



DNA-Based Nanowires

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DNA-Based Molecular Nanowires

Scientific Report

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(a) Introduction

In this report we summarize the activity, main achievements and critical issues of the project “DNA-Based Nanowires”. In our vision the work done in this frame is only a part of what we consider as a longer path towards the development of molecular DNA-based nanoelectronics, which will continue in the already running “DNA-based nanodevices” project and hopefully also in consecutive and parallel projects. We believe that the results achieved so far motivate further investments in the direction of this vision.

A scientific project as the one reported here is by definition of high risk and huge potential. We evaluate our performance by two general criteria. One is the bare comparison of the scientific outcome versus the main executive objectives. The other is in view of our scientific impact, namely the quality and quantity of the scientific production of the participating groups in terms of publications, presentations in conferences, patents and development of methods.

In short, before going into more elaborate descriptions, we have developed, as planned in the project objectives, novel DNA-based molecular nanowires of various sorts, covering double [1,2], triple [3], and quadruple [4] helical conformations. Most significant, we have continuously improved the control over their properties and quality, by optimizing the synthesis yield, reproducibility and protocols. The production task was an important, yet propaedeutic portion of the whole commitment, dedicated to the demonstration of self-assembling molecular wires. Thus, to implement our strategy, we have performed a variety of morphology and electrical measurements based on nanoscopic imaging and spectroscopy. All the characterization studies, both of electrical and structural nature, were backed up by a continuous effort towards physico-chemical understanding, carried out by means of a vast range of theoretical/computational/modeling approaches [5,6,7,8,9,10,11]. A central finding is a reproducible polarizability in G4-DNA wires when adsorbed on a hard surface on the background of "silent" dsDNA [12]. Direct measurements of conductivity, although with positive initial results, met many technical and reproducibility obstacles and are continued. In parallel we developed and applied new methods for the measurement of conductivity in short DNA wires [13,14]: The assessment of such methods is a primary achievement of this project, since they represent a new practical tool, reproducible in several equipped forefront laboratories in alternative ways. We proved that short DNA-based nanowires can support very high currents, implying a fast conduction mechanism. We also carried out a theoretical analysis of the charge conduction as a function of the structural/electrical details of the junction, posing limits on the current densities that can be achieved [9]. This analysis is still

far from a quantitative simulation, but establishes the grounds for an understanding of viable charge motion mechanisms through lead-DNA-lead nano-junctions.

Finally, we also pursued progress towards the development of DNA-based devices that go beyond the linear wire-like behavior. We realized that control over the nucleotide folding is a prominent goal in view of reproducibility of the electrical response. Thus, we have developed a protocol for the production of 4-stranded G4-DNA, in a unique folding fashion made of parallel strands, connected through biotin-avidin attachment. This approach will enable to insert sequence modifications at specifically pre-planned locations as a basis for the development of embedded nano-devices. These activities are only part of the large manifold of activities that is elaborated below.

As mentioned above, this project is of high risk and huge potential. It is important to mention, although we do not emphasize it further, that as is natural to such projects, the way towards the above and below achievements was full of obstacles and difficulties which could be overcome only by a concerted and extremely dedicated effort of the participating groups.

(b) Project objectives

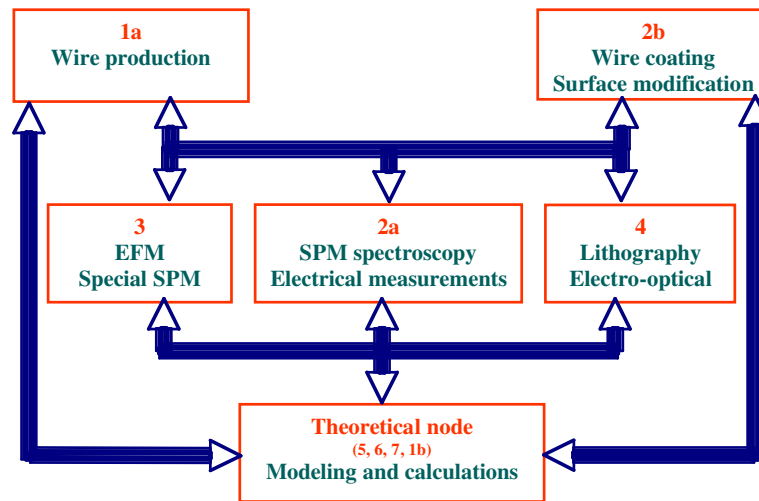
We state here the project objectives as quoted from the annex 1, our scientific contract.

“The central objective of this joint project is the development of DNA-based nanoelectronics. The project will focus on: *(i)* Production of G4-DNA, M-DNA and PC-DNA molecular nanowires and characterization of their electrical properties, *(ii)* Development of a control over the molecule-electrode and molecule-substrate interaction, *(iii)* Development of a theoretical understanding of the energetics and the conduction mechanisms in these wires, and *(iv)* Development of model nanoelectronic devices on the basis of the above DNA-Based wires. Single molecule electric conduction studies will provide insight into the so-far poorly understood mechanisms for charge mobility in DNA molecules. Finally we hope to establish first prototype single-DNA-based electronic devices.”

(c) Methodologies

- Management: Tel Aviv University (TAU-1a) – Amiram Porath; Hebrew University of Jerusalem (HUJI-2a) – Danny Porath; Italian National Research Council (CNR-5) – Rosa Di Felice.

- Enzymatic molecular synthesis, biochemistry: TAU-1a – Alexander B. Kotlyar.
- Surface chemistry, organic chemistry, polymerization techniques: HUJI-2b – Shlomo Yitzchaik; Universidad Autonoma de Madrid (UAM-3) – Felix Zamora.
- Scanning probe microscopies and spectroscopies (AFM, EFM, STM, STS) and electrical measurements: HUJI-2a – Danny Porath; UAM-3 – Julio Gomez; EPFL-4 – Eli Kapon.
- Lithography: EPFL-4 – Eli Kapon and Benny Dwir.
- Ab-initio theories and computations of the electronic and optical properties of nano-scale matter: CNR-5 – Rosa Di Felice; Universidad del Pais Vasco (UPV-6) – Angel Rubio.
- Modeling transport characteristics by empirical Hamiltonians: Regensburg Universitaet (REGU-7) – Giovanni Cuniberti.
- Kinetic theories of electron transfer: TAU-1b – Joshua Jortner.



