Functionalized DNA Materials for Sensing & Medical Applications

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ABSTRACT

The U.S. Army has strong interests in nanoscale architectures that enable enhanced extraction and controllable multiplication of the THz/IR regime spectral signatures associated with specific bio-molecular targets. Emerging DNA-based nano-assemblies (i.e., either materials or structural devices) will be discussed that realize novel sensing paradigms through the incorporation of organic and/or biological molecules such that they effect highly predictable and controllable changes into the electro-optical properties of the resulting superstructures. Results will be given to illustrate the utility of functionalized DNA materials in biological (and chemical) sensing, and to demonstrate how the basic science can be leveraged to study and develop synthetic antibodies, reporters and vaccines for future medical applications.

Keywords: nanoscale architectures, DNA materials, terahertz, infrared, spectral sensing, medical applications

1. INTRODUCTION

The U.S. Army has strong interests in developing capabilities for the engineering of specialized nanoscale architectures that allow for the enhanced extraction and controllable multiplication of the terahertz (THz) and/or infrared (IR) regime spectral signatures associated with specific bio-molecular targets [1-2]. This paper will overview a joint U.S. Army Research Office (ARO) and U.S. Army Edgewood Chemical Biological Center (ECBC) program that is supported by the U.S. Defense Threat Reduction Agency (DTRA) for the purposes of defining DNA-based nano-assemblies (i.e., either materials or structural devices) that realize novel sensing paradigms through the incorporation of organic and/or biological molecules such that they effect highly predictable and controllable changes into the electro-optical properties of the resulting superstructures. This goal of defining a new class of functionalized DNA materials is presently being addressed by the three thrust efforts: (i) Artificial molecular capture/report for biological sensing based upon DNA origami; (2) Nanofluidic sensor platforms for spectroscopic fingerprinting of bio-molecules; and, (3) Molecular-level platforms for electrical and spectral characterization of bio-molecular systems. The presentation that follows below will first (see Section 2) provide a historical overview of the program goals and summary descriptions of these three thrusts to illustrate how they are collectively working towards the development of THz/IR-sensitive material systems and associated devices with measurable macroscopic THz/IR electro-optical properties that reveal the underlying states and vibration dynamics of bio-molecular targets of interest. Here, the ability to capture, incorporate and interrogate the geometrical and dynamical properties of target bio-molecules has important ramifications for threat-agent sensing, and it also has broad implications for the future study and development of artificial antibodies, receptors and vaccines. To illustrate the potential utility of engineered DNA materials, a hybrid biological-organic functionalized (HBOF) smart material concept will be briefly discussed (see Section 3) that allows for the microscopic study of antibody and receptor mimics, and for the generation of new insights that can be used for developing biomimetic-based recognition/detection methodologies. Commentaries will also be provided on the expected future phases, and potential extrapolations, of the program (see Section 4) in regards to study and development of synthetic molecules and materials (antibodies, reporters, vaccines) that have direct relevance to medical applications. Finally, conclusions will be provided that summarize the accomplishments to date and comment on expectations for the future.

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2. MAJOR SCIENTIFIC COMPONENTS & LONG-RANGE PROGRAM GOALS

During the course of the last five years, the U.S. ARO and the U.S. Army ECBC worked jointly to lead a program entitled, "Rapid, Reagent-less Detection and Discrimination of Biological Warfare (BW) Agents using Multi-Photon, Multi-Wavelength Processes within Bio-Molecular Architectures," which organizes a diverse academic team towards the goal of developing the S&T foundation for advanced nanoelectronic architectures that will enable a new approach to biological agent detection and identification. The focus and structure of this program was motivated by earlier fundamental research results [2,3] that suggested very long wavelength (i.e., in the terahertz frequency regime ~ 0.3 to 3 THz and very far infrared) spectra signatures might be useful for the detection and identification of BW agents; and by the special challenges of the complex dynamical phenomenology associated with biological materials and agents [1]. More specifically, while it was recognized that THz (and IR) spectral information present within biological materials and agents had potential for use in detection and characterization applications, there were also very great challenges associated with extracting that information from the target samples (i.e., that arises at the molecular level) due the dependencies on microscopic/macroscopic geometries and the influences of the external environment. Hence, the program sought to organize a multidisciplinary research team that would be capable of demonstrating novel bio-architectures where a high-level functionality and sensing capability would be designed into the molecular system such that it would be possible to extract, enhance and/or multiply the available species-specific THz/IR spectral signatures.

