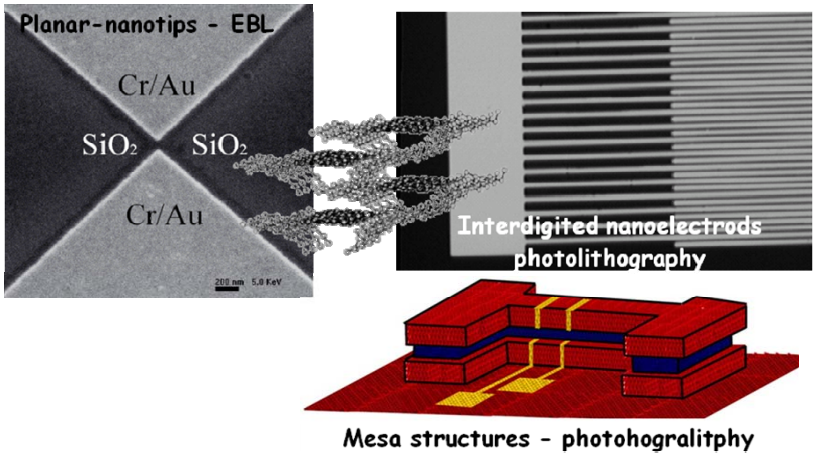
In this talk I will report on nanotechnological strategies to fabricate electronic devices based on guanosines and guanosine-oligothiophenes complexes.

Self assembled mono layers were produced by physisorption or chemisorption by cast deposition and slow solvent evaporation. Guanosine molecules were modified in order to promote their stable chemical anchoring onto gold or silicon surfaces.

Molecular self assembled monolayers were tested by Atomic force microscopy and scanning force spectroscopy in order to check the ordering and aggregation properties of immobilized guanosines. Scanning tunneling spectroscopy in UHV were performed in order to map the electronic configuration and/or modification of guanosines and guanosine-complexes deposited onto conductive surfaces.

On the base of these results both bare molecules and gold nanoparticles covered by guanosine ribbons were produced and trapped inside interdigitated electrodes and mesa junctions for I/V measurements and device performance testing.



High sensitivity electronic devices for guanosine-based molecular electronics

O-01

COMPUTATIONAL AND EXPERIMENTAL ANALYSES OF G-QUADRUPLEX FORMATION, STABILITY, LOCATION AND FUNCTION

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We use computational and experimental techniques to understand the structure and function of G-quadruplex nucleic acids on a whole-genome scale. Using the *quadparser* algorithm,(1) we can approximately predict which genomic sequences are likely to form G-quadruplexes *in vitro*. Using a Markov model, we show that humans and almost all organisms sequenced have many fewer putative G-quadruplex sequences (PQS) than expected. We have previously used this approach to investigate the prevalence of PQS in human gene promoters,(2) and have further developed this analysis to select promising drug targets. We have used a similar analysis method to identify RNA G-quadruplexes that play a role in translation regulation, and perhaps in transcription termination.(3) Human variational studies provide evidence of selective evolutionary pressures acting on G-quadruplex sequences, and we show that in general G-quadruplexes are being lost, consistent with the depletion observed. This implies that G-quadruplexes in general have deleterious functions.

There are currently only limited experimental data to predict the *in vitro* stability of G-quadruplexes, and some rules of thumb that have been developed to approximate the stability. We have developed a Bayesian predictor that can learn the important parameters from a supplied data set, and we can now predict the thermal melting stability of many G-quadruplexes to within 5 °C, and we have developed an active learning programme to improve our predictive accuracy. Our predictor is available online for others to use. (4) We are also collecting new data on more complex G-quadruplex arrangements, so as to improve our predictive ability.

We have also investigated how G-quadruplexes could form in the context of a complementary strand and chromatin, and show that they are found predominantly between nucleosomes(5), and that they are frequently associated with nuclease hypersensitive regions(2) and with regions prone to forming RNA/DNA hybrids.(6)

We developed the website quadruplex.org, which contains a wide range of information and tools for researchers in this field. This includes *inter alia* a listing of all predicted G-quadruplexes in various genomes, and online tools for *de novo* predictions. We would be grateful for any suggestions for further developments.

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O-02

MULTIFOLDING STATES OF 2 PUTATIVE G-QUADRUPLEX SEQUENCES AND EFFECT OF FREE POLYMER ON STABILITY OF I-MOTIFS

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G-quadruplexes may find applications in various fields, such as biotechnology, nanotechnology or biology. Understanding the thermodynamics and kinetics of G-quadruplex formation and their structures is necessary for development of any of these applications. For example, this will enable the development of drugs that target G-quadruplex structures involved in diseases such as cancer. Many studies have been performed on short single stranded DNA sequences, showing the effect of salt concentration, strand concentration, pH or loop length, but few have been done on long oligonucleotides, where more than one G-quadruplex may form. There is currently some controversy as to the effects of having multiple G-quadruplexes; do they interact with each other or are they independent? If they interact, do they mutually stabilize or destabilize each other? We have used biophysical techniques to study long sequences and resolve these questions in general.

We have particular studied sequences with 2 possible G-quadruplexes with 8 runs of GGG and constant loops. We investigate the effect of the number of bases separating the 2 putative G-quadruplexes, and find a strong dependence on the separation of the two possible G-quadruplexes. When the two are very close together, we find two distinct transitions by spectroscopic thermal denaturation, showing that the structure interact and that the transition is a complex process with at least two reaction intermediates. We have also used CD melting, mutation and gel electrophoresis to study the details of the structures formed, and we will present a model of the folding equilibriums for this oligomer. For slightly larger separations, we find that the stability decreases, but then stabilizes at longer separations, suggesting that any stacking interaction is small, and the structure is akin to beads on a string. This agrees with previous thermodynamics studies on varying number of GGG or GGGG runs [1, 2].

We have also investigated the properties of the C-rich strands complementary to G-quadruplex forming regions. We find that their stability depends strongly on the lengths and composition of the loops, but following different rules to G-quadruplex stabilities. We also find a strong pH dependence, as expected from the requirement to form hemiprotonated cytosine•cytosine bonds. Nonetheless, we find that with careful sequence selection we can find reasonably stable i-motifs at pH 7.4, and we further show that under appropriate molecular crowding conditions, they can be very stable under physiological conditions.

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O-03

CONTROLLING GUANOSINE ASSEMBLY WITH SYNTHETIC RECEPTORS

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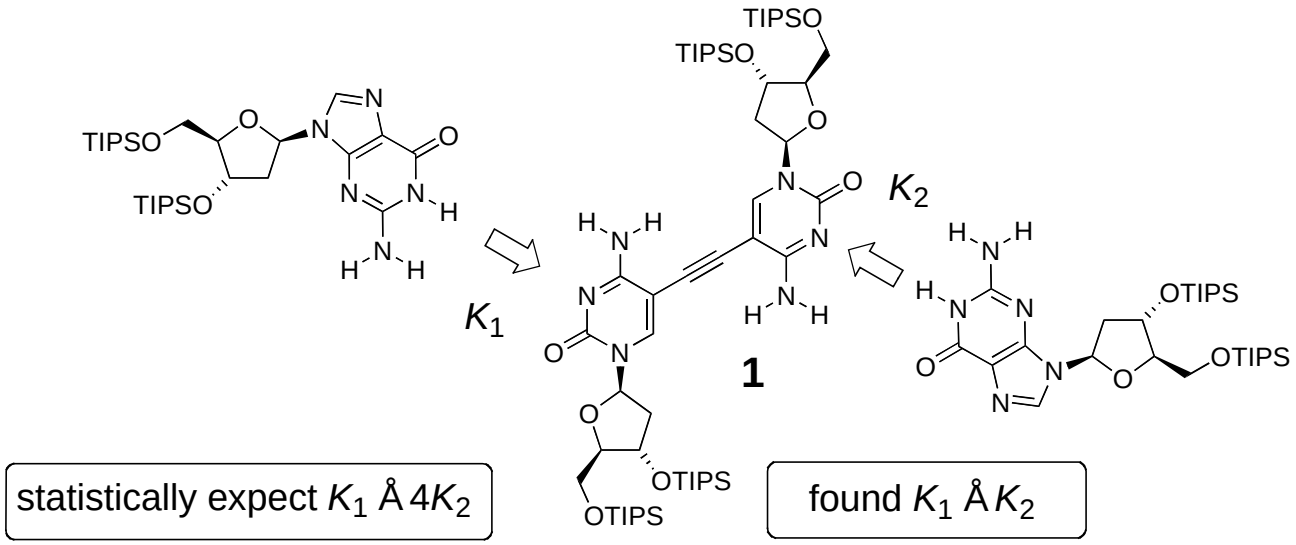
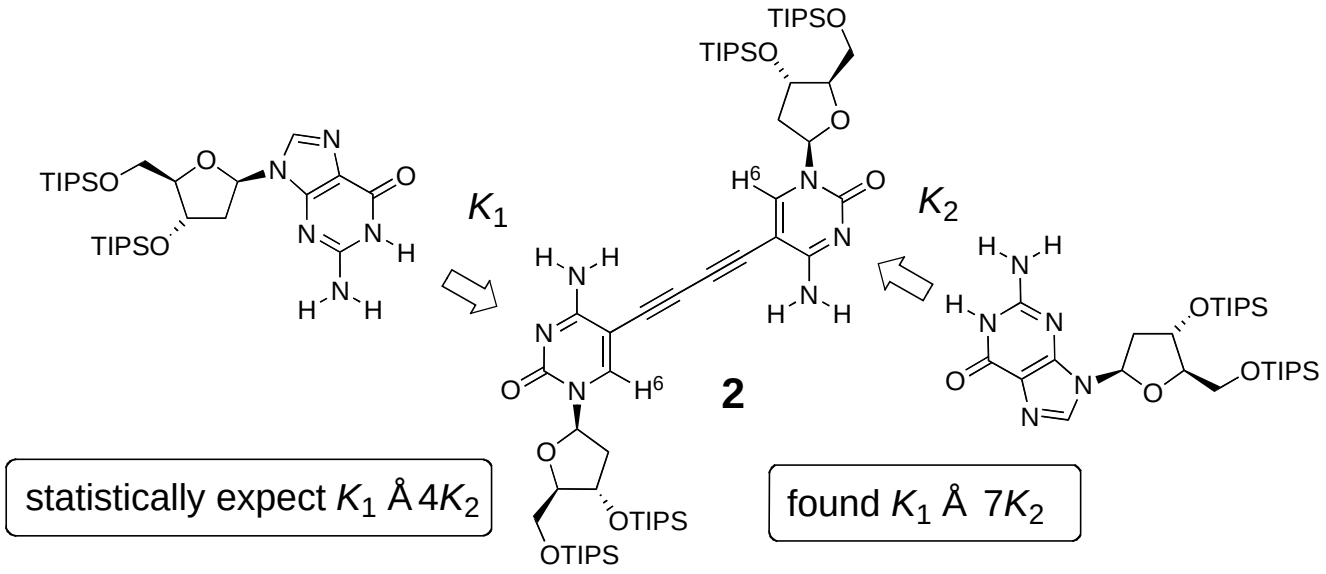
We probe the self-assembly of ditopic receptors **1** and **2** for guanosine using NMR titration, DOSY NMR spectroscopy, isothermal titration calorimetry. In only case **1** where steric effects within the receptor complex are possible, do we observe greater than statistical binding of guanosine guest.



Using a receptor wherein the two symmetric binding sites are further apart, **2**, a closer to statistical ratio of product complexes is seen.



The implications for 'cooperative' or non-statistical binding events are discussed. We conclude that one strategy for selective control of guanosine aggregation is the use of a receptor able to transmit the presence of a guest from one binding site to the next.

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O-04

G-QUADRUPLEX IN THE PROXIMAL PROMOTER OF BASIC FIBROBLAST GROWTH FACTOR

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The human fibroblast growth factor 2 gene (FGF2) codes for a protein involved in broad angiogenic, mitogenic, and neurotrophic activities. We will present evidence indicating that a double helical region intrinsic to its proximal promoter, that appears conserved across species, has innate ability to form a parallel G-quadruplex. We will show that the topological conversion is driven by potassium cation concentration, and crowding conditions. This results reinforces the thesis indicating that the transient nature of both cation concentration, as well as crowding conditions, can act as contributors to potential regulatory function of quadruplexes in biological systems.

O-05

TIME-RESOLVED STUDIES OF GUANINE QUADRUPLEXES

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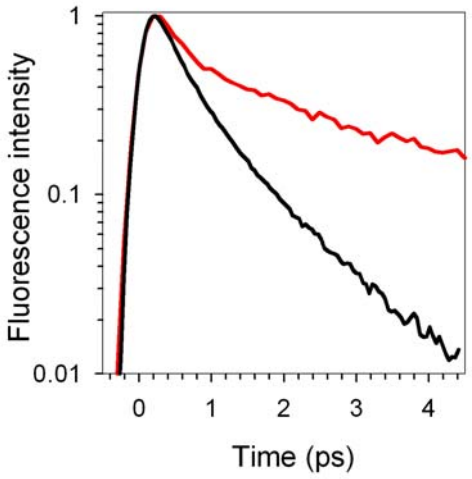
DNA bases show ultrashort fluorescence lifetimes (<1 ps) upon excitation by UV light, which is explained by efficient non-radiative relaxation of their singlet excited states to the ground state [1]. Nevertheless, the organization of bases in DNA strongly influences the nature of the excited states as shown in the case of model helices containing only A:T or G:C pairs [2,3]. Association of guanine rich sequences to a quadruplex structure is expected to a have similar effect on the excited state properties.