(d) Project main achievements

Generally, the work progressed according to the plan. We briefly describe below selected highlights and achievements of the project.

- **Novel enzymatic synthesis of poly(G)-poly(C) and its mechanistic explanation.** We developed a novel method for enzymatic synthesis of μm -long uniform and continuous polyG-polyC double stranded molecules [1,2,4]. The enzymatic mechanism for the synthesis was released and made publicly available [1]. We remark that this is a central

success of the whole project: the attainment of this synthesis protocol allows us now to have DNA-based molecules of different helical motifs (double, triple and quadruple helix, see below) of controllable quality and of length ranging between hundred nm and μm . Such lengths are necessary for the envisaged technological exploitation of the molecules. On the contrary, very short segments on which almost all the single-molecule measurements on DNA are done would not allow the successive steps towards devices and self-assembling circuits.

- Production of long monomolecular G4-DNA.** The above molecules were used as the starting point for designing new procedures for complexation with metal ions and production of μm -long monomolecular G4-DNA nanowires (see Figure 1) [4]. Synthesis of the above DNA-derivatives was optimized and complemented by characterization using standard (bio)chemical methods [4], Atomic and Electrostatic Force Microscopy (AFM and EFM) [4,12], Scanning Tunneling Microscopy (STM) [2,15] and Spectroscopy (STS) [16], electrical transport measurements, and theory [5,6,7].

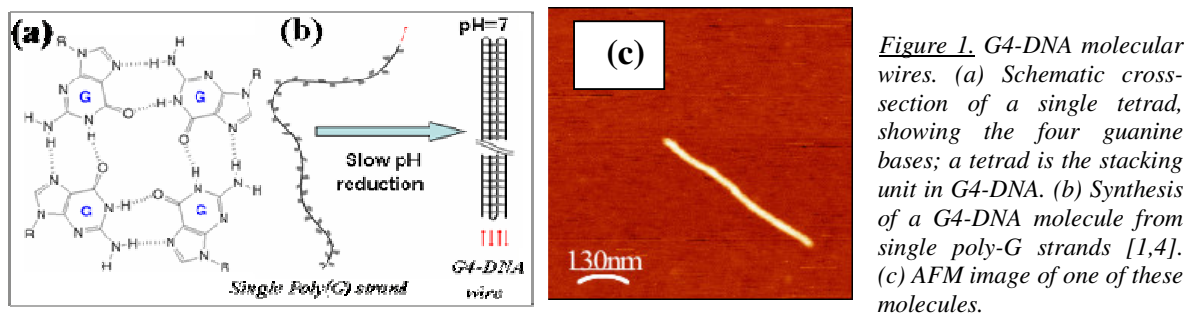
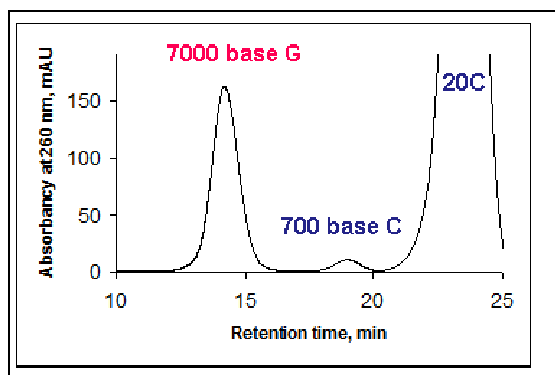


Figure 1. G4-DNA molecular wires. (a) Schematic cross-section of a single tetrad, showing the four guanine bases; a tetrad is the stacking unit in G4-DNA. (b) Synthesis of a G4-DNA molecule from single poly-G strands [1,4]. (c) AFM image of one of these molecules.

The efficiency of the method for the synthesis of G4-DNA was optimized. G4-DNA was originally synthesized from long polyG-polyC double-stranded DNA that is produced enzymatically with Klenow exonuclease minus DNA polymerase. The strands were separated chromatographically at high pH, and the G4-DNA molecules were formed upon lowering of the pH. It was found that dCTP (cytosine tri-phosphate) can be replaced during the enzymatic synthesis with an oligomer of cytosine (typically 20-mer), to obtain a double helix of polyG with unconnected oligomers of C. This improved dramatically the separation yield of the polyG from the fragmented oligomers of C under basic conditions, and hence enabled us to obtain much higher yields in G4-DNA production, although less size uniformity on the surface is observed (Figure 2) [17].



*Figure 2. Size-dependent HPLC of poly(dG)-n20(dC) at high pH. poly(dG)-n20(dC) synthesized with Klenow *exo*— as described in Materials and Method was pretreated for 15 min at room temperature in 0.1 M NaOH. 170 μ l of DNA sample was applied onto TSKgel G-DNA-PW column (7.8x300 mm) and eluted at room temperature with 0.1M KOH at a flow rate of 0.5 ml/min. Elution was followed at 260 nm. Manuscript in preparation.*

• **Synthesis of 4-stranded G4-DNA through biotin-avidin attachment.**

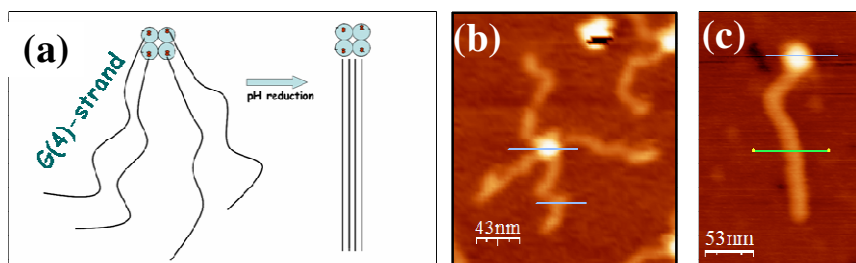


Figure 3. Formation of 4-stranded G4-DNA. (a) scheme of the formation through biotin-avidin attachment. (b) 4 monomolecular “tails”. (c) 4-stranded molecule.