The original nanosensor paradigm was based upon a new class of DNA-based components that utilized THz (and/or IR) sensitive bio-molecules as the active elements for prescribing the high-level function and enhanced sensing at the molecular-level. Here, the program sought to leverage the emerging science based in self-assembled DNA nanoscaffolds to define completely new types of electronic/photonic transduction devices that allow for the extraction of microscopic information (e.g., structure and dynamics) related to bio-molecular targets of interest. Therefore, the envisioned bio-architecture concept would seek to utilize the target bio-molecule as a strategic part of the transduction device and/or the smart material system, so one would be able to utilize multi-spectral excitation and detection for detecting and characterizing the desired molecular properties. The multi-disciplinary teamed effort, which was overviewed in a 2009 paper [4], set the long-range goal of realizing biologically-based molecular-level sensing in the context of integrated nanosensor platforms, and was initially organized around the four major thrusts: (1) DNA-based photonic bandgap (PBG) wave-guiding structures to facilitate quasi-optical control of signal propagation in integrated molecular/electronic systems; (2) bio-inspired paradigms for establishing electro-THz-optical communication channels, propagating wave control methodologies and input/output function useful for incorporation into biologically-based devices and components; (3) test beds for the measurement and characterization of THz-sensitive bio-molecular elements and multi-terminal devices; and (4) hybrid multi-terminal bio-molecular devices that can achieve (a) control and manipulation of signal propagation, and (b) enhanced sensor function, using molecular-level multi-photon and multi-wavelength processes. This particular programmatic structure was chosen because it allowed for starting with a single innovative photonic device paradigm (i.e., DNA PBG wave-guide) which was directly amenable to significant size down-scaling (i.e., one could start from larger aperture material systems and devices that could be characterized at long wavelengths) and that allowed many degrees of freedom for introducing molecular-level functionality (i.e., DNA-based nanoscaffolds could be tailored to incorporate many types of organic and biological molecules). The program was also carefully balanced so that it would contain focused efforts in each of the major science and technology challenge areas [4], which included: DNA Nanoscaffold Design and Self-Assembly; Electro-THz-Optical (ETO) Properties and Functionality (i.e., for defining novel functionality in the DNA nanoscaffolds); Physical Models and Numerical Simulation (i.e., for describing the required bio-molecular devices and systems); Novel Test and Characterization Methodologies and Technologies (e.g., fluidic chips, plasmonics, inelastic electronic tunneling, etc.); and, THz-Sensitive Device & System Demonstration (i.e., which is represented by various unifications of the work in the prior four identified efforts).

During the course of this program, it has generated a significant number of science and technology accomplishments (for some citations prior to 2010 see reference [4]) and established many of the foundation elements that will be required for achieving the long-term goal of nanoscale sensing architectures. In 2010, DTRA leadership directed the program to increase the emphasis of the research towards scientific opportunities related to characterization and development of synthetic antibodies and receptors. This slight refocusing of the program is highly advantageous because antibody and receptor mimics have dual-use relevance to both recognition-based sensing and to medical diagnostics and treatments.

To briefly explain, the original goal of the program was to innovate electro-optical based transductions, which would reveal the spectral properties (and in turn the structural and dynamical characteristics) of target molecules that had been man-engineered into DNA-based nanoscaffolds. However, this same paradigm is useful as a "smart material" where the functionalized molecules (and defined transduction) contained within the DNA superstructures can be used to sense/study the microscopic electrostatic interactions and/or binding events associated with synthetic antibody function (e.g., DNA aptamer capture or other binding moieties) and synthetic receptor operation (molecular capture and signal-based reporting). This expanded research scope has obvious utility in defining molecular components and architectures for recognition-based detection and identification, and it will provide new scientific insights into antibody and receptor mimics that can be used for inventing completely new types of recognition/detection methodologies for biological (and chemical) threats (see Section 3) and for developing new types of medical diagnostic and prophylactic/therapeutic tools (see Section 4). Hence, the scientific and technical results produced by the program will now be relevant across many more disciplines and applications. To promote these new technical goals explicitly, the research and development efforts under the "Bio-Molecular Architectures" program were reclassified into the three new thrust areas as described below.