Here we report steady-state and time-resolved optical spectroscopic study using femtosecond fluorescence upconversion of G-quadruplexes formed by four oligonucleotides (TG_4T). A comparison between single-stranded and four-stranded structures and the corresponding stoichiometric mixture of non-interacting nucleotides shows how horizontal and vertical organization affects the properties of the excited states. Emission from guanine excimers is observed only for single strands, where conformational motions favor their formation. Fluorescence of the quadruplex, whose quantum yield is 3×10^{-4}, arises from a multitude of excited states generated via electronic coupling between guanines; the average fluorescence lifetime is longer and the fluorescence quantum yield higher compared to those of non-interacting nucleotides. The fluorescence anisotropy recorded on the sub-picosecond time-scale, where molecular motions are hindered, reveal that energy transfer takes place among the bases composing the nanostructure. These results are in line with the conclusions drawn from similar studies on model DNA duplexes.



Fluorescence decay of the four stranded TG_4T (red) and that of the stoichiometric mixture of non-interacting dGMP and TMP (black) observed at 360 nm upon excitation at 267 nm.

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O-06

PROBING SELF-ASSEMBLY AND HYDROGEN BONDING IN DEOXYGUANOSINE DERIVATIVES USING HIGH-RESOLUTION SOLID-STATE NMR TECHNIQUES

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A recent publication has highlighted the unique insight offered by solid-state NMR into structure determining interactions by directly probing intermolecular hydrogen bonds in organic materials [1]. Similarly, solid-state NMR has been shown to identify distinct NH---H bonding arrangements which direct the supramolecular assembly of lipophilic guanosine derivatives in the solid state [2], supporting previous investigations of these materials within hydrocarbon solvents [3]. Specifically, it was shown that changes in the alkyl substituents resulted in different modes of assembly, namely the classical G quartet-like assembly or a 'ribbon'-like structure (the latter also having been demonstrated by single-crystal X-ray diffraction).

The current study now examines a variety of guanosine derivatives using advanced high-resolution 1H and 13C solid-state NMR methods, in combination with limited crystal structure information and DFT calculations. The ability of NMR to recognise crystallographic variations of these compounds at natural abundance is demonstrated. Moreover, 1H double-quantum (DQ) CRAMPS (combined rotation and multiple-pulse spectroscopy) techniques [4] are exploited in order to obtain structural information about proton-proton proximities and hence determine the differing supramolecular arrangements of the various derivatives.

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O-07

COMPARATIVE STUDY OF SURFACE ORGANIZATION OF AMPHIPHILIC GUANOSINE DERIVATIVES WITH LANGMUIR-BLODGETT TECHNIQUE AND ATOMIC FORCE MICROSCOPY

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Self-assembly is an intrinsic property of DNA nucleosides governed by a variety of interactions such as hydrophobic, van der Waals, ion-dipole and hydrogen bonding. Investigations focused on precise control of nucleoside self-assembly represent a prerequisite step to development of complex supramolecular architectures and nanostructures. Molecular organization can be fine-tuned by slight modifications of the molecular structure. We present a comparative method for investigation of amphiphilic guanosine based derivatives. Using Langmuir-Blodgett technique and Atomic Force Microscopy, surface films of three types of guanosine amphiphiles with one, two and three decane chains were studied. Surface pressure versus area isotherms of Langmuir films display strong correlation with theory and provide information of compressibility of such layers. Langmuir-Blodgett films on cleaved mica substrate were scanned with Atomic Force Microscope to draw parallels with the isotherms. The obtained information is useful for construction of nanoscale based electronic devices that depend on molecular organization.

The study describes the molecular organization in surface films on liquid and solid substrates for different amphiphilic guanosine derivatives. It connects the nanoscopic organization of molecules and molecular clusters in vitro with a macroscopic observable surface pressure. Guanosine amphiphiles with one, two and three decanoyl chains were observed at different conditions of self assembly on surface area. To supplement the understanding of molecular ordering, the deposited thin films were evaluated under the scanning atomic force microscope. The homogeneity and overall height of each film is discussed. The nanoscopic and macroscopic results were evaluated within the theory of molecular geometry and assembly. The results show that the derivatives with less lipophilic chains have closer packing on surfaces than those with more. Molecular geometry plays an important role in the packing process and hence for dictates the macroscopic structural organization.

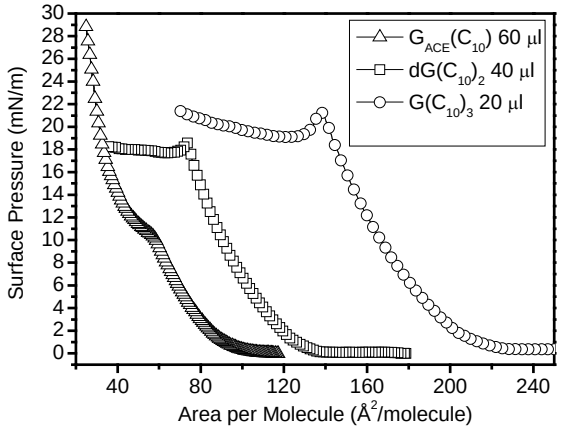

Fig 1. Surface pressure versus area isotherms for $G_{ACE}(C_{10})$, $dG(C_{10})_2$ and $G(C_{10})_3$.

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O-08

ADVENTURES IN SUPRAMOLECULAR SPACE USING 8-ARYLGUANINE DERIVATIVES

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The bottom-up approach to the construction of supramolecular nanostructures requires the availability of recognition motifs that are easy to synthesize and self-assemble with good selectivity and fidelity. The guanine base (G) stands out as an excellent candidate for such motif since it can form tetrameric structures that self-assemble in the presence of a variety of cations to form higher ordered structures known as G-quadruplexes. Our strategy for nanoconstruction relies on the use of recognition motifs made from 8-arylguanine derivatives (8ArGs) that are relatively easy to make and offer robust and reliable self-recognition properties in both organic[1] and aqueous media[2]. Our results indicate that the properties of the resulting supramolecular assemblies can be modulated by parameters that are intrinsic (i.e. structural features) or extrinsic (environmental conditions such as solvent,[3] temperature, cation template, etc.) to the 8ArGs. The use of intrinsic parameters to drive the formation enables the reliable construction of a desired assembly with relative independence from the environmental conditions.[2] Modulation of extrinsic parameters, on the other hand, enable the elaboration of responsive systems that switch their state as a function of external stimuli.[3] The usefulness of these 8ArGs will be highlighted by their use in the development of supramolecular 'soft' nanostructures like self-assembled dendrimers[4] and self-assembled ligands.

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O-09

CHAPERONE ACTIVITY OF CATIONIC COMB-TYPE COPOLYMERS FOR INTERMOLECULAR DNA QUADRUPLEX

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Introduction G-quadruplex structures are an attractive component for the elaboration of nanomolecular machines due to their self-assembly properties. The intermolecular quadruplex structures composed of short DNA oligomers, are particularly interesting because of their thermodynamic stability and robustness. However, their folding and unfolding kinetics is extremely slow[1], hampering with its application to smart nanodevices.

We have been interested in IPECs composed of comb-type copolymers having a polyelectrolyte backbone and abundant hydrophilic graft chains as one component. The copolymer comprised of more than 80 wt% hydrophilic graft chains and less than 20 wt% cationic backbone gives the totally soluble IPEC with DNA in which DNA condensation, coil-globule transition, is suppressed. We showed that the copolymer considerably increased association rates and stability of DNA hybrids including double helical and triple helical DNAs. In this study, we studied the effect of PLL-g-Dex on the kinetics of quadruplex formation. We showed that PLL-*g*-Dex accelerated both the association and dissociation of tetramolecular quadruplex

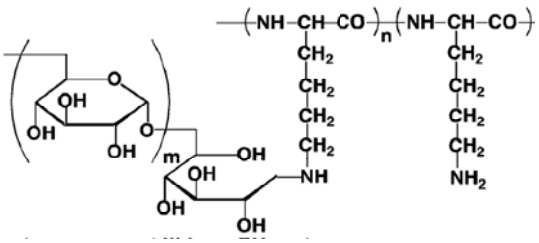

Fig. 1 Structural formula of PLL-*g*-Dex. (Mw of PLL backbone: 5.3×10^4, Mw of dextran grafts: 8.4×10^3, drafting degree: 16.2 mole%)

Result and Discussion The effect of PLL-*g*-Dex on the association of the intermolecular quadruplex was traced by circular dichromic spectroscopy and was plotted against the N/P ratio. In the absence of the copolymer, k_{on} value of 3.1×10^8 $M^{-3}sec^{-1}$ at 4°C was obtained. In the presence of the copolymer, the k_{on} value increased with increasing N/P ratio and reached a plateau at N/P = 2. At N/P = 2, the copolymer accelerated the quadruplex folding by three-order. We then evaluated temperature dependency of the quadruplex folding. The k_{on} values decreased with increasing temperature in the absence of the copolymer. The negative temperature dependency was considered to be owing to a nucleation-zipping pathway[2]. The negative temperature dependency was also observed in the presence of the copolymer, suggesting that the quadruplex folding took place through the nucleation-zipping pathway even in the presence of the copolymer.

Next, we evaluated the effect of the copolymer on the dissociation of the intermolecular quadruplex. In the absence of the copolymer, the first-order dissociation rate constant of 1.7×10^3 sec^{-1} at 60°C was obtained. Of interest, the copolymer increased the dissociation rate with N/P ratio dependent manner. The copolymer, at N/P = 2, increased the dissociation rate by 6-fold. No significant difference in the activation energies was found. Note that the copolymer increased both the association and dissociation rates of quadruplex, suggesting a nucleic acid chaperoning acticity that reduces the energy barriers need for both the dissociation and association of folding.

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O-09

TEMPLATE ASSISTED SYNTHESIS OF G-QUADRUPLEX (TASQ)

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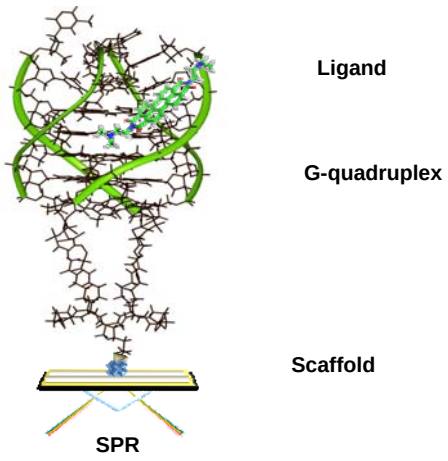
A large number of molecules are known to interact with DNA and have thus been used for various applications. In this context, the design of G-quadruplex ligands has received a great attention because these DNA conformations represent a valuable biological target. Indeed, these structures have been found to play an important role in many relevant biological processes such as telomere stabilization, oncogene activation, and regulation of the immunoglobulin switch region. However, a wide variety of topologies can be adopted by the G-quadruplex depending on the number of strands involved in the structure, the strand direction, as well as variations in loop size and sequence. Obviously, this can confuse the study of recognition phenomena with potential ligands.

We recently described the use of a peptidic scaffold as a topological template that can direct the intramolecular assembly of covalently attached oligonucleotides into a parallel G-quadruplex (see opposite). We demonstrated that the use of the scaffold allows the formation of the quadruplex motif even without the addition of cations and dramatically incrases the stability of the motif. We also showed that this molecular system can be immobilized on surface for studying by SPR (Surface Plasmon Resonance) the interactions with small organic molecules.[1] This latter result represents the first example of immobilization of parallel G-quadruplex motif for SPR studies.



The natural human intramolecular quadruplex and a duplex have been also anchored on this peptidic scaffold. In this manner by using SPR, we have investigated the interactions of various ligands with these different nucleic acid conformations (antiparallel G-quadruplex, parallel G-quadruplex and duplex). Interestingly, the various studied ligands have shown differences of behavior that could be correlated with the binding mode of the ligand. Indeed, discrimination between intercalators and groove binders can be evaluated. Thus, this novel SPR-based approach using the peptide scaffold is of interest for the screening of G_4-DNA ligands, providing key information on their affinity, selectivity and binding mode. Furthermore, this strategy could be used for the formation of other particular conformation of DNA (antiparallel G-quadruplex, *i*-motifs...).

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O-11

THE DESIGN OF QUADRUPLEX TOPOLOGIES WITH HIGH THERMODYNAMIC STABILITY

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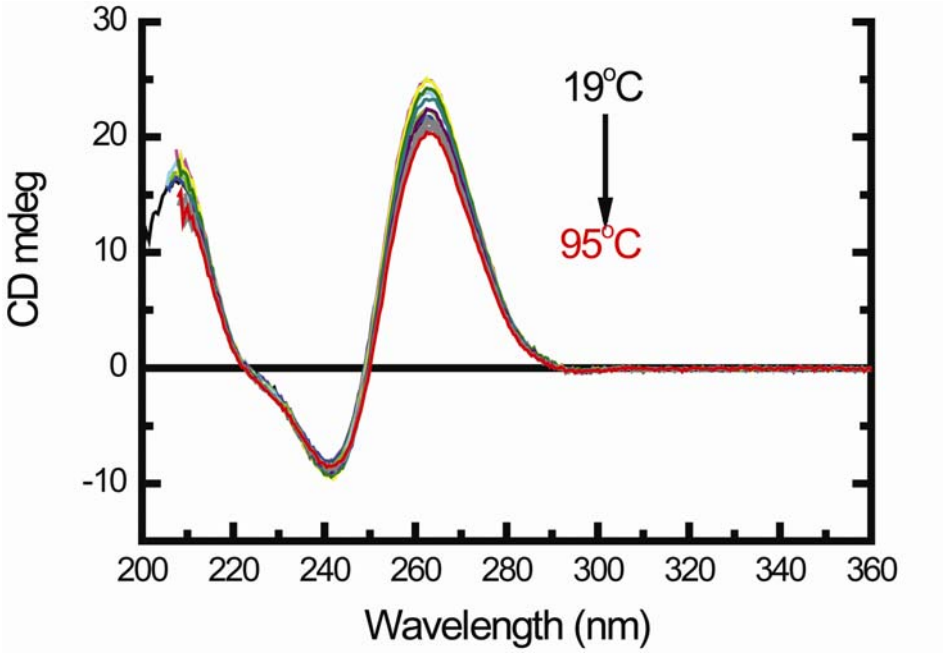
The unique thermodynamic properties of quadruplexes make them good candidates for roles as biomaterials and biotechnological tools. In order to realise some of this potential quadruplexes may be designed to be resistant to high temperatures and have unique and predictable topologies. We have endeavoured to design quadruplex topologies characterised by high thermodynamic stability. Using a DNA sequence shorter than 25 nucleotides, we succeeded in designing a topology that melts above the boiling point of normal physiological aqueous solutions. We probed its formation utilizing Laser Induced Liquid Bead Ion Desorption (LILBID) mass spectrometry and HPLC to show stoichiometry of folded oligonucleotides, and UV and CD studies, both to show quadruplex formation and their heat resistance.