Attempts to investigate the conductivity of G4-DNA with 4 parallel strands (rather than the other possible folding motifs), together with the improved control on the sequence of each parallel strand, drove us to develop a new nanostructure. This structure is composed of 4 strands that are connected through biotin molecules: A G-strand is connected at its 5' end to a single biotin molecule, and then the biotins couple to avidin tetramers, as sketched in Figure 3a. Thus, a change in the sequence of one of the strands may be used to impose an electrical non-linearity, namely an embedded nanodevice. So far we have been able to construct avidin with 4-attached molecules (either 4 poly(G)-poly(C) or 4 monomolecular G4-DNA). Then by opening the dsDNA or unfolding the G4-DNA at high pH we were able to obtain the desired 4-stranded G4-DNA. The scheme of the nanostructure and AFM images of the avidin with 4 tails and finally 4-stranded appear in Figure 3.

• **Synthesis and characterization of novel triplex nanostructures and their growth.**

We have demonstrated that the extension of the G-strand of 700 base pairs poly(dG)-poly(dC) by Klenow *exo*⁻ fragment in the presence of dGTP yields a complete poly(dG-dG)-poly(dC) triplex [3]. HPLC analysis of the synthesis products shows that the amount of dG-bases incorporated into the dG-strand during the synthesis is equal to the amount of dG-bases in poly(dG)-poly(dC); the length of the poly(dG)-strand is thus doubled during the synthesis. Direct AFM imaging of the molecular morphology (see Figure 4a) shows that the poly(dG)-poly(dC) and the poly(dG-dG)-poly(dC) have almost the same length, and that no single-stranded fragments are present in the synthesized product. These data

strongly indicate that the *de novo* G-strand is associated with the poly(dG)-poly(dC) duplex. The doubling of the poly(dG) strand was monitored by FRET analysis (Figure 4b).

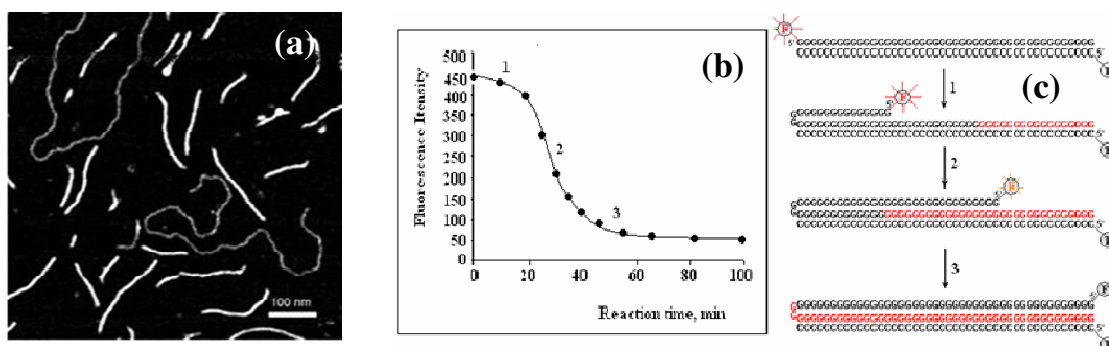


Figure 4. (a) AFM image of synthesized poly(dG-dG)-poly(dC) molecules co-deposited with a linear plasmid pSK+ DNA (scale bar = 100 nm) on mica. The plasmid DNA can be easily distinguished due to its lower height (lower gray level) and much longer length. (b) Kinetics of Flu emission change during the extension of G-strand of Flu-(dC)₂₂₀-(dG)₂₂₀-TAMRA by Klenow *exo*⁻. The reaction was started by addition of 20 μg/ml Klenow *exo*⁻ to the cuvette containing: 100 mM Tris-Acetate, pH 8.0, 1.2 mM MgCl₂, 5 mM DTT, 1.0 mM dGTP, and 0.2 μM Flu-(dG)₂₂₀-TAMRA-(dC)₂₂₀ duplex and followed in time at 37°C by monitoring Flu emission at 520 nm; excitation was at 490 nm. (c) Schematic presentation of the intermediate products of the synthesis: F denotes for Flu, T for TAMRA. Emission of Flu in 220 base pairs long poly(dG)-poly(dC) is not quenched by TAMRA attached at the opposite end of the DNA molecule.

• **Development of a new protocol for G4-DNA with K⁺ ions and strong and reproducible length dependent EFM polarizability signal.** In the course of the attempts to reproduce and control the production of molecules with the above properties we found out that molecules prepared with K⁺ ions gave a clear and strong electrostatic force microscopy (EFM) signal. This signal originates from the electrostatic interaction between a metallized tip and a polarized sample – in this case G4-DNA. Such polarization is a clear indication of possible conductivity (measurements are under way). This clear signal was found in 7 consecutive batches that were prepared with K⁺ ions, and not in G4-DNA prepared in different ways. To further support these results we used and optimized a sensitive procedure for measuring the signal (3-D mode). Using this mode we find that the EFM signal depends on the length of the molecules. It was observed for molecules longer than 70 nm, indicating very high sensitivity of our operation. This is a significant step towards the development of conducting molecules as described above. The results of the EFM measurements are shown in Figure 5.

These measurements were followed by more sophisticated measurements using the 3-D modes methods. These measurements confirmed the above results and provided a clear length dependence of the polarizability signal. Further, the results were complemented by a model that accounts for the observed length dependence of the signal.

