**Designing Amplified Electrical and Optical Sensors, Controlling Interfacial Charge Transfer, and Performing Reversible Mechanical Motion using ///////////Functional Nucleic Acids////////////**

Thesis submitted for the degree of

“Doctor of Philosophy”

By Gilad Pelossof



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Professor Itamar Willner

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Abstract

**Chapter 1-introduction**

The information encoded in the base sequence of DNA provides structural and functional meaning. Structural information includes duplex formation by complementary base pairs, pH-stimulated formation of i-motif structures from C-rich sequences, K+ ion-induced self-assembly of G-quadruplex nanostructures from G-rich sequences, stabilization of duplexes by specific metal ion bridges, such as T-Hg+-T or C-Ag+-C, and more. Functional information includes the recognition properties of single stranded DNA (aptamer) forwards low-molecular-weight substrates, macromolecules or even live cells. Sequence specific nucleotide acids exhibit unique catalytic functions (DNAzymes) such as the self-assembled hemin/G-quadruplex, horseradish peroxidase-mimicking (HRP) DNAzyme, or metal ion-dependent DNAzymes.

Biosensor devices are analytical systems that enabled the selection and specific analysis of target analytes. The sensor includes a recognition element for the target analyte and transducing element that reports the recognition events. Different elements, such as enzymes, DNA aptamers and others are used to develop specific biosensors, and different transduction means, such as electrochemical signals, optical signals, magnetic, microgravimetric, and other, are implemented to readout the recognition events.

The specific recognition properties of nucleic acids towards the complementary strands, the affinity interaction between low-molecular-weight substrates/macromolecules and the respective aptamers, and the specific bridging of duplex DNA structures by means of metal ions e.g. Ag+ or Hg2+, provide resources to develop a variety of sensor devices. Indeed, numerous sensing platforms that use nucleic acids as recognition elements for sensing DNA, aptamers or, complexes of metal ions were developed. Electrochemical, optical, microgravimetric, surface plasmon resonance, magnetic and more, were used as readout signals for the recognition events. Examples of different DNA-based sensors are presented. With the development of nanotechnology, new nano materials exhibiting unique electronic, optical and photo-physical properties emerged. For example, the size-controlled plasmonic properties of metallic nanoparticles (NPs), or the size-controlled luminescence features of semiconductor quantum dots (QDs) nanoparticles were extensively used to develop biosensor platforms. Specifically, the aggregation of metallic nanoparticles and the color changes accompanying the transitions upon aggregation and deaggregation of the NPs were broadly implemented to develop various sensing platforms. For example, the color changes observed upon aggregation of Au NPs (as a result of intraparticle plasmon-exciton), or the color changes associated upon the deaggregation of the particles (individual NPs exhibiting localized plasmon) were used to develop numerous sensing platforms.

One challenge in the development of biosensors and specifically oligonucleotide-based sensors involves the increased sensitivity of the resulting sensors. This is accomplished by the amplified detection of sensing events through the coupling of enzymes or DNAzymes to the recognition event. The catalysts transform single recognition event to numerous readout signals (electrical or optical), thus providing a method to amplify the recognition event. The introduction exemplifies several amplification methods including the rolling circle amplification (RCA), process, hybridization chain reaction (HCR) and polymerization/nicking machinery.

Beyond the use of the structural/functional information encoded in DNA for the development of variety of sensor systems, the encoded information can be used to develop DNA-based devices, such as DNA switches or DNA machines. A DNA switch is a nucleic acid assembly that can be reversibly reconfigured between two distinctive structures using appropriate inputs and anti-inputs (input=fuel, anti-input=antifuel). Different external stimuli, such as nucleic acid strands, alternating the pH, metal ion/ligand, light, or electrical signals were used as triggers to reversibly switch DNA between distinctive states in solution and on surfaces. DNA machines represent a higher hierarchical level of mechanically triggered nucleic acid assemblies. The DNA machines perform cyclic mechanical functions between two or more states using appropriate fuel and antifuel. Numerous DNA machines, such as cranes, tweezers, walkers, rotors, gears and more were reported using different triggers, such as strands, alternate pH values, metal ions/ligands, or light. The introduction exemplifies the concept of DNA machines while discussing the operation of DNA tweezers, the light-induced activation of a DNA walker, the controlled operation of a rotor, and the operation of a bipedal walker.

As the thesis involves the assembly of many DNA-based sensor systems on electrodes or metallic NPs, and the assembly of DNA machines on surfaces, the chemical modification of surfaces with nucleic acids, and the characterization of the resulting monolayer-modified surfaces are essential parts of the thesis. The introduction summarizes the mechanism of self-assembly of nucleic acids on Au surfaces, or on Au NPs or semiconductor QDs, and discusses the different spectroscopic, electrochemical, optical and microscopic characterization of the resulting monolayers. Specifically, surface plasmon resonance spectroscopy (SPR) and faradaic impedance spectroscopy (FIS) are used to, characterize the formation of monolayer-modified surfaces, in addition to following the dynamic transitions of the DNA components on the surfaces.