#1 Artificial Molecular Capture/Report for Biological Sensing Based Upon DNA Origami - DNA structural technology has made major advances in recent years, and has now evolved to a point where it is possible to design and manufacture complex structures of significant size and diversity. Starting from the early work of Ned Seeman [5] that recognized almost any shape could be programmed into a DNA system using the information encoded in the sequential order of the bases as "address codes," the basic technique has been extrapolated to where non-identical building-block units can be combined to realize finite and non-repetitive 2-D structures [6]. In the context of the goal to realize nanoscale architectures with large amounts of embedded functionality, the technique of DNA Origami as developed by Rothemund [7] is important because it provides for the reliable generation of "large" structures (~ 100 nm on an edge) that posses large numbers of unique sites (~ 200) for ready modification. DNA Origami, which is now a chemically well-understood and controllable process, uses one long DNA template strand (usually a circular plasmid) that is essentially raster (bent and folded, usually in 2D although 3D is fully possible) into predefined structural geometries by sequentially presenting many short DNA "staple" strands to the DNA template while in solution. Here, it is possible to leverage the sequence of the DNA template and the specific design of the DNA staples to realize a complicated pattern of cross-linking that leads to large and robust structures. Because the short (~ 30 base pair) staple sequences are entirely synthetic, they can be chemically modified to achieve essential unlimited (atomic level) resolution in terms of the molecular design of the functional architectures. Therefore, DNA Origami offers the potential for bridging size scales from atomic to ~ micron scale with a single technology that is also effective for manufacturing the sought after smart materials in large quantities, with high reproducibility, and at relatively low costs.

However, important molecular design and synthesis challenges must be addressed before the types of DNA tiles, arrays and crystals can be achieved that would enable the artificial molecular capture (i.e., antibody function) and report (i.e., receptor function) characteristics as required by the Bio-Molecular Architectures program. Specifically, DNA Origami design software is needed that can rapidly and reliably prescribe DNA nanostructures that are more stable and defect free as compared to the present state-of-the-art. New synthesis techniques and technologies are also needed that are extremely effective for creating large DNA Origami based superstructures that possess significant functional diversity across the entire architecture. Requirements for design and synthesis will build upon and extend DNA architectural concepts (see Fig. 1) of the type recently achieved under the program by the Norton Group of Marshall University [8]. Figure 1(A) illustrates an example of a pattern designed to realize a 72 X 98 nm rectangular DNA with an open 20 X 26 nm aperture. As illustrated in Fig. 1(B), these blocks can be docked along the 72 nm edge to produce 1D arrays. This design has already been extended to include biotin/streptavidin adapter molecules for the insertion and study of polymeric molecules. The basic designs and AFM images before and after modification with Streptavidin are presented in Fig. 2. This particular system has

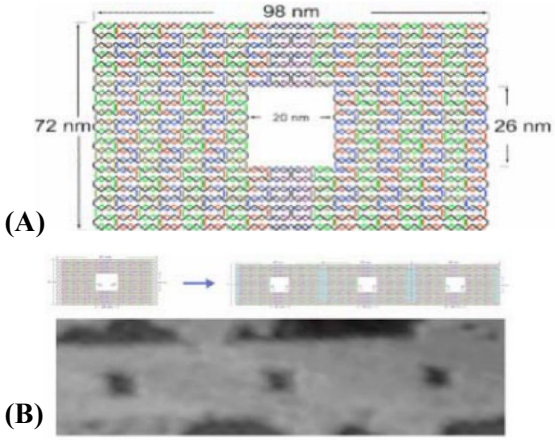

Fig. 1. Linear arrays of rectangular origami constructs (A) DNA folding pattern for 98 X 72 nm construct with 26 X 20 nm aperture, (B) AFM images of assembled constructs.