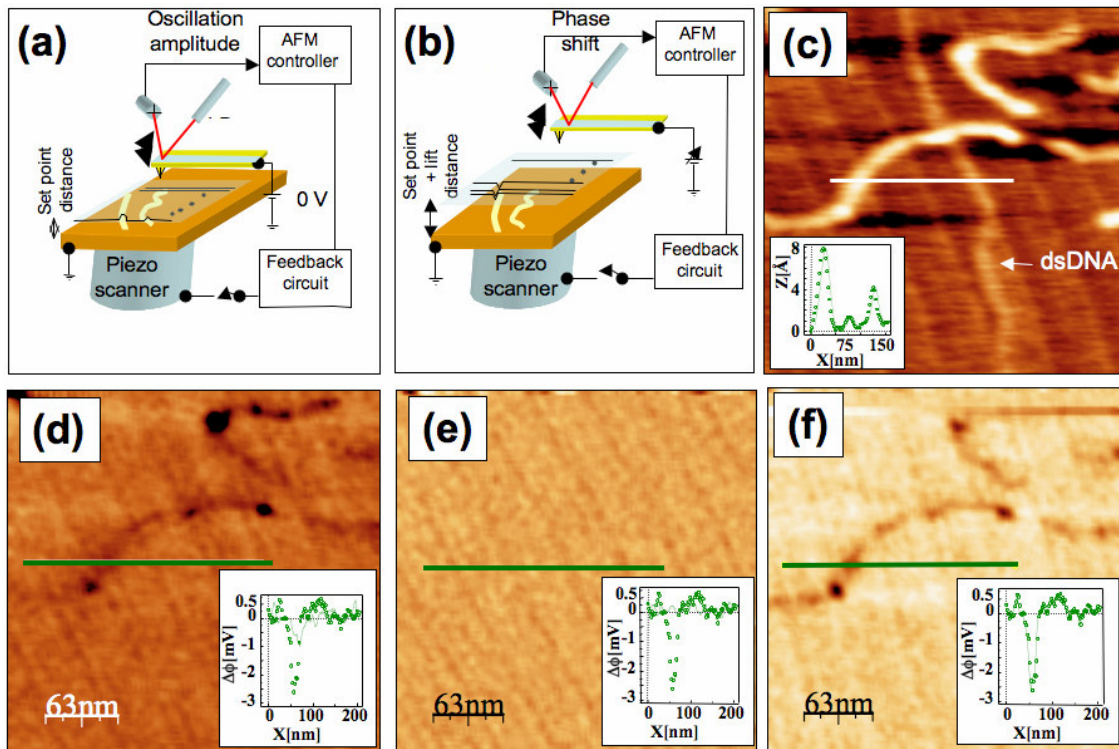


Figure 5. (a) and (b) show schematic views of the EFM “plane mode” method. (a) A topography image is acquired and its plane parameters are recorded. (b) Then, the tip is lifted and the phase shift is measured with disconnected feedback at lifted height parallel to the previous image plane, with various bias voltages applied to the tip. (c) AFM topography image of the measured co-adsorbed G4-DNA (a batch of G4-DNA molecules made of ~3200-base poly(G) strands was used) and dsDNA with a cross section showing the height profile of the molecules. The G4-DNA appears about twice higher than the dsDNA. (d-f) Phase shift images of the same area in “plane mode” at -3 V (d), 0 V (e) and +3V (f), showing clearly that the phase signal shifts only above the location of the G4-DNA and only when applying bias voltage. The tip was lifted by 14 nm above the set point value, which was ~20 nm above the surface, as extracted from the force-distance (F-Z) calibration. The negative phase shift, presented in mV, indicates a decrease in the frequency of the tip oscillations. Line profiles show the magnitude of the signals at the different voltages (insets).

- High currents in DNA by the “standing DNA” method.** Direct electrical transport measurements on a complex sequence of 26 base-pair double-stranded DNA showed high currents (220 nA@2V) [13,14]. These experiments were performed when the ssDNA molecules are arranged in a monolayer on a gold surface. Some of them hybridize to a complementary strand that is attached to gold nanoparticles at the opposite ends and no non-specific interaction along the molecule with a hard surface occurs. A conductive AFM tip is utilized to both scan the sample and contact the gold nanoparticles that are connected to the surface through the dsDNA. The experimental configuration, an AFM image of the surface covered with ssDNA and dsDNA connected to nanoparticles and a collection of typical I-V measurements is shown in figure 6 below. A comprehensive set of control experiments, including I-V curves obtained while stretching the DNA molecules, confirms these results. The results of these experiments have been recently published [13]. Further

experiments utilizing this experimental method are under way to characterize sequences of varying length, sequence and of G4-DNA.

Further measurements were performed on monolayers of ssDNA, dsDNA connected with thiols only on one end to the surface and on dsDNA thiolated on both ends. These measurements were done without the gold nanoparticles, confirmed the above results and demonstrated the crucial role of the covalent attachment of the molecules to the electrodes [14].

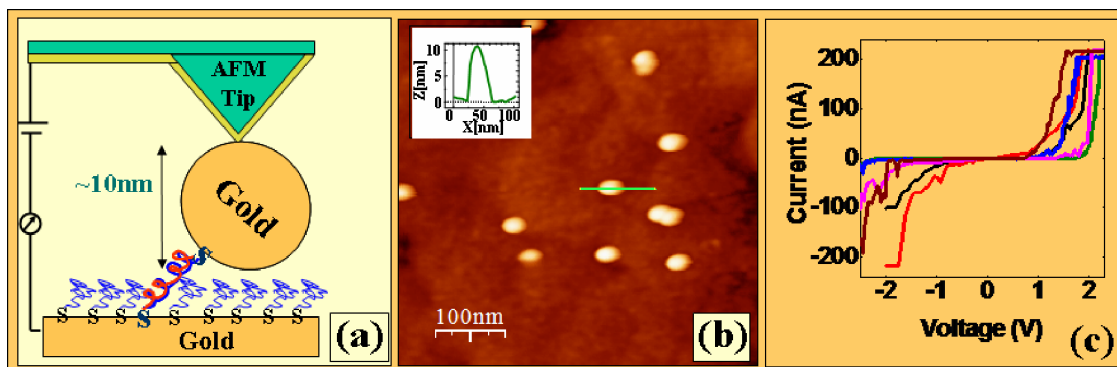


Figure 6. 26 base-pairs long dsDNA of complex sequence connected to a metal substrate and a 10 nm gold particle on opposite ends using thiol groups. (a) Schematic presentation of the measurement configuration. (b) AFM image of the gold nanoparticles ($250 \times 250 \text{ nm}^2$). and (c) collection of I-V curves, showing high current density measured on different samples and in different occasions.