**Research goals**

The research focused on the application or functional information (structural and reactivity) encoded in the nucleic acid polymers to develop sensitive sensing platforms and functional assemblies on surfaces that control electron transfer at the liquid-solid interfaces and can be used as DNA machines operating on surfaces.

The specific goals of the research included:

i) Applying hemin/G-quadruplex structures associated with electrodes as electrocatalyst for the reduction of H2O2 to water. Within this topic, the hemin/G-quadruplex structures were implemented as electrocatalytic probes for the detection of DNA, sensing the formation of aptamer-substrate complexes, the detection of metal ions (Pb2+), and the implementation of the catalyst to identify H2O2 generated by oxidases (glucose oxidase).

ii) Applying surface plasmon resonance as a physical method to sense the formation of aptamer-substrate complexes and for the detection of hazardous metal ions (Pb2+, Hg2+). Within these efforts, we would like to implement the hemin/G-quadruplex formed as a result of the different sensing process as an optical label for the amplification of the sensing events involved. The hemin/G-quadruplex formed on the surface that contained the sensing matrix was implemented as an optical label that amplified the surface plasmon resonance transduction signals. Furthermore, G-quadruplex optical labels were immobilized on Au NPs, and these were further assembled on a Au surface. The resulting system was probed using surface plasmon resonance spectroscopy (SPR). By this way, the dielectric changes occurring on the surface as a result of the sensing processes, and the formation of the hemin/G-quadruplex label, were amplified by the coupling of the localized plasmon of the NPs with the surface plasmon wave. As a result, minute dielectric charges (small concentrations of the analyte target) led to measurable changes in the surface plasmon resonance signals.

iii) Electrode surfaces were modified with programmed nucleic acids that demonstrated reversible transitions between random coil to organized structures, such as i-motif or G-quadruplex. The state of the DNA structures was triggered by external stimuli, such as pH (to form, or dissociate, i-motif structures) or by the addition of K+ ions (to stimulate the formation of G-quadruplexes). The structure changes of the nucleic acids associated with the electrode led to the cyclic control of the interfacial electron transfer (ITF) properties at the solution electrode interface. The ITF properties were monitored by faradaic impedance spectroscopy.

iv) We constructed on electrode surfaces programmed DNA assemblies that acted as mechanical "arms" which were mechanically driven across specific sites on the DNA scaffold. The mechanical arms were activated by external stimuli and their operation was monitored by electrochemical means with a response of a redox label associated with the moving arm.

All of the functional systems operating as sensors or DNA machines were characterized by electrochemical, spectroscopic and microscopic methods. Special efforts were directed to bridge between the structural and microscopic properties of the systems and their macroscopic functional activities. In addition, the implications of the study and the future applications of the different systems are discussed.

**Chapter 2 – Research Methods**

This chapter describes the different instruments and experimental setups used in the research. The detailed description of the experimental systems related to the results are detailed in the experimental sections of the respective set of scientific papers attached to the thesis.

**Chapter 3 – Research results**

This chapter includes the scientific reports derived from the research projects, and these are separated into subsections that include 5 scientific papers:

3.1 “Amplified Biosensing Using the Horseradish Peroxidase-Mimicking

 DNAzyme as an Electrocatalyst”

 Pelossof G., Tel-Vered R., Elbaz J., Willner, I. *Anal. Chem.* **2010**, *82*, 4396.173\*

3.2 "Amplified Surface Plasmon Resonance-Based DNA Biosensors, Aptasensors,

 and Hg2+ Sensors Using Hemin/G-Quadruplexes and Au Nanoparticles"

 Pelossof G.; Tel-Vered, R.; Liu, X.Q.; Willner, I. *Chem. Eur. J.* **2011**, *17*, 8904.174\*

3.3 “Amplified Surface Plasmon Resonance (SPR) and Electrochemical Detection of Pb2+ Ions Using the Pb2+-Dependent DNAzyme and Hemin/G-quadruplex as a Label”

 Pelossof, G.; Tel-Vered, R.; Willner, I. *Anal. Chem.* **2012**, *84*, 3703.175\*

3.4 “Controlling Interfacial Electron Transfer and Electrocatalysis by pH- or Ion-

 Switchable DNA Monolayer-Modified Electrodes”

 Pelossof, G.; Tel-Vered, R.; Shimron, S.; Willner, I. *Chem. Sci.* **2013**, *4*, 1137.176\*

3.5 “Switchable Mechanical DNA “Arms” Operating on Nucleic Acid Scaffolds

 Associated with Electrodes or Semiconductor Quantum Dots”

 Pelossof, G.; Tel-Vered, R.; Liu, X.; Willner I. *Nanoscale* **2013**, *5*, 8977.177\*

**Chapter 4 – Discussion related to the research results**

This chapter summarizes the research results described in the collection of the papers appearing in the thesis.

The discussion includes:

* A short description of the research concepts associated with the specific scientific reports, while addressing the experimental methods associated with the studies.
* A description of the major accomplishments derived from the research activities.
* Major conclusions derived from the research results and their implications on future development of on the relevant research topics.

**Chapter 5 – References.**

This chapter includes literature references related to the thesis.