O-12

SHEDDING LIGHT ON THE INTERACTION BETWEEN TMPYP4 AND HUMAN TELOMERIC QUADRUPLEXES

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G-quadruplex structures can inhibit the activity of telomerase, the enzyme that adds the telomeric repeats to the ends of chromosomes favoring the proliferation of cancer cells (1). Inhibition of the telomerase could stop tumor growth, thus, small-molecules capable of interfere with telomere maintenance inducing the formation of quadruplex structures and/or binding them, are considered as potential anti-cancer agents (2).

Among all, cationic porphyrins have emerged as a promising class of quadruplex-binding molecules with potential applications as anti-tumor therapeutic agents (3,4). Particularly, the cationic *meso*-tetrakis-(*N*-methyl-4-pyridyl)-porphyrin (TMPyP4) has been the subject of extensive investigations.

The binding mode and stoichiometry of the TMPyP4 cationic porphyrin to G-quadruplex structures have been controversial and they are not so clear so far, especially for the intramolecular G-quadruplexes from the human telomeric sequence. We have performed, by the use of isothermal titration calorimetry and circular dichroism, a systematic study to characterize the binding reaction between TMPyP4 and four G-quadruplexes formed by different truncations of human telomeric DNA, with 5'- or 3'-flanking bases. The results clearly indicate that all the studied G-quadruplexes are able to bind up to four TMPyP4 molecules, while two out of the four sequences show a ten-fold higher affinity for the porphyrin molecule. Circular dichroism studies showed that interaction of the TmPyP4 promotes the conversion of the hybrid structures to an antiparallel conformation. ITC results reveal that the binding process is composed of two sequential events, a first event where one molecule of TMPyP4 interacts with the quadruplex structures, and a second one where other three molecules bind to the structures.

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O-13

NOVEL G-QUADRUPLEX-BASED APTAMERS AS POTENTIAL ANTI-HIV AGENTS

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Recently, the synthesis and characterization of G-rich oligonucleotides as potential anti-HIV agents has emerged as an extremely interesting and active research area. In the course of our studies on modified and/or conjugated oligonucleotides, the sequence TGGGAG, found to be active at submicromolar conc. if 5'-conjugated with large aromatic groups, was taken as a model for further synthetic elaborations. Novel mono- and bis-conjugated oligonucleotides carrying this sequence have been synthesized and characterized in order to elucidate their structure-activity relationships. From a complete biophysical characterization of the resulting tetramolecular G-quadruplex structures, the role of the 5'-substituents was defined, proving that the kinetically and thermodynamically favored formation of G-quadruplex complexes is a pre-requisite for the *in vivo* antiviral activity (*1*,*2*).

Current efforts are aimed at developing mini-libraries of novel aptamers, able to fold into stable G-quadruplex complexes, as potential antiviral agents (*3*). Particularly, mini-libraries of G-rich oligonucleotides, derivatized at the 5'-end with various large aromatic groups, and at the 3'-end with biocompatible hydrophilic tethers (*e.g.* polyethylene glycol chains or mono-, di- or oligosaccharides), have been prepared aiming at enhanced pharmacokinetic profile. For the realization of these mini-libraries we have adopted synthetic protocols - recently developed in our laboratories – allowing 5',3'-bis-conjugated oligomers following a straightforward, versatile and efficient on-line solid phase approach, in which the conjugating agents are connected to the sugar-phosphate backbone through stable phosphodiester linkages (*4*). The rational search for tailored conjugations to be inserted into the model oligonucleotide platform will be then coupled to a detailed biophysical characterization and biological evaluation of the synthesized compounds, thus furnishing a clear picture for a further drug optimization process.

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O-14

A COMBINED APPROACH TO STUDY THE AFFINITY OF THE ANTITUMORAL DRUGS DOXORUBICIN AND MEN 10755 FOR THE G-QUADRUPLEX STRUCTURE OF HUMAN TELOMERIC DNA

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During the last decade it has become evident that the G-quadruplex structure may play a crucial role in many biological events. Human telomeres consist of repeats of the guanine-rich sequence 5'-d(GGGTTA)-3' capable to form the G-quadruplex. During cell replication telomere shortening is one of the factors accounting for cell senescence. A telomere maintenance mechanism is mediated by the protein telomerase. Overexpression of this protein has been observed for rapidly replicating tumor cells guaranteeing their "immortality". The G-quadruplex structure seems to play a significant role in this mechanism. Moreover, guanine-rich sequences are also found in promoter regions of many oncogenic genes, and transcription of these genes passes through the recognition of the guanine-rich sequence and its presence or absence as the G-quadruplex. For these reasons guanine-rich sequences have become a very attractive target for the development of new antitumoral strategies. In this frame we started to study the binding of some anthracycline-based antitumoral drugs to guanine-rich sequences. It is known that some drugs of this family target DNA and form complexes with the duplexes via intercalation of the aromatic part between the stacked bases. Up to now data on the affinity of the anthracycline drugs for the G-quadruplex structure are missing. We used an approach that combines various techniques, mainly spectroscopic, to obtain information on the binding process, already applied successfully to the study of the interaction of other drugs to serum albumins. In the case of doxorubicin and Men10755 we found that binding occurs to the G-quadruplex of the telomeric sequence 5'-dGGG$(TTAGGG)_3$-3' and two complexes of 1:1 and 2:1 (drug/G4) stoichiometry coexist in solution. Further complexation of the G-quadruplex caused an increase of the melting temperature of the structure of ca. 6°C.

O-15

ELECTRONIC STRUCTURE OF DNA-MODIFIED ASSEMBLIES COMPLEXATED WITH TRANSITION METAL IONS

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M-DNA is a type of metalated DNA obtained from the complexation of one metal ion per base pair. Experiments carried-out on Zn-DNA suggested a metallic behavior, with an insulator-metal transition from the natural DNA.

In our work, we inspected if and how the possible electronic coupling between the metal and the nucleic acid bases may be responsible for such a transition [1]. The effect of other metals such as Cu and Ag [1], is also taken into account to inspect which metal elements are most suitable to perturb the electronic structure of DNA in a desired way.

An alternative way to go beyond the limits of native-DNA for nanotechnology impact is to design and syntesize more conductive structures by chemical modifications of the nucleobases. One particular approach relies on the expansion of each natural base with a benzene ring that is covalently bonded to the base and co-planar with it: xDNA [2]. Recent findings reported enhanced stacking interactions in xDNA duplexes and suggested they might be suitable molecular-wires.

Here we investigate possible ways of tailoring the DNA electronic structure by combining the metal-doping and aromatic insertion, namely M-DNA and M-xDNA. DFT calculations of the electronic structure and stability properties of salient selected structures, are performed with several localized basis sets, different exchange-correlation functionals, with and without implicit solvent.

Results indicate that Cu-modified GC and xGC complexes are the most promising candidates for nanowires with enhanced electron transfer and also for on-purpose modification of DNA double-helix for signal detection.

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O-16

G-QUADRUPLEX SELF-ASSEMBLY REGULATED BY COULOMBIC INTERACTIONS

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The importance and complexity of self-assembly in biological systems, such as the structure of viruses, the recognition of small molecules by proteins, or the polymorphism of DNA, represent a continuous inspiration to scientists in their quest for the construction of nanostructures with a well-defined size and shape. If such nanoobjects are built from molecules with a particular function, we can expect unprecedented properties arising from cooperative interactions between the organized molecules that may be of use in several applied fields, ranging from optoelectronics to nanomedicine. Controlling the size and the structure of self-assembled systems is, however, not always easy to achieve. For such a goal, one must be able to modulate the interplay between multiple noncovalent interactions so that they work in concert to impart stability to the assemblies and, at the same time, limit their growth to a certain extent.

In our work, we profited from the singular characteristics of guanosine (G) self-assembly into G-quadruplexes in the presence of alkaline salts.[1] The G-quadruplex is a biologically relevant architecture that plays a fundamental role in telomeric DNA during cell replication. G-quadruplex formation is templated by alkaline metal ions (usually sodium or potassium) that are strongly complexed within the cavities formed between hydrogen-bonded cyclic G-tetramers. The counteranion, on the other hand, is left out of the quadruplex structure, exposed to the surrounding media. The noncovalent forces that cooperatively hold the G molecules together (i.e. π–π stacking, hydrogen-bonding and cation-dipole interactions) give an extraordinary stability to these assemblies that, in most cases, tend to form long polymeric fibers.[2]

However, it is well-known that, working in organic solvents with lipophilic G derivatives, G-quadruplex growth can be in some cases restricted to the formation of discrete objects, assembled from a well-defined number of guanosine molecules. In our work,[3] we have managed to achieve an exceptional size control of the complexes, via the modulation of a thermodynamically unfavorable component in the overall self-assembly process: the Coulombic interactions between the complexed cation and the "free" anion. Coulomb's law states that the energy of such interactions is inversely proportional to the solvent dielectric constant and the distance between charges. In fact, we show that some factors, such as the solvent polarity, the nature of the anion, or the cation-anion distance, have a decisive role in the growth of lipophilic G-quadruplexes. Therefore, by adjustment of these parameters, we have been able to construct, selectively and quantitatively, assemblies comprising 8, 12, 16, or even 24 guanosine molecules.[3]

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O-17

DIELECTROPHORETIC TRAPPING AND ELECTRICAL CONDUCTIVITY OF DNA ORIGAMI STRUCTURES

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O-18

A CONTRACTILE ELECTRICAL SWITCH MADE OF DNA

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Double-helical DNA has been shown to conduct both electrons and electron holes, the latter over distances of >200 Å. DNA is thus a material of significant interest for the bottom-up construction of nano-circuitry. Although a small number of DNA-based devices have been reported, DNA structures capable of serving as general-purpose electrical junctions have proved elusive. Here, we report the construction of a contractile DNA nano-switch for electron hole conduction, one which is "off" while in a structurally extended state and "on" in a "contracted" or "pinched" state. To achieve its "on" state, two stretches of guanine-guanine mismatch motifs placed within a double helix synapse together to form a hole-conductive G-quadruplex, bypassing an insulating DNA element sandwiched between them. This switch can be turned on an off by sequential treatment with quadruplex-promoting cations such as K^+ or Sr^{2+}, and by chelation of these cations. This contractile nano-switch defines a prototype for the construction of increasingly sophisticated electrical devices made of DNA.

O-19

BINARY GUANOSINE GELS FOR SCALABLE DISPERSION AND ALIGNMENT OF SINGLE WALLED CARBON NANOTUNES

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The unique properties of single walled carbon nanotubes (SWNT) including mechanical strength, electron mobility and nanoscale dimensions make them excellent candidates for use in conductive films, sensors and transistors. Efforts to realize their commercial potential have been impeded by the lack of convenient, economical methods for mass production of individually dispersed and aligned tubes. Here we present an easy, cost effective and scalable method for solubilization of SWNTs that results in their alignment without any assistance. The method employs a gel medium that is formed through reversible self-association of a binary mixture of guanosine compounds in aqueous solution [1]. These materials exhibit unique thermoresponsiveness and tunability as a function of the ratio and total concentration of the guanosine compounds, cation content, buffer and pH. The gels readily solubilize high concentrations of SWNTs with little or no sonication to form solutions of individually dispersed and in some cases aligned SWNTs that are stable for months and even years. By changing the composition of the gel, we can tune the selectivity towards SWNTs of different chirality or diameter. The gels can also be used to selectively enrich SWNTs as a function of their electrical properties.

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O-20

ELECTROCHEMICAL ATOMIC FORCE MICROSCOPY CHARACTERISATION OF GUANOSINE AND GUANINE SELF-ASSEMBLIES ONTO A HOPG ELECTRODE SURFACE

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The interaction mechanism of guanosine and guanine with the surface of a highly oriented pyrolytic graphite (HOPG) electrode was studied using atomic force microscopy (AFM) in an electrochemically controlled environment. Both guanosine and guanine spontaneously self-assemble onto the HOPG electrode, forming stable thick multilayer films with similar structure and morphological characteristics, which cover the surface uniformly and almost completely. The molecules adsorb onto HOPG in a horizontal position, with the aromatic rings parallel to the surface, establishing hydrophobic interactions between the hydrophobic nitrogen-containing rings and the hydrophobic HOPG. Additionally, the stability of the films is enhanced by multiple hydrogen bonding interactions between adjacent guanine moieties. The process of adsorption of guanosine and guanine can be controlled by adjusting the potential of the HOPG electrode; the molecules are arranged on the surface of HOPG in a thick molecular film, but less compact and uniform, with a morphology resembling polymeric structures. The electrochemical deposition of guanine leads to guanine adsorption, together with dimers, trimers and other oxidation products. The dissolution of the multilayer films formed by electrochemical assisted adsorption is achieved in only one potential cycle between 0 and +1.3 V. However, re-adsorption of guanine molecules to form a new film immediately occurs.