- **STS of single poly(G)-poly(C) DNA and scaling behavior in DNA.** Scanning tunneling spectroscopy is a unique tool for investigating, nearly in a direct way, the density of states in single molecules. This was demonstrated for various molecules and nanoparticles in the past but in a very limited way for DNA, in spite of the immense interest in this molecule, due to the complexity and technical difficulty in performing STS on single DNA molecules. We were able to measure reproducible and informative STS of poly(G)-poly(C) and extract large statistics on the energy levels and on the width of the energy gap of the molecule [16]. An STM image of poly(G)-poly(C) molecules (taken before the electrical STS measurement), and the measurement scheme, are shown in Figure 7.

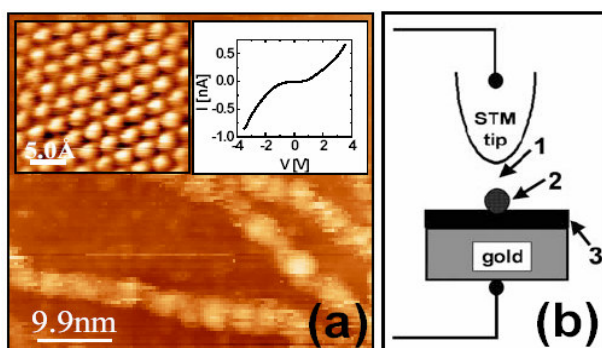


Figure 7. (a) STM image of poly(G)-poly(C) DNA molecules on which STS measurements were performed. (b) Schematic illustration of the double barrier tunnel junction configuration of the experimental layout. The first tunnel junction is formed between the STM tip and molecule (indicated as #1). The DNA molecule profile is marked as #2, and the second junction (marked as #3) is between the molecule and the gold surface. The left inset shows gold atoms and the right inset shows I-V on bare gold.

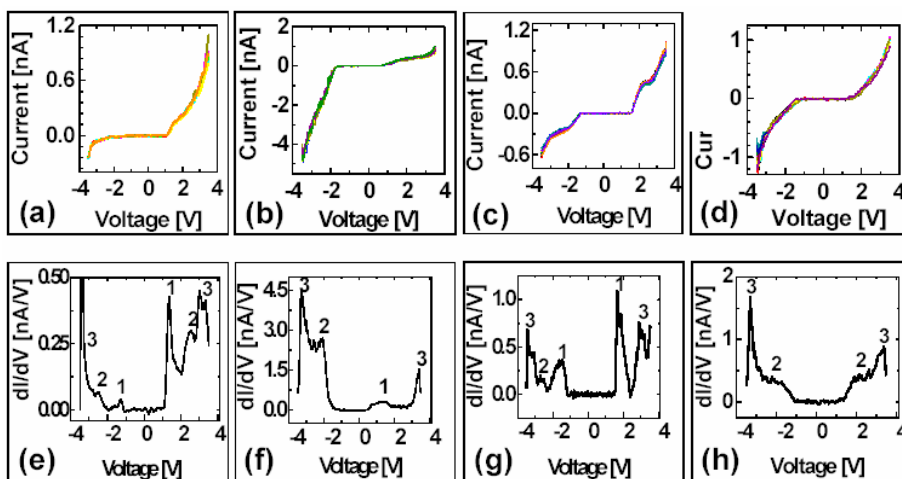


Figure 8. Results of the STS measurements are presented in the following graphs of the I - V and the corresponding derivative (dI/dV - V) characteristics. The tunnel junction parameters in all the measurement were set to V_{bias} of 2.8 V and I_{set} of 0.5 nA. (a-d) Each panel shows 10-14 I - V curves; each panel refers to measurements on a single molecule, so measurements on 4 different molecules are shown in the different panels. An excitation gap is evident in each set of measurements. (e-h) Each panel shows the derivative of the average I - V of the corresponding set in the upper row.

The STS results are shown in Figure 8, where we present the I - V and corresponding dI/dV , which is related to the density of states.

We used *ab-initio* calculations to relate the peaks in the dI/dV to the electronic level structure of the molecules [16]. The next step toward a more complete theoretical description of the STS data consists of going beyond the approximate DOS-based description of the tunneling current. This approximation is exact only at $T=0$ and if the current depends solely on the molecular features, not affected by the presence of the substrate and by the specific coupling between molecule and substrate (coupling strength, orientation, etc.). We are aware that this is certainly not the case, but we are limited by the fact that the description of the real experiment in an *ab initio* fashion is beyond present computational resources. Therefore, we are also performing model calculations with empirical tight-binding Hamiltonians to take into account: (i) the non-equilibrium situation under applied voltage, for which the measured conductance may deviate from the ground-state DOS; (ii) environment-induced effects due to the presence of the substrate. By sacrificing on one hand the *ab initio* accuracy and absence of assumptions concerning the electronic structure, we thus gain the flexibility of exploring in a more qualitative way situations that may be closer to the reality. Non-equilibrium calculations that address the effect of the surface on the measurements through the frontier orbitals are underway.

The STM characterization lead to the observation of contrast reversal in STM imaging (also in a controlled way) and the development of a theoretical model to account for the phenomenon [15].

- **Apparent reduction of the energy gap upon metallization measured by STS.** Preliminary scanning tunneling spectroscopy of polyG-polyC molecules indicated a band gap of about 2.5 eV, which was lowered by about 0.5 eV upon complexation with Cu^+ cations (Figure 9).

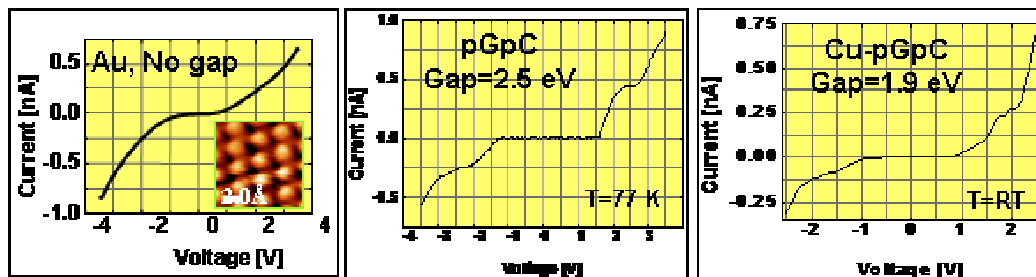


Figure 9. (a). Scanning tunnelling spectroscopy on bare Au (a) (inset shows an STM image with atomic resolution), on a poly(G)-poly(C) DNA molecule and on a poly(G)-poly(C) DNA molecule metallized with Cu.

