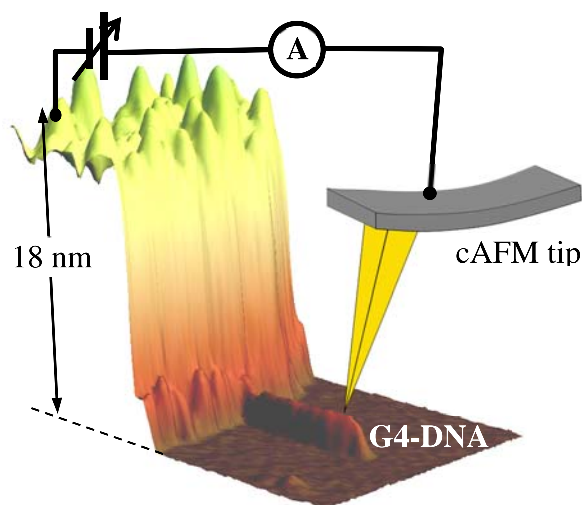
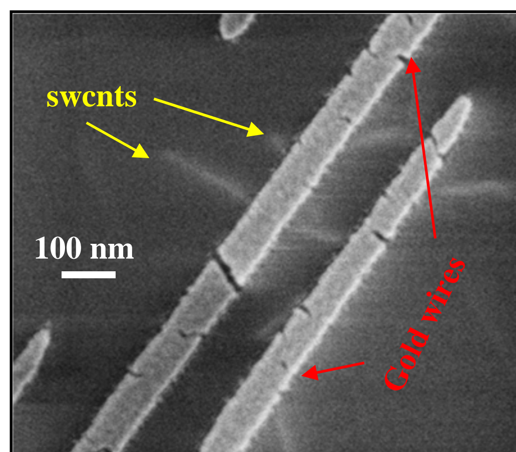
Development of novel electrical characterization methods and measurements of DNA derivatives

The structural and electronic properties of DNA have inspired its possible role as a conductive wire in solid-state molecular electronic devices, with sufficient delocalization for the transport of charge along the molecule. This oversimplified view, however, does not take into account the deleterious effect of the surroundings on this soft biomolecule. Moreover, the variability of published data, both in molecules and experimental designs, has produced a wide range of partial or seemingly contradictory results, highlighting the challenges to perform well-controlled and reproducible experiments, as well as to transport significant current through individual DNA molecules.

In this talk, I describe my research in the lab of Prof. Danny Porath at the Hebrew University of Jerusalem to address these challenges. I report a new platform for electrical measurements of individual biomolecules, where strong reproducible electrical coupling to the molecule is achieved by very precise metal deposition through electrostatically-clamped stencil masks. We applied this technique to study various types of molecules: micron-long dsDNA, guanine-quadruplex DNA (G4-DNA) [1], short segments of single-wall carbon nanotubes (SWCNTs), multi-wall carbon nanotube (MWCNTs) bundles and networks, SWCNT-dsDNA-SWCNT hybrid nanostructures, and a new form of metallized DNA, called E-DNA. In particular, conductance along G4-DNA displayed non-trivial length dependence, compatible with a long-range thermally-activated hopping mechanism between multi-tetrad segments [2]. Improvements in biochemical synthesis and electrode design have enabled higher currents over longer distances [3]. I discuss these preliminary results, as well as the mechanisms for nanometer-sharp patterns and metal penetration.





References

[1] Livshits, G. I. *et al.* Comparative electrostatic force microscopy of tetra- and intra-molecular G4-DNA, Adv. Mater. **26** 4981, (2014).

[2] Livshits, G. I. *et al.* Long-range charge transport in single G-quadruplex DNA molecules, Nat. Nanotechnol. **9** (12), 1040-1046 (2014).