O-21

SELF-ASSEMBLY PROCESS OF GUANOSINE 5´-MONOPHOSPHATE ON THE SURFACE

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Controlled deposition of biomolecules on various surfaces is essential for manufacturing specific surface architectures for broad range of applications in nanoscale molecular electronics. Promising candidates for such application are Guanosine molecule and its derivatives. They are known to have the property to self-assemble under specific conditions into highly ordered structures, such as quartets and wires [1].

We made comparative analysis of surface self-assembly of di-sodium and di-ammonium salt of Guanosine 5´-monophosphate (5´-GMP). The selected compound was first dissolved in pure distilled water at several mass concentrations of the solution (0.02wt%, 0.2wt% and 2wt%) [2]. Afterwards the material was deposited from solution on two different solid substrates (Au(111) and muscovite mica) and the developed surface structures were investigated by Atomic Force Microscopy (AFM) in combination with Sum-frequency generation (SFG) spectroscopy. SFG spectroscopy is a second order non-linear optical technique particularly sensitive to surfaces and interfaces. We recorded SFG spectra for different polarization combinations in the range of C-H vibrations (2800-3100 cm^{-1}). For concentrations of 0.02wt% and 0.2wt% the SFG and AFM results indicate formation of similar surface structures for both types of the GMP salts. On contrary to this, at higher concentrations (c = 2wt%) the SFG spectra are profoundly different. We substantiated that the SFG signal at high concentrations originates from the bulk and not from the surface. Therefore the discrepancy of the SFG signals from the two types of the GMP salts originates from very different bulk organization of the adsorbed films [3].

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P-01

SYNTHESIS AND SUPRAMOLECOLAR CHARACTERISATION OF N9-SUBSTITUTED GUANINES

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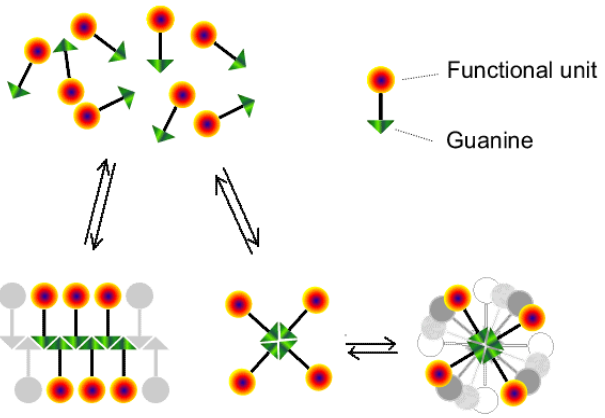
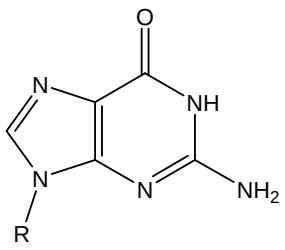
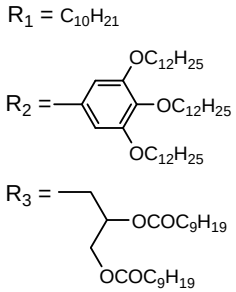
Self-assembly relies on the association of pre-programmed building blocks through non-covalent interactions to give complex supramolecolar architectures. Previous studies provided evidence for the unique self-assembly properties of semi-synthetic lipophilic guanosine derivatives which can sequestrate ions from an aqueous phase, carry them into an organic phase where they promote the generation of well-defined supramolecolar assemblies.[1] In the presence of cations lipophilic guanosines form columnar aggregates while in their absence they generate supramolecolar ribbons. The principal aim of the present work has been the synthesis and supramolecolar characterization of N9-substituted guanines. By using guanine instead of guanosine, while maintaining all the hydrogen bond acceptor and donor groups required for supramolecular aggregation, the steric hindrance to supramolecular aggregation is notably reduced because of the absence of the sugar (i.e. guanines with groups in N9 different from sugar are expected to have a greatest conformational freedom even in presence of bulky groups in C8).



The following alkyl-substituted guanine derivatives have been prepared:





The characterization of their self-assembly at the supramolecular level has been accomplished in solutions by NMR and CD spectroscopy and on surface by STM technique. In analogy with guanosine derivatives, also the N9-substituted guanines form either ribbon-like aggregates or cation-templated G-quartet based columnar structures.

1 J. T. Davis, G. P. Spada SUPRAMOLECULAR ARCHITECTURES GENERATED BY SELF-ASSEMBLY OF GUANOSINE DERIVATIVES *Chem. Soc. Rev.* **2007**, *36*, 296-313

P-02

PERYLENE CONJUGATED G-QUADRUPLEX FORMING OLIGONUCLEOTIDES

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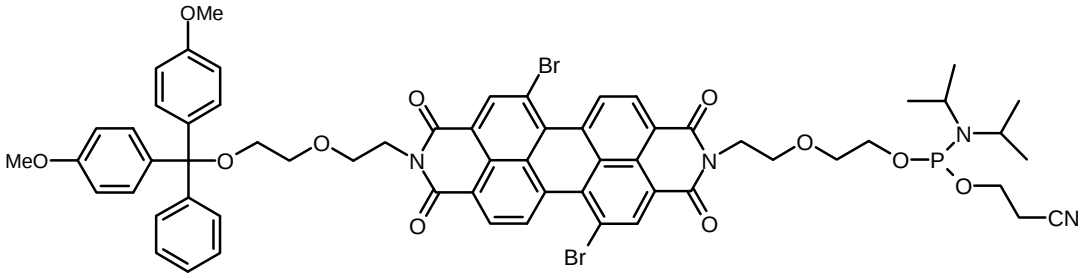
Perylene diimides present very interesting spectroscopic properties, in particular a high quantum yield fluorescence [1], and can interact with different DNA structures, with particular attention to G-quadruplex structures [2]. For these reasons, several synthetic approaches are present in the literature to bind perylene derivatives to synthetic oligonucleotides, in order to obtain fluorescent probes [3] or to stabilize specific DNA structures [4]. The possibility to conjugate perylene diimides to G-rich oligonucleotides is particularly tempting, since it is expected to stabilize the derived G-quadruplex structures. Aptamers with these characteristics have been shown to be efficient HIV integrase inhibitors, and thus potential anti-AIDS drugs [5].

Due to the poor solubility of perylene diimides, in the synthesis so far reported the total yields for the preparation of the ligands are quite poor (5-12%). Moreover, in most cases, it is necessary to perform the conjugation step in solution, detaching the growing oligonucleotide from the solid support. This implies several limitations of the ligand position in the sequence [4]. In any case, such low yields are not compatible with a large scale synthesis and the obtained products result difficult to purify.

On the basis of our previous knowledge of the physico-chemical properties of perylene derivatives modified on the bay-area by bromination [6], we have synthesized bromine-substituted perylene diimides with oxydryl-ended side chains, with a good solubility in common organic solvents. So, we have developed a new approach to obtain with a good yield (29%) an asymmetric perylene derivative as a “phosphoramidite building-block” (Figure), presenting the two typical groups of the nucleotides precursors (DMT and phosphoramidite), useful for the solid-phase synthesis of conjugated oligonucleotides. This compound, due to its good solubility, can be efficiently inserted in the desired step of the oligonucleotides solid-phase synthesis, according to well-established procedures. By modifying the coupling times, coupling yields have been optimized to 64%. The obtained conjugated oligonucleotides have been purified and characterized, and the G-quadruplex structure formed by these conjugates have been studied.



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P-03

NEW HYDROPHILIC THREE SIDE-CHAINED TRIAZATRUXENE DERIVATIVES AS STRONG AND SELECTIVE G-QUADRUPLEX LIGANDS.

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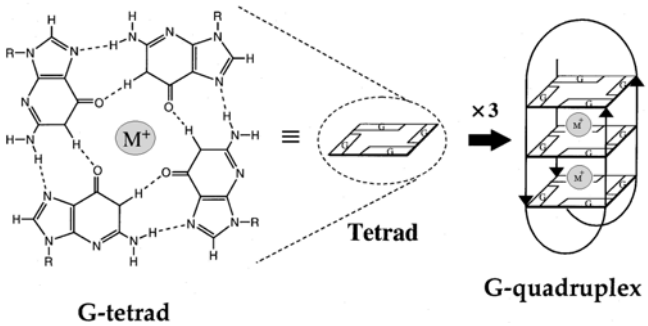

Fig.1 G-quadruplex formation

G-quadruplexes are a family of nucleic acids secondary structures stabilized by G-tetrads, coplanar quartets of guanines held together by a cyclic arrangement of eight unconventional hydrogen bonds[1] (Hoogsteen bonds) (*fig.1*). DNA G-quadruplex can be formed by G-rich strands as the telomeric sequences.Telomeric single-strand DNA is the substrate of telomerase, an enzyme necessary for telomeric replication, which is over-expressed in most cancer cells and participates in tumors genesis[2]. The formation of a telomeric G-quadruplex blocks telomerase activity and offers an original strategy for new anti-cancer agents. In the last 10 years, several families of compounds have been identified which specifically bind to the telomeric quadruplex. These derivatives, called "G-quadruplex DNA ligands", are able to block telomeric replication in cancer cells and to cause replicative senescence and/or apoptosis after a few cell cycles.

In this field, we have identified an interesting core as a precursor for new and selective G-quadruplex ligands: the triazatruxene moiety. In recent years, triazatruxene derivatives have been of great interest in supramolecular chemistry and in particular in organic electronics[3], nevertheless they have not been reported for important pharmaceutical applications. So, we thought that this kind of compounds could represent a useful basis to develop new G-quadruplex ligands, provided suitable hydrophilic side chains were added to the triazatruxene moiety. The triazatruxene core consists of a C3 symmetric cyclotrimer of indoles, which presents a wide aromatic surface with three useful points for the attachment of side chains, namely the three indolic NH at 5, 10 and 15 positions 5. So, we found a convenient synthetic strategy to obtain the unsubstituted triazatruxene core and, subsequently, the three-substituted triazatruxene derivatives with side chains of different length and basicity. These compounds, which present optimal molecular features for the interaction with the G-quadruplex (*fig.2*), have shown a strong G-quadruplex binding affinity and a good selectivity with respect to duplex DNA, as derived by mass spectrometry studies. Considering their DNA binding properties and high water solubility, these new compounds are very promising in terms of biological and possible pharmacological effects, not only at telomeres but also with respect to several oncogenes regulation[4].

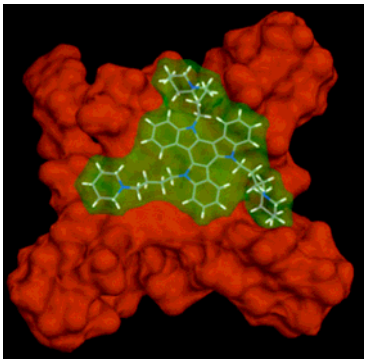

Fig.2 Simulated interaction between AZATRUX and G-quadruplex DNA.

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P-04

STUDY OF BINDING AFFINITY AND SELECTIVITY OF PERYLENE AND CORONENE DERIVATIVES TOWARDS DUPLEX AND QUADRUPLEX DNA BY ESI-MS.

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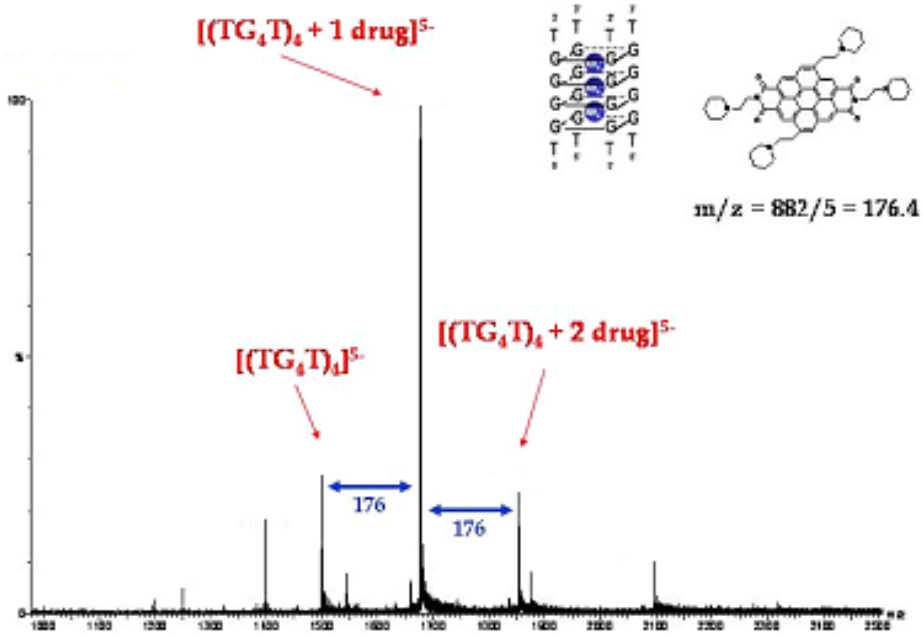
Among G-quadruplex interactive compounds, perylene and coronene diimides represent two well known classes of telomerase inhibitors [1]. Our research group has synthesized a large number of perylene [2] and coronene [3] derivatives, which have been extensively studied by means of several biophysical and biological assays. Here we report an extensive study of the noncovalent interactions between different inter- and intramolecular G-quadruplex structures and several perylene and coronene ligands by ESI-MS, a suitable and powerful technique for the determination of stoichiometries and relative binding affinities of such complexes [4].