- **Empirical modeling of current-voltage characteristics of DNA-based wires between electrodes.** As a preliminary experimental-theory collaboration before the beginning of the project, a simple fishbone model was initially formulated and applied to investigate the role of nucleobase-backbone coupling in the electrical behavior: the evidence of an excitation gap in measured transport curves was explained [18]. Later, the model was refined to take into account the environmental effects, in particular the presence of a solution represented as a thermal bath. The results show important modifications of the quantum conductance at the Fermi level due to electron-phonon coupling, and allow for an interpretation of recent experiments [10,11]. We remark that this theoretical activity, devoted to environmental effects, was undertaken as a follow-up to reviewers' comments at the first-year evaluation, which encouraged a tighter experiment-theory connection and concerted research efforts.
- **Modeling of STM images.** To explain experimental evidences of contrast inversion in DNA imaging, an empirical model to compute the tunneling current in term of Fermi distribution and scattering amplitude was formulated [15]. The phenomenon of contrast inversion can be described by accounting for indirect tunneling through virtual states between the STM tip and the DNA molecule, which are created by the curvature of the field lines due to irregular charge distribution.

- **Classical molecular dynamics calculations of G4-wires of different lengths and inner cation coordination.** It is found that the metal species in the inner channels affects the overall stability of the quadruple helical conformation. The shorter the quadruplexes, the more sensitive they are to the degree of coordination and they may unfold when they are not completely filled with metal cations. Instead, long quadruplexes can remain in the folded state even in the absence of inner cations or in the presence of the smallest Li ions [7] (Figure 10). These simulations were undertaken to explain some aspects of the synthesis.

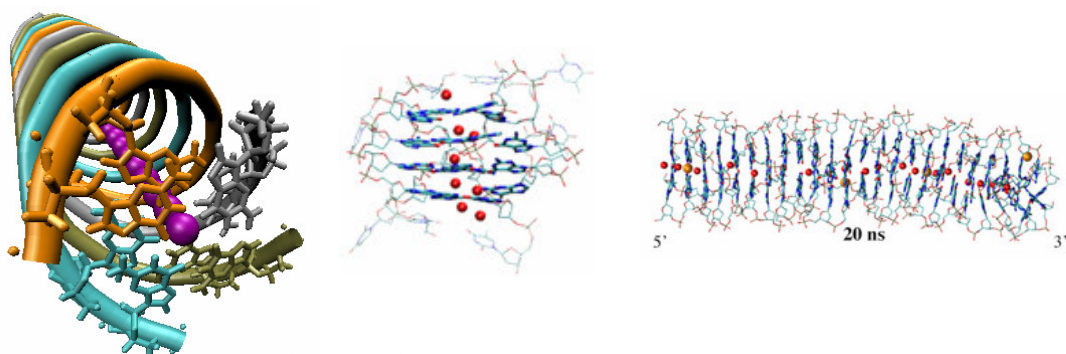


Figure 10. Left: three-dimensional representation of a G4-wire, where the purple spheres represent the inner cations. Middle: a short 4-plane G4-wire becomes distorted at the edges and tends to unfold in the absence of inner cations on a time scale of 3.5 ns (the counterions in solution do not enter the channel and are not shown, the red spheres represent water molecules). Right: an initially empty long 20-plane G4-wire persists in the regular quadruplex conformation on a time scale of 20 ns (few Li ions and several water molecules enter the channel).

- **First-principle DFT calculations of the electronic and optical properties of various DNA-based assemblies, including G4-wires.** The electronic structure of periodic G4-wires indicates that a pure band-like conduction mechanism is unlikely. However, the π - π overlap between consecutive planes originates channels for helectron/hole motion [6,19] (see Figure 11). The electronic structure parameters (band gaps and widths) are very sensitive to various geometrical factors, such as twisting and axial strain [5]. The optical properties of nucleobases and their assemblies were computed from first principles in the framework of Time-Dependent DFT for the first time, and compared to available

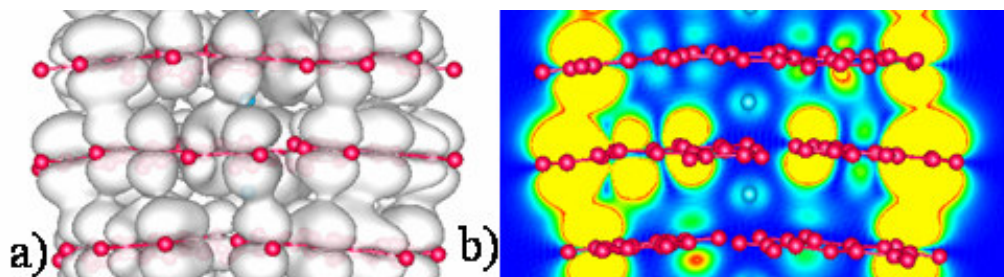


Figure 11. (a) Isosurface plot of the convolution of a manifold of electron states derived by the HOMO of guanine. (b) Contour plot of the same convolution, on a plane perpendicular to the tetrameric planes and containing the inner K ions: no significant metal-guanine coupling is revealed.

experimental data with success [8]. The optical investigations are currently continuing with the implementation and test of circular dichroism (CD) spectra at the same level of theory: reliable CD spectra computed in this way can be employed for the interpretation of in-situ post-synthesis data, since CD is often used for routine characterization.