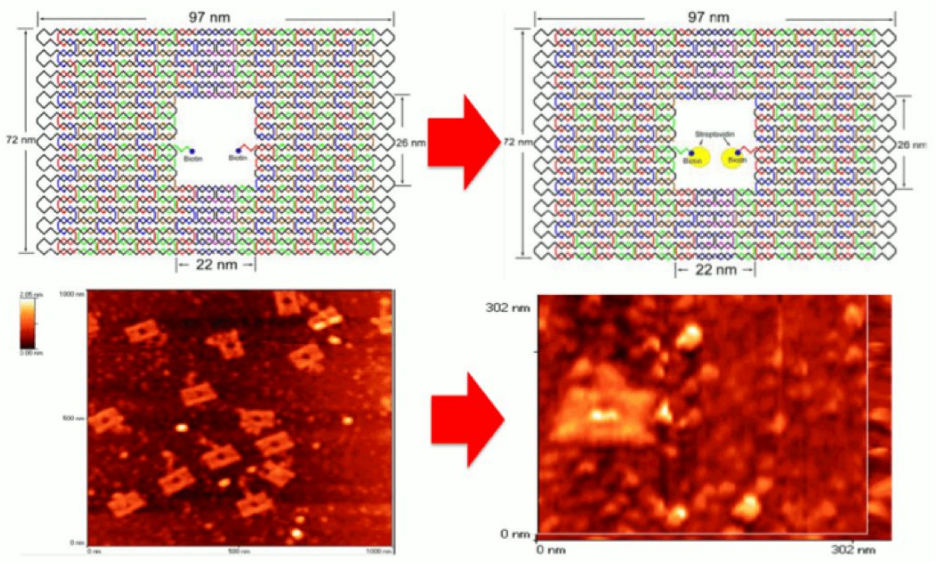

Fig. 2. Design (Top) and image (Bottom) of Origami functionalized to enable "plug and play" addition of biotinylated polymers to Origami frameworks. Left: Origami without Streptavidin molecules. Right: Origami construct containing Streptavidin.

been designed to enable the installation of any 14 nm long polymeric molecule terminated at both ends with biotin. Such a modular "DNA cassette" will enable rapid integration of functional molecules without significant modification of the substrate design. For example, the Norton group has also placed dye molecules and protein molecules at specific locations on the surfaces of Origami constructs [9]. Going forward in the program, work will be performed to identify and develop novel biological and organic molecular concepts that can be embedded into the DNA cassettes to realize antibody and receptor mimics (see Section 3 for examples). Work will also be performed to further expand the size and diversity of these DNA architectures through the use of hybrid 2D (and possibly 3D) arraying techniques where DNA origami structural blocks are designed so as to allow for their "sticky-end" assembly into larger superstructures [10]. Hence, this research thrust seeks to produce DNA Origami structures and hybrid DNA superstructures with surface charge distributions, hydrophobic/ hydrophilic surface properties and controllable electro-optical transduction such that they exhibit artificial antibody (capture) and receptor (capture and report) functionality that can be monitored (and possibly controlled) by external ETO signals. This work is expected to enable novel investigations into the reaction dynamics of these artificial systems with target antigens related to biological (and chemical) warfare agents or their simulants. Here, research will be used to assess the affinity and recognition potential of these artificial aptamers to known antigens, and the results will be used to refine both the capture and reporting mechanisms. These results will have relevance for defining new types of artificial molecular capture/report for use in detection and identification sensors, and for use in developing artificial vaccines (see Section 4). Therefore, the main goal of this thrust is to define a new class of artificial molecular systems that can be affordably and reliably mass-produced for battlefield applications related to the recognition/detection, medical diagnosis and prophylactic/therapeutic treatment of threat agents.

#2 Nanofluidic Sensor Platform for Spectroscopic Fingerprinting of Bio-Molecules - The successful development of DNA architectures with the desired artificial antibody and receptor characteristics as prescribed above will required highly effective and accurate measurement capabilities for the spectroscopic fingerprinting of the constituent molecular components at very long wavelengths (e.g., THz frequencies). Recent progress demonstrates [11-12] the importance of controlling the orientation of the molecular targets in achieving highly repeatable spectral characteristics, and produced very sharp THz spectral signatures (i.e., 10 GHz) that rival any ever reported for solids or liquids at room temperature. Hence, these results motivate the implementation and refinement of state-of-the-art microfluidic techniques for sample injection, processing and control into a THz-compatible spectroscopic sensor platform that will provide for highly sensitive and repeatable fingerprinting of bio-molecules. Here nanofabrication will be applied to produce the required nanoscale portals, control structures and channels useful for the introduction, stretching and processing of bio-molecules (e.g., such as genomic DNA) and their accurate spectroscopic characterization. In order to uniformly stretch and/or process chain-like DNA molecules, the dimensions of the nanofluidic structures should be of the order or smaller than