The selectivity for these structures with respect to duplex DNA, a fundamental topic for the biological evaluation and the pharmacological application of these ligands as potential chemotherapeutic agents, has also been investigated. After exploring this topic according to the classical approach based on the very simple duplex model of an autocomplementary dodecamer, we extended our analysis reporting for the first time a competition ESI-MS experiment in the presence of genomic DNA. Whereas those ligands showing a high level of selectivity between quadruplex and duplex oligonucleotides, in terms of binding constants and percentage of bound DNA, confirmed their selectivity in the competition experiment, the opposite was not always true. This result suggests that artificial interactions are possible with a short duplex oligonucleotide, which are not biologically significant [5].

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P-05

PHOTOMODULATION OF G-QUARTET BASED ARCHITECTURES

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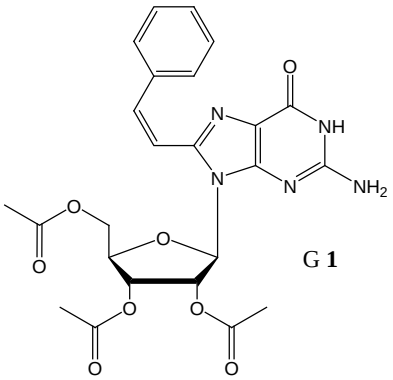
Reversibility is a hallmark of supramolecular chemistry. By exploiting the information stored in the molecule, in particular, its preprogrammed propensity to undergo self-recognition and self-association pathways, in combination with the reversibility of its self-assembly under external stimuli, it is possible to implement molecule-sized prototypes of dynamic chemical devices. Lipophilic guanosines, in the presence of certain cations, can form G-quartet-based octamers or columnar aggregates (supramolecular polymers) depending on the concentration of the cation and nucleobase. The metal cations are located between the quartets and act as templates. In order to control the self-assembly of G-quartet-based columnar aggregates, we report here on a lipophilic guanosine derivative (G **1**) who bear a C(8) photo-responsive substituent.



G **1**

Through ^{1}H-NMR spectroscopy and circular dichroism we demonstrated that when the ethenyl double bond is trans-configurated, G **1** forms, in the presence of KPF_6, G-quartet-based octamers; their symmetry, C_4 or D_4, depend on the solvent used. After trans-to-cis photo-isomerization, the octamers disassemble and G **1** remains dissolved in the monomer state. The process of self-assembly and disassembly is reversible. In summary, we have shown the photo-modulation of the reversible interconversion between an octamer and a monomer of a guanosine derivative. This supramolecular dynamer can be of importance as a model system to mimic the formation-annihilation of G-quartet-based architectures, which might be of biological significance, in the frame of nucleic acid telomerase.

P-06

TETRA-END-LINKED-ODN APTAMERS AS POTENTIAL ANTI HIV-1 AGENTS

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Many synthetic oligonucleotides (ODNs) have been found to be potent antiviral agents and a number of modified ODNs have been selected as promising candidates against HIV. The mechanism of action is based on the inhibition and/or the interference with the viral cycle at different levels, including HIV binding to target cells.

Hotoda and co-workers identified the lead sequence d(5'-TGGGAG-3', **1**) active against HIV-1 and they demonstrated that this specific anti-HIV activity was correlated with the ability of G-rich sequences to form stable quadruplex structures.(1) These latter have been postulated to be the ultimate active species directly interacting with the *in vivo* targets. A variety of 5'-substituted d(5'-TGGGAG-3') have been synthesized. Among these, the ODN **1** bearing the 3,4-dibenzyloxybenzyl (3,4-DBB) group at the 5'-end and the 2-hydroxyethylphosphate group at the 3'-end (R-95288) showed the most potent anti-HIV activity and the least cytotoxicity among the synthesized analogues. The antiviral activity of 5'-modified ODNs was attributed to the presence of aromatic groups of the native ODN backbone, which could produce hydrophobic effects stabilizing quadruplex structures.

Recently we reported the synthesis of a novel class of parallel monomolecular quadruplexes indicated as Tetra-End-Linked (TEL)-quadruplexes.(2,3) The main structural feature of TEL-quadruplexes is the presence of four ODN strands whose 3′- and/or 5′-ends are attached to a non-nucleotidic tetra-end-linker. The resulting parallel TEL-quadruplexes are characterized by better kinetic and thermodynamic parameters when compared with the natural counterparts. On the basis of the assumption that the formation of a kinetically and thermodynamically favoured quadruplex complex is a prerequisite for efficient antiviral activity, we synthesized a series of some representative TEL-analogs of Hotoda's anti-HIV aptamer, in which the 3'-ends of four d(5'-TGGGAG-3') strands are linked to the four arms of the TEL (two different size of TEL have been tested). The resulting TEL-quadruplexes have been analyzed by UV and CD experiments and their biological activity has been evaluated also in comparison with the unmodified counterparts. The faster kinetic of TEL-quadruplex formation was found to correlate with better IC_{50} values, thus furnishing quantitative evidence that the G-quadruplex structures are the ultimate active species able to inhibit both virus-to-cell and cell-to-cell HIV-1 transmission.

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P-07

SOLUTION AND SURFACE ASSEMBLY OF GUANOSINE-RICH OLIGONUCLEOTIDES

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Due to the distinct charge transfer properties of guanosine derivatives, surface deposited guanosine wires (G-wires) are believed to be very promising as building blocks for molecular electronic devices. Two main steps have to be resolved to attain basic understanding of wire formation from G-rich DNA oligomeric sequences: (a) self-folding and self-assembly of the macromolecular aggregates in solution as a function of molecular composition (i.e. base sequence), and (b) the effect of surface interaction on the deposition from solution phase and growth of wire-like aggregates on various surfaces.

Utilizing principles of self-assembly based on the geometric formalism of quadruplex folding we derived structures of possible macromolecular G-quadruplex assemblies. The main goal of our study was to find out how particular details of the folding geometry, like for instance the number and orientation of the loops, affect the ability of the G-rich oligonucleotides to form G-wires. The second goal was to resolve a relationship between the G-wires formed on surfaces and the G-wires formed in solution used for surface coating (drop coating, dip coating, spin-coating, etc.).

The growth of G-wire structures formed by different G-rich DNA oligomeric sequences in aqueous solution and on the surface of mica was investigated by combining dynamic light scattering (DLS) measurements with atomic force microscopy (AFM) imaging. The former provides information on the size and shape of the self-assembled aggregates assembled in solution, while the later provides information on the structure of aggregates formed after surface deposition. Various solution concentrations, as well as different deposition procedures were investigated. Comparison was made between different sequences designed to assemble into "slipped" and "sticky-end-joined" quadruplexes.

P-08

RADICAL-ARMED GUANOSINE ARCHITECTURES

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Among the bases occurring in DNA and RNA, guanosine represents a versatile molecule for the presence of H-bond donors and acceptors which allow its self-assembly into regular supramolecular architectures. Depending on their substitution pattern, solvent, and cation availability, lipophilic guanosine derivatives spontaneously self-associate to give either H-bonded ribbons or quartet-based columnar structures (Figure 1). Recently we have shown that the scaffolding of the persistent radical unit 4-carbonyl-2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPO), is achieved by taking advantage of the self-assembly templated by potassium ions of a guanosine derivative (GaceTEMPO, Figure 2a) into a H-bonded networks.[1] Here we report the self-assembly behaviour, in the presence and in the absence of cations, of a new radical-armed derivative (dGTEMPO, Figure 2b) where two paramagnetic units are connected to the guanosine deoxynucleoside. Compared to our previous communication, in presence of a templating cation[2], our challenge was to increase the spin exchange difference between the two states giving rise to drastic magnetic changes before and after addition of the metal. In absence of cations dGTEMPO can form a compact birefringent gel-like phase mediated by the formation of ribbon-like assembled species. The self-assembly processes are investigated by the use of ESR spectroscopy and Circular Dichroism.

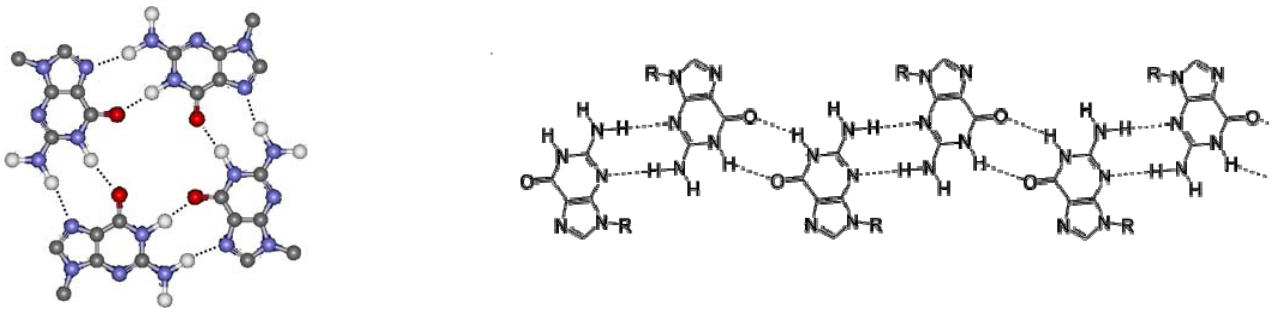

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Figure 1

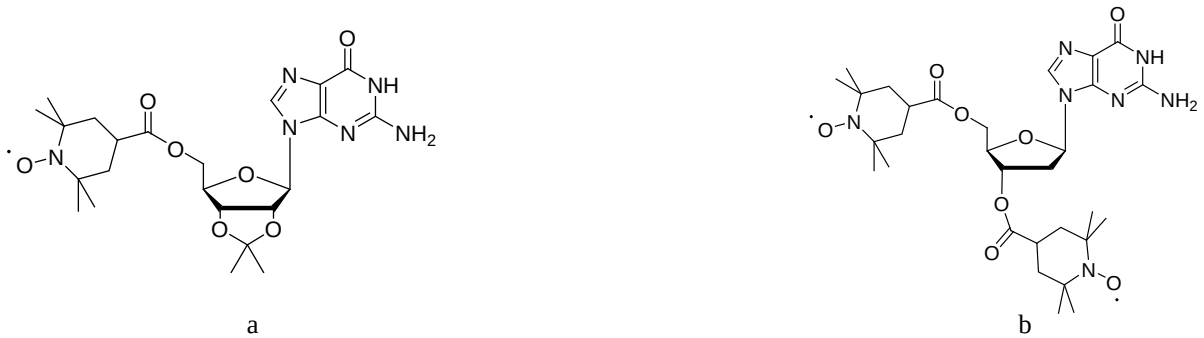

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Figure 2.

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2) P. Neviani, E. Mileo, S. Masiero, S. Pieraccini, M. Lucarini, G. P. Spada, Manuscript submitted

P-09

NMR STUDIES ON QUADRUPLEX STRUCTURES CONTAINING ABASIC SITES

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Genome integrity is of vital importance to cellular survival and replication. However, a broad variety of causes, ranging from the effect of genotoxic chemicals to error-prone cellular processes, render DNA rather vulnerable to damage and mutation. The most common types of DNA defects are single base mismatch, abasic site, single base bulges and oxidized bases. Among these, abasic sites (AP sites) are expected to be one of the most frequent lesion in DNA. They can arise by the spontaneous hydrolysis of the *N*-glycosidic bond (generally depurination) or the removal of altered bases by DNA glycosylases. Damaged bases can be produced by processes of alkylation, oxidation and deamination. They include both alkylated and oxidised bases, such as N^7-methylguanine, 5,6-dihydroxy-5,6-dihydrothymine, uracil, 3-alkyladenine, O^6-alkylguanine and 8-oxo-7,8-dihydroguanine. The accumulation of unrepaired AP sites can be lethal because they hinder DNA replication. Moreover, even when bypassed by DNA polymerases, AP sites frequently lead to the insertion of mutagenic bases opposite them. Finally recent studies have shown that the aldehyde residue formed by an AP site can generate an inter-strand cross-link with the exocyclic N^2-amino group of a guanine residue on the opposite strand of the double helix.

Since many of the events generating AP sites involve guanines the presence of such lesions result particularly important in G-rich tracts. These have been observed in critical segments of eukaryotic and prokaryotic genomes, promoter regions, both short microsatellite and longer minisatellite repeats, ribosomal DNAs, as well as telomeres in eukaryotes and immunoglobulin heavy chain switch regions of higher vertebrates. Guanine-rich tracts have the potential to form G-quadruplex structure. Although the above considerations clearly suggest a relationship between AP sites and G-quadruplex structures, at the best of our knowledge, only one report concerning AP sites containing quadruplex structures has appeared in literature so far.

In an effort to investigate the effects of AP sites in quadruplexes, we have undertaken a systematic study concerning the structural features of oligodeoxynucleotides containing residues mimicking AP sites, potentially able to form quadruplex structures. In this communication, we report the preliminary NMR data concerning the quadruplex complexes formed by five ODNs, all based on the parallel quadruplex forming sequence TGGGGGT, in which all guanines have been replaced, one at a time, by an AP site mimic. A tetrahydrofuranyl analogue was employed instead (dS). The main structural properties of quadruplexes formed by dS containing ODNs have been investigated by both NMR and CD spectroscopy.