- **Incoherent charge hopping and conduction in DNA and long molecular chains in the context of electron-transfer theory.** Some relations have been established between the kinetics of incoherent hopping charge transport in bridged large scale chemical systems or in a single-component duplex DNA, and the electrical properties of these systems connected between two electrodes [9]. The kinetic model can be applied for the description of incoherent charge transport in a donor-acceptor pair connected by two electrodes. In summary, different behaviors of the current are found in the low-voltage and high-voltage regimes. In both limits the current intensity depends on the details of the voltage drop through the junction and on the lead-molecule interfaces, which are still to be unraveled before a quantitative theory is disclosed for the direct simulation of experimental conditions.
- **Ongoing theoretical efforts.** (i) Collaboration with experimental groups to explain STM spectroscopy data in terms of molecular Density of States. (ii) Collaboration between

different theory groups to interface the ab-initio and the model-hamiltonian calculations for G4-DNA. (iii) Ab-initio TDDFT computation of CD spectra for GC pairs, GC pairs complexed with metal ions, double helical short poly(G)-poly(C) fragments.

- **Additional activities.** The above results are only a handful of highlights extracted out of the large amount of scientific activity performed during the “DNA-based nanowires” project by its partners. This work was accompanied by many other important results: lithography on graphite to direct the positioning of the DNA, high resolution imaging and investigation of various DNA derivatives, measurements of electrical transport through molecules between planar electrodes, attachment of molecules to metal surfaces by phosphotioate groups along the DNA backbone, polymer coating of DNA by polyaniline and many more activities.

Side by side with these activities we performed a lot of routine synthesis and characterization feedback loops between the synthesis and measuring groups. Similarly an intense collaboration took place between the experimental groups and theory groups to model the structures, analyze and fit the measurements results and to guide the experimental activity.

(e) Critical activity survey in view of the project deliverables

In this part of the report we provide a short critical review of the planned deliverables and add a statement of what has been achieved and what not. Then we shortly summarize the current status of the progress towards achieving conducting DNA-based nanowires.

D1.1, D1.2 – M-DNA (DNA doped with metal ions in each base-pair) molecules with metal ions and their preparation procedure:

This route was not fully explored in spite of promising initial results in the first periods of the project (electrical transport on a large number of molecules and HOMO-LUMO gap reduction upon metallization as observed by STS). The main reason is the focusing of efforts and resources in what seemed (and still does) a more promising direction of the G4-DNA. Among the variety of tested candidates that were suggested in the proposal (M-DNA, PC-DNA, G4-DNA), the choice of G4-DNA to go forward is motivated by the following results: higher stiffness relative to double-stranded molecules, higher regularity due to the sequence uniformity, success in optimizing the synthesis and possibility of driving the synthesis through recognition capabilities (biotin-avidin and others), easiness of inserting non-linear embedded devices by just changing one or few bases in the parent poly(G) strand. We note that metal doping is relevant to G4-DNA as well.

D1.3, D1.4 – G4-DNA molecules with metal ions and their preparation procedure:

This direction was one of the major successes of the last year. We developed a procedure for reproducible production of G4-DNA molecules with K^+ ions that show a clear length dependent EFM signal [16]. It is not yet clear why this specific procedure leads to the desired signal and this issue continues to be investigated. We check the correlation between modifications of this preparation procedure and the resulting EFM signal. Direct electrical transport measurements in these molecules were not yet successful due to technical problems in the evaporation procedure that is very delicate.

D1.5, D1.6 – PC-DNA molecules of various length, intercalators, and metal-ion composition:

The progress along this route was limited in the first two years of the project. In the last year we were finally able to produce and characterize alternating monolayers of DNA and polyaniline (PAN) and also polymerize single molecules [20]. The observations are supported by AFM, impedance and CV measurements. No electrical transport or EFM was measured on the molecules yet. This work is in progress: we expect that it will bring more scientific insight, but it will not be a central effort, as explained above.

D2.1, D2.3 – Metal surface monolayer coating for SPM and transport characteristics:

The original aim of this deliverable was to create an anchoring layer on metal substrates for STM imaging and on electrodes for controlling the molecule-electrode contacts in transport measurements. In practice this was achieved by two different approaches. In the first approach the DNA was anchored to a gold surface using an ATP_h layer for the alternating DNA/PAN monolayers. In the other approach the poly(G)-poly(C) was grown while using nucleotides with phosphothioates (PT) connected along the phosphate backbone. This way the PT enable the molecules to efficiently attach to metal surfaces and electrodes. Molecules prepared in this way are also folded to the G4-DNA structures. This way the charge injection to the molecules can be improved.

This deliverable was also implemented within the preparation procedure of another system – the "standing DNA", where the molecules are connected to the metal surface through thiols and $(CH_2)_3$ linker. The charge injection is indeed well optimized in this way.

All these routes will be useful and further developed along the way towards DNA-based nanodevices.

D2.2 – Insulator surface monolayer coating for SPM and transport characteristics:

One aim of this deliverable was to create a "soft monolayer" in between the electrodes to minimize the effect of deforming surface forces on the molecules and on electrical transport through them. This was not yet achieved.

The other aim of this procedure was to create a buffer layer between the hard surface and the molecules for imaging. This was achieved on HOPG surfaces (not suitable for transport) and indeed the molecules' "apparent height" is larger, thus suggesting that the coating monolayer is indeed effective in minimizing the deforming influence of the hard surface. This procedure may be exploited in our future activities towards devices.

D3.1 – SPM spectroscopy of the various molecules:

SPM spectroscopy of the various molecules was performed by both STM and AFM. We have now excellent STS characterization of poly(G)-poly(C). This characterization lead also to the observation of other very interesting scientific phenomena: contrast inversion and scaling behavior. We do not yet have satisfactory STS for G4-DNA and partial STS on M-DNA. The AFM spectroscopy (standing DNA) is described in another deliverable.

D3.2 – SPM imaging characterization of the various molecules:

This task has been one of the most resource-consuming tasks all along the project and requested a lot of effort by all the measuring groups in order to meet the growing need for imaging feedback by the synthesis groups. The efforts were directed to imaging and also statistical analysis of the parameters describing the molecules and also to high resolution imaging with special sharp tips. We initially underestimated this task and had to face the unexpected demands, as much as possible without affecting the other activities. This synthesis-morphology feedback work was emphasized because it was found extremely effective to select the synthesis conditions that produced the molecules most suitable to be deposited on substrates in such a way that the structural features of the helical motif were conserved at best.