the persistence length of double stranded DNA (i.e., 50-nm to 100-nm). To sufficiently enhance the interaction of the THz radiation with the absorption signature it is expected that the bio-chip platforms must possess high-density arrays in excess of 10-million nanofluidic channels within the THz spectroscopic window. The main goal of this thrust is the development and demonstration of a functioning prototype device that will: (1) completely validate the effectiveness of nanofluidic sensor platforms for the label-less "THz-frequency spectroscopic fingerprinting" of biological molecules; and, (2) enable a detailed assessment of THz-based detection, identification, and classification of biological materials and agents. A successful technology demonstration will represent a major enabler to biological (and possibly chemical) sensing and monitoring in defense, security, biomedical, pharmaceutical, food quality, and environmental relevant scenarios for civilian, military, government, and commercial sectors around the world.

An excellent example of the important advances being made in the nanofluidic sensors area by the program is the recently reported accomplishment [13] by the Brown Group at Physical Domains, LLC of the first demonstration of a "Sweep & Zoom THz Interrogation of Nanofluidic Cells." More specifically, the Brown Group was able to establish a new modality in a THz photomixing-transceiver based sensor platform whereby the spectrometer is first operated in "sweep" mode, scanning in frequency regime from ~100 GHz to 1.5 THz (or higher) and seeking signatures with high resolution (< 1 GHz) and high sensitivity (minimum detectable transmission difference ~1%). After the successful identification of a signature, the transceiver can then be adjusted to fixed-frequency mode at the center frequency of the signature to execute ultrasensitive monitoring of that particular signature over long periods of time. This new capability was first demonstrated on the si-RNA biomolecules in solution as they have previously been shown to exhibit very strong THz signatures. These reported experimental results are summarized in Fig. 2 where (a) shows the results of the broadband sweep-mode interrogation of the si-RNA and the positive identification of a signature just below 850 GHz, which is a signature that has been observed in siRNA many times before [11]. Fig. 2(b) then shows the result of "zooming" in on this signature by adjusting the spectrometer to fixed-frequency mode and monitoring the THz signal transmission over time as the si-RNA solution flows into the nanofluidic chip and is subjected to various levels of electrophoretic bias voltage applied to the chip. There is a clear change, well out of the noise floor, in the transmitted signal over time with a significant dependence on the electrophoretic bias. This work, which was accomplished in collaboration with Dr. Ed Mendoza under an Army SBIR Phase I project, demonstrates a first-time THz nanofluidic sensor platform capability for signature detection, tracking over time, and control with electrophoretic bias voltage, and this signal processing capability is expected to make important impacts to molecular component characterization.