P-10

LOOP STRUCTURE CONTROLS ION MOVEMENT WITHIN $d(G_4T_3G_4)_2$ G-QUADRUPLEX

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The prerequisite for the formation of G-quadruplexes is the presence of mono- or divalent cations, which counteract the electrostatic repulsions of the four carbonyl groups in the center of each G-quartet. In some cases the presence of different cations can lead to changes in the topology of a G-quadruplex. Recent X-ray crystallography (Neidle et al.) and solution state NMR studies (Wu et al.) have shown that the oligonucleotide $d(G_4T_3G_4)$ forms several G-quadruplex structures in the presence of K^+ and Na^+ ions, respectively. Our NMR studies in solution have also shown that the oligonucleotide forms several structures in the presence of K^+ ions. Interestingly, a single bimolecular G-quadruplex is formed in the presence of $^{15}NH_4^+$ ions. The antiparallel G-quadruplex features four G-quartets with two T_3 loops spanning along the edges of the outer G-quartets on the opposite sides of the G-quadruplex core. This topology is identical to the topology of the head-to-tail G-quadruplex found in the crystal structure in the presence of K^+ ions. With the use of ^{15}N-heteronuclear 2D NMR experiments we were able to locate three $^{15}NH_4^+$ ion binding sites between pairs of adjacent G-quartets. Ions inside G-quadruplex structures are usually not static, but move between binding sites and bulk solution. However, no $^{15}NH_4^+$ ion movement within $d(G_4T_3G_4)_2$ could be detected at temperatures up to 25 °C. Raising the temperature to 35 °C enabled us to observe only a very slow movement of ions between the outer binding site and bulk solution. On the basis of our NMR data we have constructed a low-resolution model of the T_3 loops than span the outer G-quartets of dimeric $d(G_4T_3G_4)_2$ quadruplex. Two of the thymines are positioned over the central cavity of the outer G-quartets and thus represent a steric barrier for the movement of ions between the interior of the G-quadruplex and the surrounding solution. It appears that the structure of the loops is the main factor which determines ion movement within $d(G_4T_3G_4)_2$ G-quadruplex.

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P-11

TEMPLATE ASSISTED SYNTHESIS OF G-QUADRUPLEX (TASQ)

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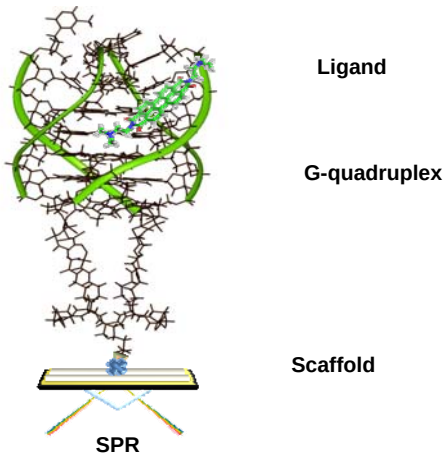
A large number of molecules are known to interact with DNA and have thus been used for various applications. In this context, the design of G-quadruplex ligands has received a great attention because these DNA conformations represent a valuable biological target. Indeed, these structures have been found to play an important role in many relevant biological processes such as telomere stabilization, oncogene activation, and regulation of the immunoglobulin switch region. However, a wide variety of topologies can be adopted by the G-quadruplex depending on the number of strands involved in the structure, the strand direction, as well as variations in loop size and sequence. Obviously, this can confuse the study of recognition phenomena with potential ligands.

We recently described the use of a peptidic scaffold as a topological template that can direct the intramolecular assembly of covalently attached oligonucleotides into a parallel G-quadruplex (see opposite). We demonstrated that the use of the scaffold allows the formation of the quadruplex motif even without the addition of cations and dramatically incrases the stability of the motif. We also showed that this molecular system can be immobilized on surface for studying by SPR (Surface Plasmon Resonance) the interactions with small organic molecules.[1] This latter result represents the first example of immobilization of parallel G-quadruplex motif for SPR studies.



The natural human intramolecular quadruplex and a duplex have been also anchored on this peptidic scaffold. In this manner by using SPR, we have investigated the interactions of various ligands with these different nucleic acid conformations (antiparallel G-quadruplex, parallel G-quadruplex and duplex). Interestingly, the various studied ligands have shown differences of behavior that could be correlated with the binding mode of the ligand. Indeed, discrimination between intercalators and groove binders can be evaluated. Thus, this novel SPR-based approach using the peptide scaffold is of interest for the screening of G_4-DNA ligands, providing key information on their affinity, selectivity and binding mode. Furthermore, this strategy could be used for the formation of other particular conformation of DNA (antiparallel G-quadruplex, *i*-motifs...).

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P-12

SELF-ASSEMBLY OF ALKYL-SUBSTITED GUANINES ON GRAPHITE SURFACE

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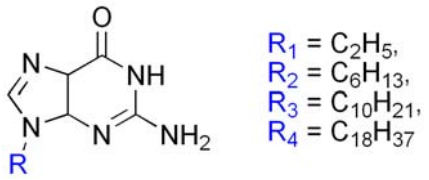
The self-assembly of small molecules into non-covalently bonded polymeric architectures at surfaces is a subject of continuous interest [1]. These supramolecular nanostructures can be used as active building-blocks in a wide range of applications, e.g. in (opto)electronics [2]. We have previously thoroughly investigated the self-assembly of various guanosine derivatives at surfaces [3]. As an extension of our work, we have focused our effort on a new series of guanine molecules functionalized with alkyl chains. Their characterization on surfaces has been accomplished with a sub-molecular resolution by Scanning Tunneling Microscopy (STM) at the solution-graphite interface.



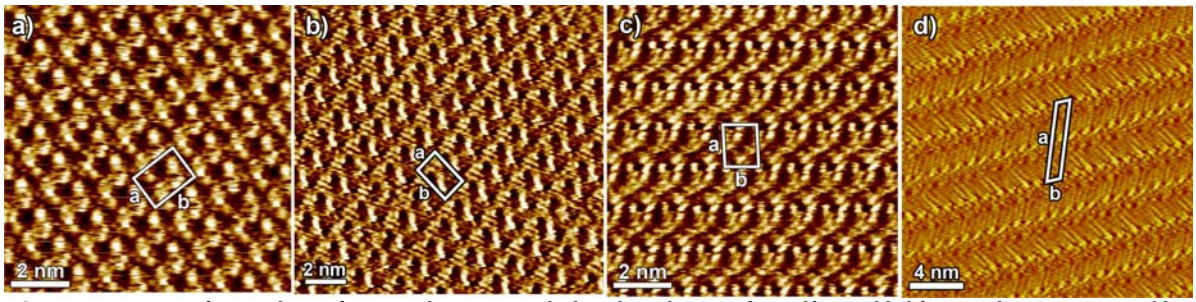


Figure 1. STM curent images of a monolayer of guanine derivatives at the liquid-graphite interface self-assembled from a solution in 1,2,4-trichlorobenzene. Monolayers corespond to : a) ethyl-guanine, b) hexyl-guanine, c) decyl-guanine and d) octadecyl-guanine.

Our STM study revealed that ethyl- and octadecyl-guanine derivatives form hydrogen bonded ribbons at the solution-HOPG interface, whereas the hexyl- and decyl-guanine derivatives self-assemble into tightly packed crystalline monolayer where the single molecules are not interacting through H-bonds. We have then extended our studies to the concentration dependence physisorption of the various derivatives. We observed that only ethyl-guanine self-assembly behavior is concentration dependent. While at high concentration H-bond ribbons have been observed, at low concentration H-bonded tetramers have been found. Upon addition of 1 equiv. of a potassium picrate solution in 1,2,4-trichlorobenzene on the top of the $C_{18}H_{37}$-guanine monolayer a the structural re-organization from the supramolecular G-ribbon into G-quartet structure have been monitored. Surprisingly, the formation of G-quartets was observed only in case of $C_{18}H_{37}$ guanine derivative.

The well-defined and highly ordered guanine supramolecular structures can be used as scaffolds for attaching functional units in 2D with a sub-nm precision, paving the way towards technological applications, e.g. as (supra)molecular nanowires. Moreover, the G-quartets are of great interest because they hold potential in anticancer drug design as they can act as enzyme telomerase inhibitors [4].

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P-13

SELF-ASSEMBLY AND GROWTH OF dGMP QUADRUPLEXES IN SOLUTION

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Guanosine molecule and its derivatives show an unusual self-aggregation ability to form highly stable G-quadruplex structures even without a linking sugar-phosphate backbone. The extend of self-assembly and consequently the length of the quadruplex depends on many parameters like temperature, pH value of the solution, added ion species and its concentration.

The dimensions of the G-quadruplex in solution can be determined by Dynamic light scattering (DLS) and Small-Angle X-ray Scattering (SAXS). DLS primarily probes the dynamic properties of the solution, which are expressed as apparent diffusion coefficients of various diffusive dynamic modes. With a corresponding hydrodynamic model for rodlike objects the length of the quadruplex is obtained. Therefore the size and the shape of the scattering objects are determined indirectly. On the contrary, SAXS probes directly the size and shape of the scattering objects, independently of their solution dynamics.

In order to clarify the complex mechanism of G-quadruplex formation in dilute aqueous solutions we studied the self-assembly of deoxyguanosine 5'-monophospate (dGMP) in the form of ammonium salt. The experiments were performed as a function of dGMP concentration and as a function of the concentration of added KCl (Fig. 1). Both techniques, DLS and SAXS, indicate that the self-assembling process is largely affected by excess potassium ions in the solution [1-3]. Interestingly however, the two methods give very different results for the length of self-assembled quadruplex. The origin of this discrepancy is investigated and discussed.

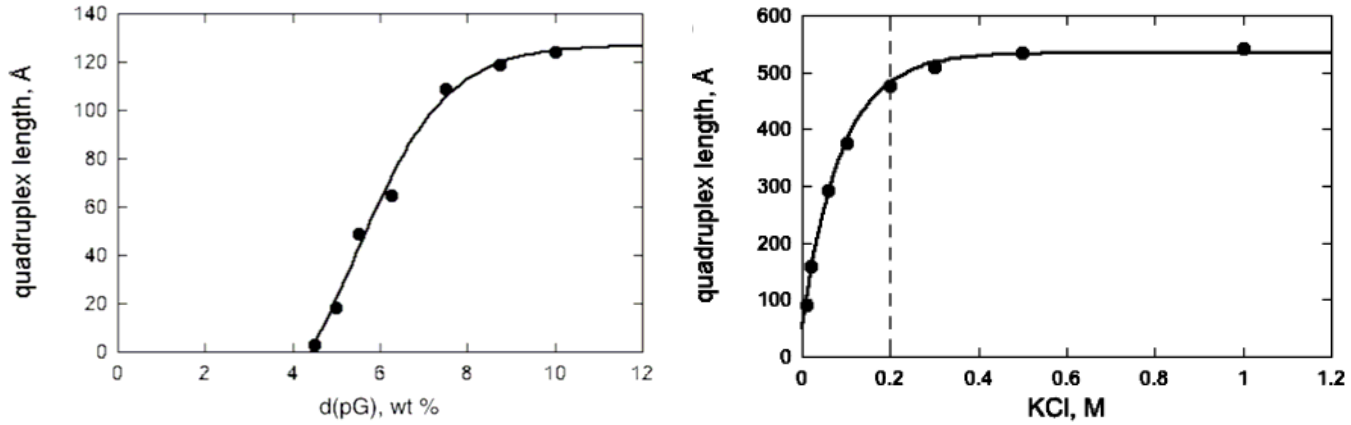

Figure 1: The length of G-quadruplexes as determined by SAXS: as a function of dGMP concentration (left) and as a function of added KCl in solutions with a fixed c = 4 wt% dGMP concentration (right).

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P-14

THE NOVEL LONG G-4 DNA NANOSTRUCTURE

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We developed the method for preparation of novel long (hundreds of nanometers), inter-molecular G4-DNA molecules composed of four parallel G-strands. The only long continuous G4-DNA reported so far, are intra-molecular structures made of a single G-strand. To enable the assembly of four G-strands into a long quadruplex structure, we developed a novel technique based on avidin-biotin molecular recognition. The new four-step procedure includes: 1. Enzymatic synthesis of long poly(dG)-poly(dC) molecules with biotinylated poly(dG)-strand; 2. Formation of a complex between avidin tetramer and four biotinylated poly(dG)-poly(dC) molecules; 3. Separation of poly(dC) strands from poly(dG)-strands that are connected to avidin; 4. Assembly of the four G-strands attached to avidin into tetra-molecular G4-DNA. The average contour length of the formed structures, as measured by AFM, is equal to that of the initial poly(dG)-poly(dC) molecules, suggesting a tetra-molecular mechanism of the G-strands assembly. The height of the tetra-molecular G4-nanostructures is larger than that of mono-molecular G4-DNA molecules. The characteristic CD spectra of the tetra- and mono-molecular G4-DNA are significantly different, suggesting a different structural organization of these two types of molecules. The tetra-molecular G4-DNA nanostructure shows clear electrical polarizability. This particular property may offer new prospects for the parallel-stranded G4-DNA in the field of molecular electronics.