D3.3 – EFM analysis of the polarizability of the molecules:

As mentioned above in D1.3, we observed a clear and reproducible, length dependent, EFM signal for several batches of K^+ G4-DNA [12]. The polarizability signal suggests that these molecules are good candidates for conducting nanowires, although we still have to prove the conductance in a reproducible way through direct measurements.

In addition, EFM has been used as one of the routine characterization tools for samples that appear to be promising.

D4.1 – Conductance measurements through molecules between electrodes:

Measurements were performed between planar electrodes that are tens of nanometers away on large numbers of molecules. Conductivity was observed in some cases but we focus on measurements on single molecules before drawing conclusions from these measurements. We are now developing new methods to perform conductance measurements when a single molecule is connected to two metal nanoparticles. This way we will be able to optimize the contacts and avoid surface deformations on single molecules.

D4.2 – Conductance measurements through molecules between electrodes:

We have established and exploited the method of "standing DNA" measurement configuration and measured relatively high currents through 26 bp long dsDNA (>200 nA@2V). We also measured the conductance of ssDNA, dsDNA and dithiolated dsDNA using a 3D mode of AFM. An interpretation of these measurements in terms of viable "transport" mechanisms was attempted with the help of the theory groups, by interpreting the existing literature and by new modeling developments.

Attempts to perform measurements between a fixed metal electrode covering part of the molecules (lying flat of the surface) and a conducting AFM tip were made, so far with no conclusive results, probably due to evaporation problems.

D4.3 – Conductance measurements under optical excitations - canceled:

D5.1-5.3 – Theory:

Ab-initio methods to compute the quantum conductance of molecular wires between electrodes, within scattering and TDDFT approaches, were developed. Due to their huge computational demands, these are not yet applicable to DNA molecules. Therefore, the theoretical investigation of DNA molecules between electrodes was limited to semi-empirical models, whereas atomistic calculations were done to determine the conformation-dependent electronic structure. The computation of optical properties by TDDFT was delayed from the first to the third year, because of computational difficulties, and was not yet feasible on guanine-tetrad structures.

In a critical view, we realized that ab-initio theories in the framework of DNA electronic structure calculations still need much progress. To compensate for this limitation, several additional tasks were added to the original plan, in order to meet the experimental demands

for interpretation and planning in an efficient way. We believe that the connection between the various theory groups, and between theoretical and experimental activities, were tightened, and we currently have several joint activities that bear a promising potential into the project continuation. For instance, we are extracting ab-initio tight-binding parameters to plug into model Hamiltonians for double-stranded molecules and for G4-wires; *ab initio* DOS calculations for a direct comparison to the measured STS spectra are ongoing, in parallel to model calculations of the tunneling current under applied bias to evaluate the effects of non-equilibrium conditions and of the measurement setup; circular-dichroism spectra for feedback to synthesis procedures will be computed by TDDFT to unravel the role of metal-DNA complexation, after an ongoing method assessment.

What we feel is still missing and deserves much attention in the future developments is the establishment of a common framework for electron-transfer-theory and bandstructure-theory, which would finally allow a conciliation between measurements of transfer rates in solution and measurements of conductivity in device configuration. Although we are working in this direction, an ultimate deliverable “product” of this effort can only be envisaged but not promised. Intermediate steps are clear and pursued: theoretical advances and software packages that can be made available to the scientific community. This goal is of invaluable importance for the whole scientific community interested in molecular nanoelectronics, and will continued to be pursued in the following phases of our collaboration.

D6.1 – Lithographically prepared electrodes:

Electrodes were prepared and used in the various tasks and deliverables.

D6.2, D6.3 – DNA-based devices and procedures:

We did not develop DNA-based devices in the original planned manner and this task is to be performed in the frame of "DNA-based nanodevices" project. We did, however, make progress in this direction by constructing complexes in which 4 G-strands are connected through biotin to an avidin. This will enable to construct embedded devices in the wires already in the synthesis process. Note that our definition for devices here was and still is different from the "classical" device definition and reflects our conceptual approach to nanoelectronics: it consists of “devices **embedded** in the wires”.

Summary of deliverables survey:

This survey summarized the project activity (what was done and what not) within the frame of the promised deliverables. In addition, we performed additional tasks which were not

originally planned and may deviate from the deliverables or be complementary to them, *e.g.*, the production of triplex DNA, MMX polymers that are planned to be hybridized with DNA etc..

We note that the scientific outcome in terms of publications, presentations, workshop and general knowledge and procedures was an important and massive outcome of the activity in the project, resulting in a large number of publications and presentations.

To summarize the current status in view of the goal of producing DNA-based nanowires: We have developed several candidates for nanowires and characterized (not fully) their properties. The leading candidate is G4-DNA with improved stiffness and persistence length. The K^+ -G4-DNA that shows reproducible polarizability may be the wire that we need or it may be the basis for further improvement of its properties towards conductivity. Therefore, on the basis of this result, we believe that the task of producing DNA-based conducting nanowires is achievable. We note that the other candidates were not fully explored in spite of promising initial results due to priority order.

(f) Summary and Future vision

DNA is a fascinating soft material that naturally expresses two of the three main features required from molecular nanoelectronic components, namely recognition and specific structuring (sequence, length). The third additional property that is needed in order to implement DNA-derivatives for electrical device applications is conductivity, still quite disputable in native-DNA.

The project “DNA-based Nanowires” demonstrated the development of novel DNA-based nanowires, evolved from modifications of the native double helix, that show encouraging polarizability signals while preserving the structuring and recognition qualities of natural nucleic acids. The same core partners now proceed to the more ambitious goal of realizing DNA-based nanodevices on the ground of the disclosed nanowires, by maturing to a full control over the molecular structure and electric response. Our strategy is to use specific alterations of the sequence and inclusions of hybrid inorganic elements to pre-planned locations in the wire. A device resulting from this approach will be nanometric in size and embedded in the wire itself. The central material of choice for the following stages towards DNA-based nanoelectronics is G4-DNA, for the reasons explained above, although we will continue to devote attention to other possibilities.

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