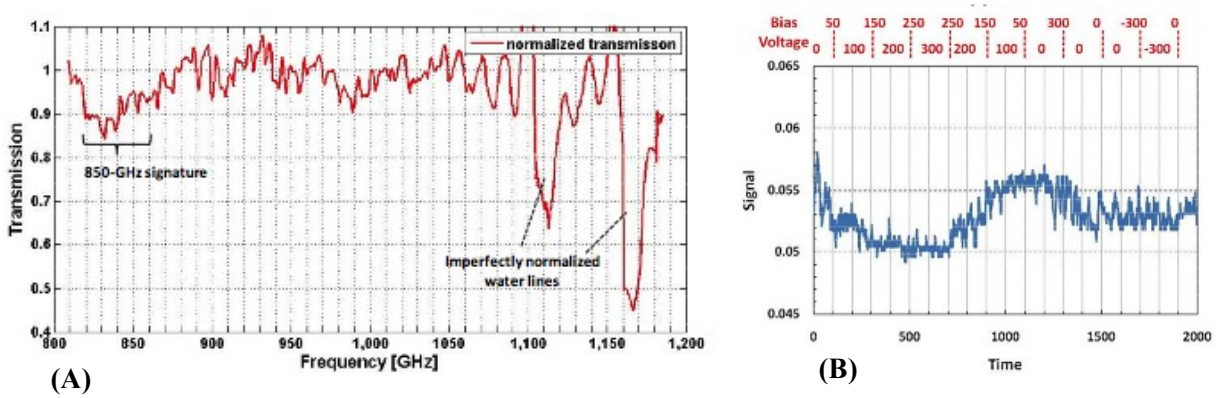

Fig. 3. (A) Normalized transmission function through a nanofluidic chip bearing a si-RNA solution obtained in "sweep" mode and showing a clear absorption signature just below 850 GHz (B) Transceiver receive signal in "zoom" mode for fixed-frequency operation at 838 GHz through the same nanofluidic cell as in (A). The transmitted signal was sampled once per second, and electrophoretic bias was varied according to the schedule (in red) at the top of the plot.

#3 Molecular-Level Platforms for Electrical and Spectral Characterization of Bio-molecular Systems - Robust experimental platforms are an important and necessary element for the electrical and spectral characterization of bio-molecular elements, devices and architectures as targeted by this program. Indeed, the demonstration of molecules as the active region of electronic devices has recently generated considerable interest in both the basic transport physics and potential technological applications of "molecular electronics." However, even DC measurements on molecular devices

exhibit significant dispersion in their characteristics, due to the highly sensitive nature of molecule-contact coupling. Therefore, this thrust seeks to implement novel characterization approaches (e.g., plasmonics, inelastic electronic tunneling, subwavelength microscopy, nanotube/nanowire probes, etc.) within nano-platforms to extract novel THz/IR spectroscopic information regarding the properties and functionality of novel DNA architectures. Such techniques are potential tools for understanding the spectral signatures of the molecules and molecular structures discussed earlier, and for studying the complex processes associated with synthetic antibodies, receptors and vaccines. Hence, research under this thrust will examine novel methods for the spectral and electrical characterization of biomolecules at the nano-level. This work will also explore new methods for interfacing from the nanoscale to the macroscopic worlds via novel electrical and optical interfaces. The development of new molecular-level platforms will be critically important for achieving the level of test and evaluation as will be needed for realizing the applications discussed above.

A very good example of the molecular-level type characterization that is being pursued under the program is illustrated by recent accomplishments of the Reed Group from Yale University in the area of "*Single Molecule* Inelastic Electron Tunneling Spectroscopy (IETS)." While the Reed Group had previously pioneered IETS in molecular junctions, the work under this program sought to develop planar platform embodiments that would be effective for executing the characterization of molecules while under plasmonic or optical excitation. Here, the challenge was to demonstrate IETS on single molecular junctions so as avoid situations where the arrangement multiple molecules might confuse the experimental observations. As a part of this program, the Reed Group was able to successfully demonstrate single molecule IETS using a transistor structure that allowed the orbital energies of the target molecule to be modified [14]. The planar geometry of the single molecule transistor structures used in these studies is illustrated in Fig. 4 (A). One of the important discoveries was the observation of "resonant enhancement" of the IET signals that arose during the appropriate gate alignments (see Fig. 4 (B)). Hence, the creation of these 3-terminal nanostructures allowed for developing insights into the energetic alignments of orbitals of the molecules in the junction, and yielded dramatically difference results for aliphatic versus aromatic molecules (specificallty, alkane dithoils versus benzene dithiol). Therefore, this new single molecule IETS capability offers potential for a spectral-based interface between the nanoscale and macroscopic for use in the characterization and refinement of the envisioned bio-molecular architectures.