P-15

CATION INTERACTIONS AND MOVEMENT WITHIN DNA THROMBIN BINDING APTAMER IN SOLUTION

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G-quadruplexes have received attention for their biological roles as well as applicability in biochemical technology. Oligonucleotide d[GGTTGGTGTGGTTGG] (noted as TBA for Thrombin Binding Aptamer) which exhibits anticoagulant properties with high binding affinity to thrombin has been extensively studied in recent years. TBA has been shown to form an intramolecular antiparallel G-quadruplex with two G-quartets and three lateral central TGT and two TT loops. We studied ability of $^{15}NH_4^+$ and Na^+ ions to induce TBA to fold into G-quadruplex and were interested in locating cation binding sites in the interior of 3D architecture. Folding in the presence of $^{15}NH_4^+$ ions proved useful not only for determining the number and location of cation binding sites but also enabled study of kinetics of $^{15}NH_4^+$ ion movement.

Titration of $^{15}NH_4^+$ ions into aqueous solution of TBA resulted in the formation of one major species, while titration of Na^+ ions led to formation of one major and several minor species. TBA G-quadruplex in the presence of $^{15}NH_4^+$ ions adopted the same topology as in the presence of K^+ ions.1-3,5 The CD spectra of TBA in the presence of $^{15}NH_4^+$ or K^+ ions are consistent with formation of an antiparallel G-quadruplex, while the CD spectrum in the presence of Na^+ ions deviates from the two in peak positions and ratios of their magnitudes. Denaturated TBA G-quadruplex in the presence of $^{15}NH_4^+$ ions did not regain initial structure even after one week at 4 °C.

NMR data suggested that loops in the TBA G-quadruplex adopt similar structures in the presence of $^{15}NH_4^+$ and K^+ ions as far as T4, T13 and T9 residues are concerned. We examined pH and temperature-dependant changes in the presence of $^{15}NH_4^+$ ions and found that they were reversible and most pronounced for imino proton signals of T3, G8 and T12. ^1H-^{15}N-filtered HSQC spectrum of TBA revealed two well resolved cross-peaks, which corresponded to $^{15}NH_4^+$ ions in bulk solution and bound within TBA G-quadruplex. ROESY spectrum was used to assign and localize bound $^{15}NH_4^+$ ions between the two G-quartets.

P-16

SELECTIVITY IN TELOMERIC G-QUADRUPLEX LIGANDS AND TELOMERASE INHIBITORS: HYDROSOLUBLE PERYLENE DIIMIDES (HPDIs)

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The search of telomerase inhibitors has been widely explored in the last few years, since telomerase activity in somatic cells can be considered as a general cancer mark. One of the possible strategies is the capping of telomere 3' end, the enzyme substrate in a conformation not available to the telomerase, in particular G-quadruplex. Small organic molecules, able to induce and/or stabilize G-quadruplex structure, have been synthesized and studied in many different research groups. We will illustrate the hydrosoluble perylene diimides, HPDI class, studied mainly in our research group, which offers the intriguing possibility to fix the molecule mojety (perylene) able to bind to the terminal G-quartet of telomeric G-quadruplex, while widely variating the number and features of side chains, which bind to the four G-quadruplex grooves. We will show that it is possible to significantly improve the HPDIs efficiency and the selectivity for G-quadruplex with respect to genomic DNA, using this strategy.

A new assay of telomerase inhibition (competitive TRAP assay) will also be illustrated.

The obtained results, mainly by FRET melting and CD spectroscopy, allow us to propose a model which derives the selectivity of HPDI for intramolecular G-quadruplex DNA at molecular level (see Figure).

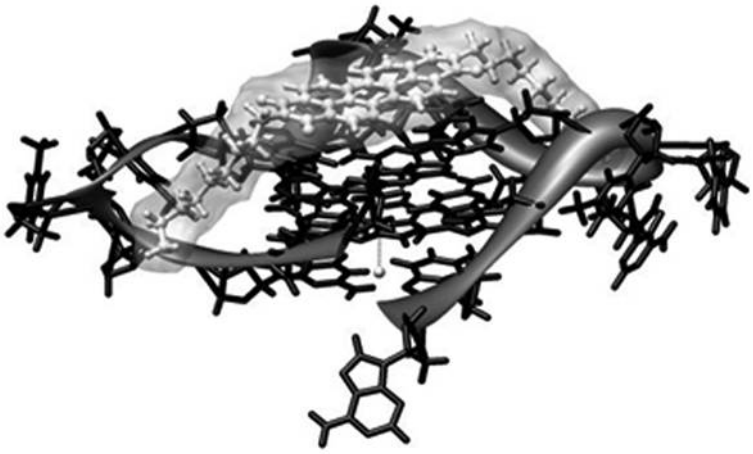

Figure. Representative models of a HPDI with G-quadruplex DNA. DNA backbone is in black with dark gray ribbons following strands conformation, while ligand molecules are ball-stick with light gray surfaces.

P-17

PUTATIVE INTRAMOLECULAR G-QUADRUPLEX STRUCTURES WITHIN A REGULATORY ELEMENT OF THE HUMAN TELOMERASE (hTERT) PROMOTER

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G-rich nucleic acid sequences have the potential to fold into four stranded structures, called G-quadruplex. Telomeric DNA, with its tandem repeats of guanine-rich sequences, comprises the best-studied source of quadruplex-forming nucleic acids, but there has been increasing interest in whether non-telomeric regions of G-rich DNA in the human genome are inherently capable of forming stable G-quadruplex structures, in which runs of guanine bases are separated by short (1-6 bases) loop sequences. Intramolecular G-quadruplex structures are important not only from physico-chemical but also from the biological point of view. In fact, there are satisfactory evidences for their significant role in telomeres functioning and more recently for the transcription regulation of a number of protooncogenes. Recent studies have shown that G-rich sequences located in a number of non-telomeric genomic sequences, including those of the oncogenes c-myc, c-kit and KRAS, Rb and VEGF genes, have the ability to form intramolecular G-quadruplex structures, which, if stabilized by some small organic molecules or by proteins, regulates the gene transcription.

In our research group, we are studying the structural features of the promoter of hTERT gene, responsible for the expression of telomerase, that occurs in most cancer cells (80-90%). We have found, by theoretical analysis, that the hTERT promoter is characterized by the presence of nine putative G-quadruplex sequences (PQS) belonging to the most accessible G-rich region of the hTERT promoter, that is unfavourable for nucleosome formation.

We show here, using a combination of PAGE (polyacrylamide gel electrophoresis), polymerase stop assay, chemical footprinting, UV/Vis and circular dichroism spectroscopy that the promoter presents two sequences that do have the capability to fold into an intramolecular G-quadruplex. These two structures are characterized by a different thermodynamic stability and their folding inhibits the DNA polymerase catalyzed reaction.

The possible equilibrium of this promoter region between the canonical duplex and the G-quadruplex structure could represent an interesting element for the gene transcription regulation by means of suitable chemical ligands and/or nuclear proteins binding.

P-18

DIRECTING GUANOSINE SELF-ASSEMBLY VIA N^2-MODIFICATION

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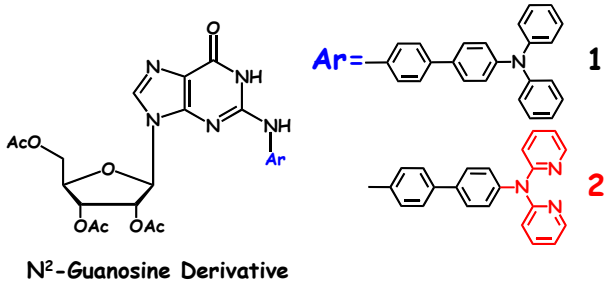
New natural or "unnatural" nucleobases have been the focus of attention, recently, due to their potential applications in biotechnology and medicinal chemistry, as well as in materials science. Many interesting supramolecular architectures have been designed by using the hydrogen bonding and electrostatic interactions of guanine-based building blocks. Hence, we are interested in tailoring the photophysical properties of guanosine, without affecting its hydrogen-bonding ability required for G-quartet or guanine-cytosine pair formation. We have synthesized two fluorescent lipophilic N^2-biphenyl-NAr_2 guanosine derivatives (**1** and **2**) that are capable of stereoselective self-assembly into exclusive octamers (G_8). By chemical modification of guanosine at the N^2-site, we have introduced stereoselectivity into the G-quartet-based structure which has not been previously shown. Octamers (G_8) of compounds **1** and **2** have been identified by CD, NMR and ESI-MS methods. A discrete all *syn* tail-to-tail left-handed octamer is exclusively formed, for all N^2-guanosine derivatives, regardless of the nature of the metal ions (Group I or II). Moreover, an interquartet π-π stacking involving aromatic N^2-substituents of guanine has a stabilizing effect and in turn permits octamer formation even in the absence of metal ions, giving rise to an unprecedented "empty" octamer, as evidence by our ESI-MS data.



P-19

G-QUADRUPLEX IN THE PROXIMAL PROMOTER OF BASIC FIBROBLAST GROWTH FACTOR

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The human fibroblast growth factor 2 gene (FGF2) codes for a protein involved in broad angiogenic, mitogenic, and neurotrophic activities. We will present evidence indicating that a double helical region intrinsic to its proximal promoter, that appears conserved across species, has innate ability to form a parallel G-quadruplex. We will show that the topological conversion is driven by potassium cation concentration, and crowding conditions. This results reinforces the thesis indicating that the transient nature of both cation concentration, as well as crowding conditions, can act as contributors to potential regulatory function of quadruplexes in biological systems.

P-20

THE DESIGN OF QUADRUPLEX TOPOLOGIES WITH HIGH THERMODYNAMIC STABILITY

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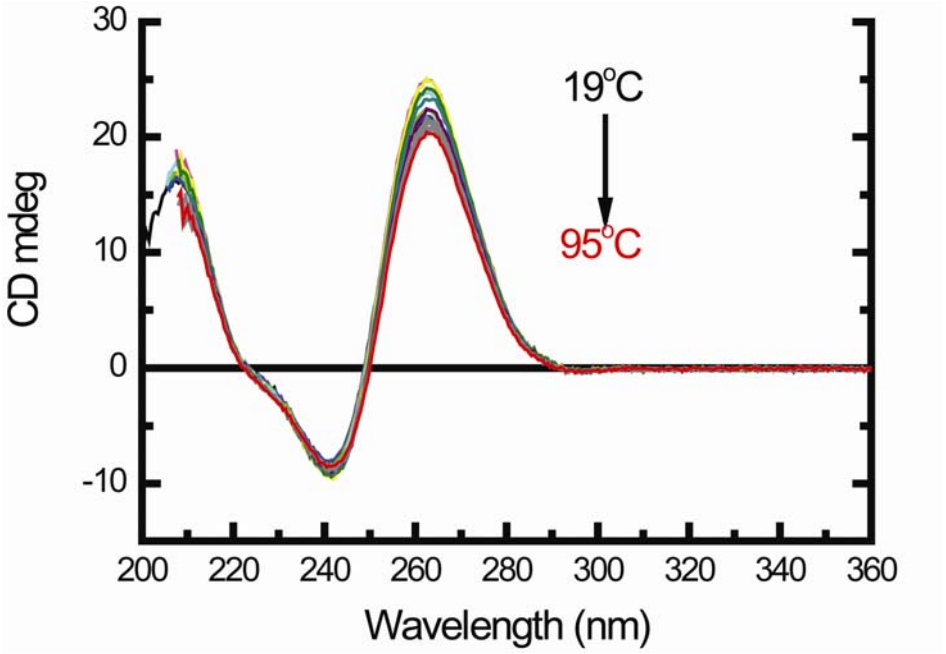
The unique thermodynamic properties of quadruplexes make them good candidates for roles as biomaterials and biotechnological tools. In order to realise some of this potential quadruplexes may be designed to be resistant to high temperatures and have unique and predictable topologies. We have endeavoured to design quadruplex topologies characterised by high thermodynamic stability. Using a DNA sequence shorter than 25 nucleotides, we succeeded in designing a topology that melts above the boiling point of normal physiological aqueous solutions. We probed its formation utilizing Laser Induced Liquid Bead Ion Desorption (LILBID) mass spectrometry and HPLC to show stoichiometry of folded oligonucleotides, and UV and CD studies, both to show quadruplex formation and their heat resistance.



P-21

VERSATILE APPLICATIONS OF A SIMPLE GUANOSINE NUCLEOSIDE COMPOUND

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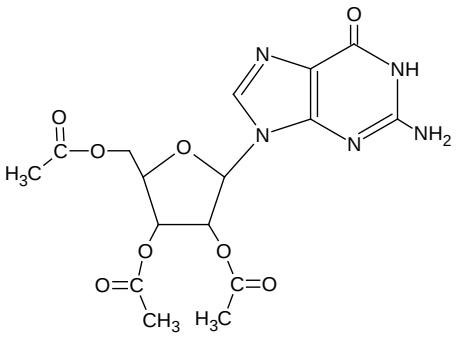
A variety of applications towards G-quartet studies are demonstrated using a simple guanosine nucleoside: 2',3',5'-*O*-triacetylguanosine (TAG). Self-assembly of TAG in the presence of metal cations M^{n+} (n = 1,2,3) and chloroform are observed. Formation of G-quartets is confirmed using ^{1}H and NOESY NMR experiments and sizes of G-quadruplex are determined using positive ion-mode electrospray-ionization mass-spectrometry (ESI-MS) as well as tandem MS/MS.

(1) We found that monovalent cations (Na^{+}, K^{+}, Rb^{+}) promote polymeric aggregate formation, while divalent cations (Sr^{2+}, Ba^{2+}, Ca^{2+}, Pb^{2+}) promote discrete octamers formation, and a combination of dodecamer and octamers are formed in the presence of trivalent cations (La^{3+}, Eu^{3+}, Tb^{3+}, Dy^{3+}, Tm^{3+}). This is the first example of stacking G-quartet formation assisted by trivalent lanthanide metal ions. A triple-decker model for $[TAG]_{12}M^{3+}$ is proposed where M^{3+} is in-plane with the middle G-quartet instead of being sandwiched. We also suggest that the various population distribution of dodecamers and octamers formed by $[TAG]M^{3+}$ are related to the ionic radii of the trivalent cations.