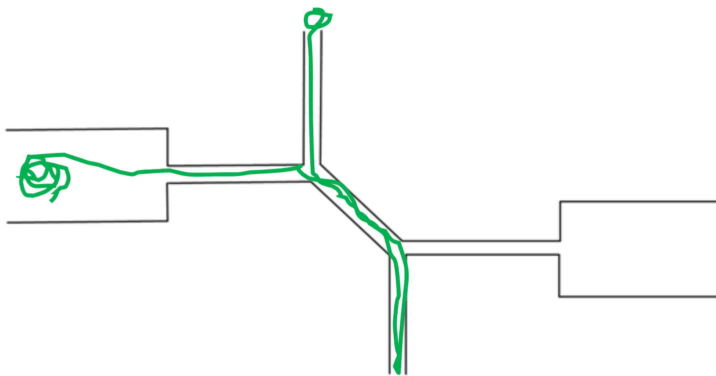
[3] Livshits, G. I. Development of novel electrical characterization methods and measurements of DNA derivatives, PhD dissertation, Mar. 2015, The Hebrew University of Jerusalem.

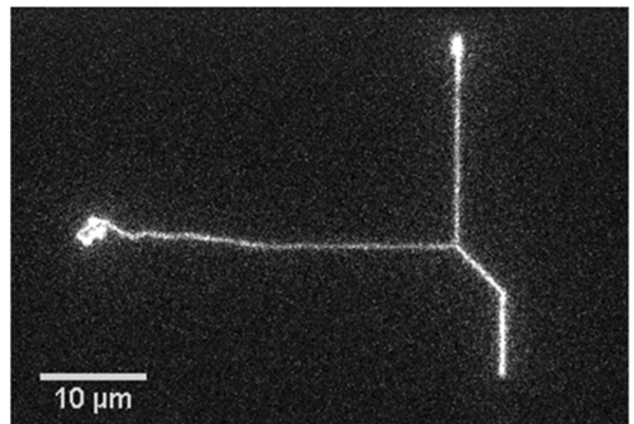
Protein-DNA interactions confined to nano-channels: diffusion and compaction

Due to self-avoiding interactions, large dsDNA molecules (>50 kb) stretch out when confined to long (~10-100 μm) nanofluidic channels with cross-sectional dimensions comparable to the persistence length (~100×100 nm^2). Such confined environments increase the likelihood of DNA-DNA pairing and DNA-protein interactions, and reveal effects that are only accessible at these length scales [1]. In this talk, I report on preliminary results in two projects in which I was involved during my post-doc in the labs of Profs. Riehn and Wang in the Physics Department at North Carolina State University.

In Project 1, we observed motor-like DNA motion due to an ATP-hydrolyzing protein. Specifically, λ-DNA confined to quasi-1D nanochannels exhibited super-diffusive motion under the action of the enzyme T4-DNA ligase in the presence of all necessary co-factors. This is possible due to cooperative interactions of the proteins with the DNA and the channel walls, inducing directed motion of the DNA molecule inside the nanochannel. Here we show directed motion in this configuration for three different proteins (T4-DNA ligase, MutS, E. Coli Ligase) in the presence of their energetic co-factors (ATP, NAD+) [2].

In Project 2, we observed real-time compaction of a synthetic telomeric DNA substrate (>100 kb) in the presence of the duplex TTA-GGG repeat binding factor-1 (TRF1). By fluid flow, we manipulate the DNA into a cross junction, formed by two intersecting nanochannels perpendicular to each other. Allowing the TRF1 protein to diffuse into the junction from a third nanochannel, we monitor its reaction with the DNA. Clear evidence of irreversible compaction is recorded for high concentrations of the protein, whereas lower concentrations slow its motion inside junction or affect its local geometry, but have no irreversible effect.





Example of telomeric DNA looping inside Double-Y junction

References

[1] Roushan, M. *et al.* Probing transient protein-mediated DNA linkages using nanoconfinement Biomicrofluidics **8**, 034113 (2014).
[2] Roushan, M. *et al.* Motor-like DNA motion due to an ATP-hydrolyzing protein (**In Preparation**).
[3] Lin, J. *et al.* TRF1 and TRF2 use different mechanisms to find telomeric DNA but share a novel mechanism to search for protein partners at telomeres Nucleic Acids Res **42**(4): 2493-2504 (2014).