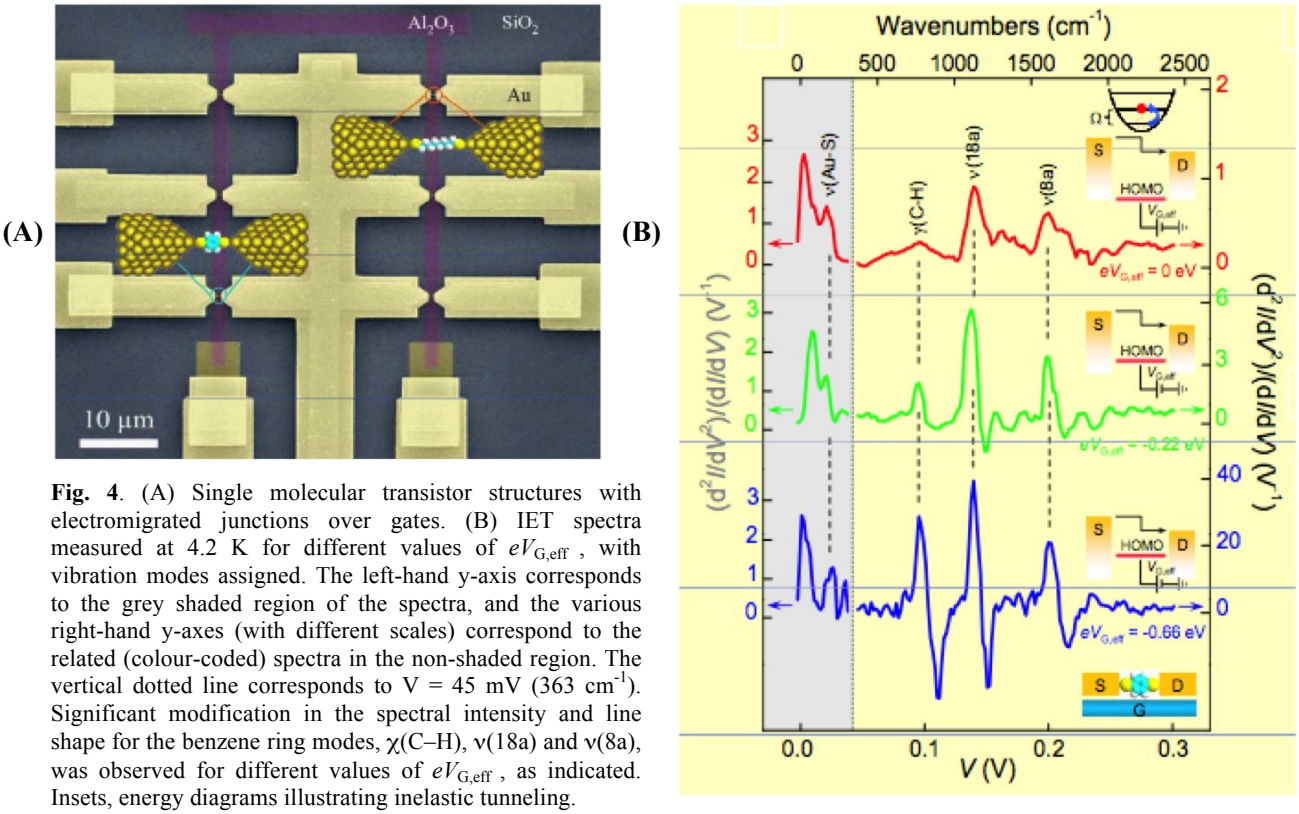

Fig. 4. (A) Single molecular transistor structures with electromigrated junctions over gates. (B) IET spectra measured at 4.2 K for different values of $eV_{G,eff}$, with vibration modes assigned. The left-hand y-axis corresponds to the grey shaded region of the spectra, and the various right-hand y-axes (with different scales) correspond to the related (colour-coded) spectra in the non-shaded region. The vertical dotted line corresponds to V = 45 mV (363 cm^{-1}). Significant modification in the spectral intensity and line shape for the benzene ring modes, χ(C–H), ν(18a) and ν(8a), was observed for different values of $eV_{G,eff}$, as indicated. Insets, energy diagrams illustrating inelastic tunneling.

The three previously discussed research thrusts are presently under support of the U.S. DTRA and there is an expectation that this program (or some modified version of it) will be continued for numerous years in the future for the purpose of developing a science and technology base in the area of functionalized DNA materials. In addition, the results from the active "Bio-Molecular Architectures" program have motivated a number of other science and technology projects that are providing leveraging opportunities for achieving the long-term applications goals. Example include, but are not necessarily limited to, multiple Army STTR projects that are providing THz and Far IR technologies and specialized test beds; multiple Army CB SBIR projects that are developing tools for designing and manipulating molecular systems; and a new Army MURI program that seeks development very broadband (i.e., RF to light wave) near and far-field interfaces to DNA-based nanostructures [15]. These existing research and development efforts, and most probably additional ones that will emerge with more focus on biomedical issues, will collectively define the scientific knowledge and technological capabilities for enabling functionalized DNA materials for sensing and medical applications. The next section will provide one example molecular paradigm that is being pursued to define smart materials of this basic type.