(2) Using 2D ^{1}H NMR techniques, we determined that the $[TAG]_{8}M^{2+}$ octamers is composed of an all-*anti* G-quartet stacking on top of an all-*syn* G-quartet in a tail-to-head fashion, and the divalent cation is sandwiched between the two G-quartets. We also report the first solution-state ^{43}Ca NMR characterization of Ca^{2+} ion binding to G-quartets at the channel site (^{43}Ca = -43 ppm).

(3) The NMR signatures for alkali metal cations (^{23}Na, ^{39}K, ^{87}Rb) residing inside the G-quartet channel are also determined: ^{23}Na = -18.9 ppm, ^{39}K = 10.4 ppm, ^{87}Rb = 60.9 ppm. Quantum chemical calculations showed remarkable agreement between experimental and calculated chemical shieldings. This is the first time that experimental NMR assignment for this type of alkali metal ions is confirmed by calculations.



2',3',5'-*O*-triacetylguanosine (TAG)

P-22

NEW NANOMATERIALS BASED ON COMPLEXES OF G4-DNA WITH INTERCALATORS

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Self-assembled DNA nanostructures were suggested to have key potential in nanotechnological devices and applications. DNA nanostructure like Guanine tetrads were proposed as building blocks of molecular nanowires. The wires that we invented and used in this work comprise a large number (hundreds) of stacked guanine tetrads, providing better conditions for π-overlap compared to base pairs of the canonical double stranded DNA. High content of guanines, which have the lowest ionization potential among DNA bases, also makes charge migration through G4-wires highly probable. We have recently demonstrated that the wires are characterized by higher charge mobility as compared to double-stranded DNA. This observation makes G4-DNA a promising candidate for nanoelectronic applications. The goal of this research was to produce long conductive wires based on G4-DNA and complexes of the DNA with various intercalators. Intercalation of the aromatic molecules into the core of the DNA might increase the π -stacking among the aromatic G-tetrad planes, and as a result to improve the conductivity of the wires.

Here we present the absorption, CD and fluorescence spectroscopy data on interaction of cationic porphyrin, TMPyP (*Lubitz, I., Borovok, N. and Kotlyar, A.B. 2007 Biochemistry. 46, 12925-12929*) and Thiazole Orange with long monomolecular wires. The results clearly show that the molecules intercalate in-between the tetrads in the wire. The estimated ratios between the tetrad and the intecalator are 0.5 for TMPyP and 1 for Thiazole Orange respectively. TMPyP and Thiazole Orange are photoactive, their triplet states are characterized by a low redox potential and are capable of abstracting an electron at electrical potentials much lower than those of the ground state. This property of the dyes will allow current to flow through the DNA-intercalator complexes at relatively low applied electrical potentials in the presence of light. Developing of stable complexes of G4-wires with intercalators which are capable of reversibly changing their electrical conductivity upon photoirradiation is useful for application of the wires in electro-optical devices.

P-23

CATIONIC COMB-TYPE COPOLYMER AS A NUCLEIC ACID CHAPERONE: EFFECT OF THE COPOLYMER ON QUADRUPLEX FOLDING

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Nucleic acids are highly charged polymers owing to anionic phosphate groups in their backbone. The .anionic charges strongly influence folding properties of nucleic acids so that the folding properties can be regulated by shielding the electrostatic interaction among the anionic charges. We have been interested in the interpolyelectrolyte complexes (IPEC) between DNA and the cationic comb-type copolymers, poly(L-lysine)-graft-dextran (PLL-g-Dex, Fig. 1), consisting of a polylysine backbone and abundant hydrophilic graft chains of dextran. Unlike homopolycations which induced condensation of DNA, the copolymer formed a totally soluble complex without DNA condensation. We have previously reported that the comb-type copolymers increased stability of a double stranded (ds) DNA and a triple helical DNA under physiological relevant conditions by shielding electrostatic repulsion among the phosphate groups. Our kinetic studies indicated that acceleration of on rates rather than off rates for DNA assembling is a stabilization mechanism of the copolymer. Furthermore, we found that the copolymers accelerated by four orders the strand exchange reaction between a ds DNA and its complementary single-stranded (ss) DNA. It is unique that the copolymer activates strand exchange reaction while stabilizing DNA hybrids. These results suggested that the copolymer acts as a nucleic acid chaperone that reducing the energy barrier for dissociation and association of base pairs and catalyze the folding of nucleic acids into the thermodynamically most stable forms. Since G-quadruplex has polymorphic characters, it is interesting to examine effect of the copolymer on G-quadruplex folding.

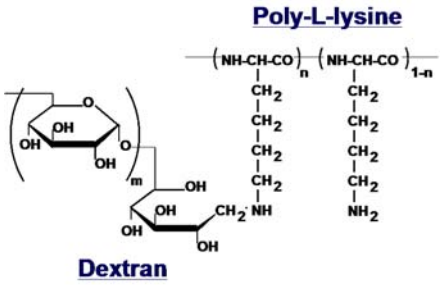

Fig. 1 Structural formula of PLL-*g*-Dex

First, we evaluated by CD measurements the secondary structure of a three and half repeats, d(GGGTTA)3GGG (G3.5), of telomeric sequence. The antiparallel quadruplexes have the characteristic positive and negative CD signals, respectively, at 295 nm and 260 nm, while the parallel quadruplex has the positive and negative signals, respectively, at 265 nm and 240 nm1. The CD spectra of G3.5 in 10 mM potassium-phosphate buffer (pH 7.2) containing 0.5 mM EDTA and 150 mM NaCl or KCl are measured. In the presence of Na^+, G3.5 shows the CD signals featuring the antiparallel conformation. In the presence of K^+, positive CD signals at both 295 nm and 265 nm were observed, suggesting mix structures or coexisting of the antiparallel and parallel orientation. These observations coincided with previous reports. In the buffer containing Na^+, the copolymer hardly influenced the CD signals of G3.5, indicating that the copolymer did not alter the antiparallel structure. In the buffer containing K^+, a drastic effect of the copolymer was observed. The positive peak at 295 nm was virtually disappeared and the strong positive peak at 265 nm was appeared. The thermal melting of the structure accompany two isochromic points in CD signal, indicating monomorphic holding of G3.5 in the presence of the copolymer. Similar results were also obtained with longer telomeric repeats. It was suggested that the copolymer stabilized a parallel structure.

We also evaluated effect of the copolymer on inter molecular G-quadruplex. The copolymer was found to accelerate significantly intermolecular folding of $[TGGGGT]_4$.

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SPECTROSCOPIC STUDIES ON BINDING OF CATIONIC AND NEUTRAL PHEOPHORBIDE-A DERIVATIVES TO FOUR-STRANDED POLY(G)

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In the last years a considerable interest and research efforts in the life science have been given to elaboration of anticancer approach based on telomerase inhibition and stabilization of telomeric G-quadruplexes by porphyrin dyes. Therefore design of various novel porphyrin derivatives and investigation of their binding to four-stranded structures formed poly(G) is very actual.

Pheophorbide-a (Pheo) is an anionic porphyrin derivative which is widely used as a photosensitizer in photodynamical therapy of tumors because of their high photosensitizing activity in vitro and in vivo. Modification of Pheo with the trimethylammonium group was carried out to obtain a cationic water-soluble dye derivative (CatPheo) capable of nucleic acids binding. In this work binding of CatPheo as well as neutral pheophorbide-a methyl ether (MePheo) to four-stranded poly(G) was monitored using absorption and polarized fluorescent spectroscopy techniques in a wide range of molar phosphate-to-dye ratios, P/D. The investigation was carried out in buffered aqueous solutions (pH6.9) of low ionic strengths (2mM Na^+).

From fluorescent titration curves it was revealed two competitive mechanisms of CatPheo binding to the quadruplex poly(G): (i) chromophore intercalation between the nucleic bases; (ii) highly cooperative outside electrostatic binding of the dye to polynucleotide backbones accompanied by the chromophore stacking. Fluorescent technique was revealed to be efficient for recognition of the binding type, because of CatPheo emission intensity increases upon its intercalation and quenches strongly upon the external complex formation. It was established that under low P/D values the outside binding was predominant, while the P/D increase results in disintegration of external complexes and prevalence of the intercalative binding. That was confirmed by substantial rise in fluorescence polarization degree and characteristic transformations in absorption and fluorescence spectra. To study pure intercalative binding mode we have investigated the interaction of neutral MePheo to four-stranded poly(G). Under high P/D values 30-fold increase in the dye fluorescence intensity was observed in comparison with those for free dye, as well as substantial rise of the fluorescence polarization degree to 0.25.

Thus, CatPheo and MePheo can be used as a fluorescent probes for recognition of G-quadruplex structure. The improved photodynamical activity for CatPheo in comparison with that for Pheo is expected because of a good water solubility of CatPheo and the efficient dye binding with polyanionic biopolymers. Besides, the strong binding of these porphyrin derivatives to poly(G) probably allows to use them in anticancer applications for targeting of G-quadruplexes of telomeric DNA.

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ON ELECTRICAL CONDUCTANCE OF GUANOSINE 5' MONOPHOSPHATE NANOWIRES

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Gunosine 5' monophosphate quadruplexes deposited on mica surface form oriented linear structures – nano-wires, composed of single qudruplex stacks. We measured the conductance of the wires as a function of temperature in air and in vacuum. In air the measured conductance shows activation energy behavior. In vacuum the conductance drops to an undetectable low value, but returns to the original one when the sample is again exposed to air. This shows that adsorbed water is necessary for the conductivity of the guanosine monophosphate nano-wires on surfaces.

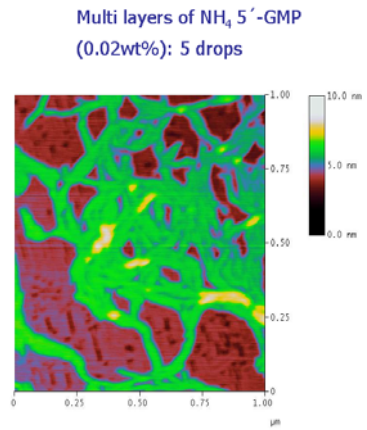

Fig. 1 AFM image of multilayer GMP film deposited on mica surface.

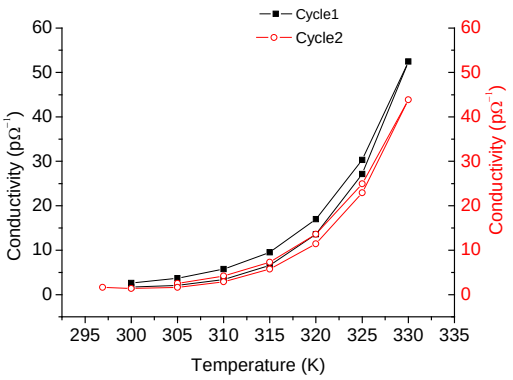

Fig. 2 Conductivity of the film as a function of the temperature.

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INTERACTION OF DISTAMYCIN A AND ITS DERIVATIVES WITH DNA QUADRUPLEX STRUCTURES

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The ends of the chromosomes in all eukaryotic species have specialized non-coding DNA sequences that, together with associated proteins, are known as telomeres. Telomere protects the ends of the chromosome from damage and recombination and its shortening has been implicated in cellular senescence. Telomeric DNA consists of tandem repeats of simple short sequences, rich in guanine residues. In the presence of metal ions such as K^+ or Na^+, telomeric DNA can form structures of potential biological significance, the G-quadruplexes.[1] Telomerase, the enzyme which elongates the G-rich strand of telomeric DNA, is active in about 85% of tumors, leading the cancer cells to infinite lifetime. The inhibition of telomerase has become an attractive strategy for the anticancer therapy and, because telomerase requires a single-stranded telomeric primer, the formation of G-quadruplex complexes by telomeric DNA inhibits the telomerase activity. Furthermore, small molecules that stabilize G-quadruplex structures have been found to be effective telomerase inhibitors and, then, the use of drugs to target G-quadruplexes is emerging as a promising way to interfere with telomere replication in the tumors cells and to act as anticancer agents.[2]

In this frame, the interactions between distamycin A and its two carbamoyl derivatives (compound 1 and 2) and DNA quadruplexes have been studied by ^{1}H NMR spectroscopy and isothermal titration calorimetry (ITC). In particular, the binding to the target $[d(TGGGGT)]_4$ and $d[AG_3(T_2AG_3)_3]$ quadruplexes from the *Tetrahymena* and human telomeres, respectively, will be reported. The interactions were examined using two different buffer solutions containing either K^+ or Na^+ at a fixed ionic strength, to evaluate any influence of the ions present in solution on the binding behaviour. Experiments reveal that distamycin A and compound 1 bind the investigated quadruplexes in both solution conditions; conversely, compound 2 appears to have a poor affinity in any case. Moreover, these studies indicate that the presence of different cations in solution affects the stoichiometry and thermodynamics of the interactions. The three-dimensional structure of the 4:1 distamycin A / $[d(TGGGGT)]_4$ complex has also been determined by an in-depth NMR study followed by dynamics and mechanics calculations.

[1] Neidle, S.; Parkinson, G.N. (2003) The structure of telomeric DNA. *Curr. Opin. Struct. Biol.* 13: 275-283.

[2] Kelland, L.R. (2005) Overcoming the immortality of tumour cells by telomere and telomerase based cancer therapeutics - current status and future prospects. *Eur. J. Cancer*. 41: 971-979.