3. A HBOF SMART MATERIAL PARADIGM FOR BIO-BASED SENSING

The development of novel molecular-based functionality paradigms that can be incorporated into DNA-based nannoscaffolds for the purposed of altering and controlling the THz and/or IR regime properties of the material superstructures has long been a goal of the U.S. Army program due the potential use of such smart material systems in sensing applications [1,4,16] In recent years, the Woolard research group at North Carolina State University has been conducting theoretical modeling and design studies on both organic molecular switches (QMSs) and biological molecular switches (BMSs) that may be used in DNA-based architectures to enable the precise extraction of nanoscale information (e.g., composition, dynamics, conformation) through electronic/photonic transformations to the macroscale. Here, the motivation was to realize "THz/IR-sensitive" materials that offered novel spectral-based sensing modalities that would be useful for detecting, identifying and characterizing select bio-molecules that were used to define the functional systems. The previously considered OMSs [17] consist of very long-chain, single-bonded carbon atoms that are made semi-periodic through the introduction of groups of aromatic (carbon-based) ring structures (i.e., with a conductivity sensitive to polar molecules), and the BMOs [17,18] consist primarily of stilbene-derivative bonded to DNA (i.e., that offer the potential for defining light-induced changes to conformation and spectral absorption). While work is presently ongoing to define and refine OMSs and BMOs with the type of electron conductivity properties and the spectral absorption characteristics that can be altered and controlled so as to achieve the envisioned sensing transductions [17], it is already possible to illustrate the relevance of these molecular concepts to the study and characterization of antibody and receptor mimics. More specifically, as the subsections that follow below will show, these OMSs and BMSs are directly compatible with the modular "DNA cassettes" discussed in Section 2, and therefore can serve as the needed molecular elements for realizing hybrid biological-organic functionalized (HBOF) smart material. Furthermore, these HBOF smart materials can be designed so as to enable electro-photonic transduction of information regarding the microscopic mechanisms occurring within antibody and receptor mimics, and therefore can provide new insights for important biomimetic innovations in the future.

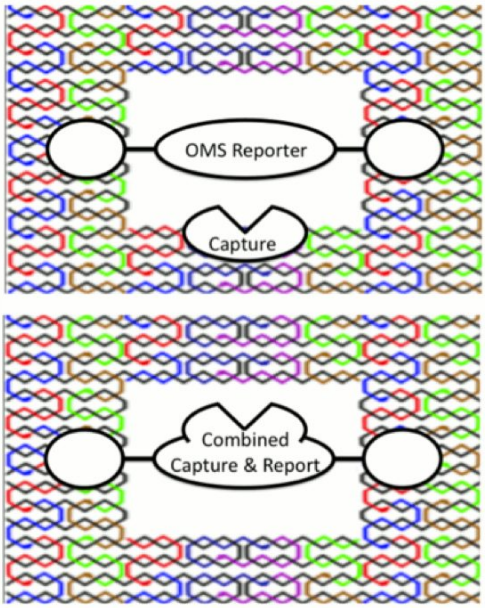

Fig. 5. Block architectures for Antibody mimic (upper) and Receptor mimic (lower).

3.1 Example Unit-Cell Concept for Antibody (Capture) and Receptor (Capture & Report) Mimics

The previously discussed OMS and BMS concepts are important because if the desired electronic and photonic properties can be man-designed and chemically-synthesized, then it becomes possible to envision compound DNA unit cells of the type shown in Fig. 5 where the resulting dielectric properties (i.e., electron conductivity and/or spectral absorption) of the unit cell will have a strong dependency on microscopic interactions between the functional molecules in the system. Furthermore, if techniques such as those discussed earlier (see Fig. 1) are used to produce smart material

systems by replicating this unit cell (i.e., in 2D or 3D) then it becomes possible to use external stimuli (i.e., chemical or electromagnetic) to control certain nanoscale processes and also to execute electronic/photonic-based transductions to monitor the state of these processes. For example, modeling results reported in reference [17] suggests that it is possible (i.e., using one or a few OMSs per cell) to define OMS-based reporters that exhibit large changes in the relative dielectric constant when they are exposed to the dipole moment of polar molecule. Hence, the OMS of [17] could potentially be used as a reporter (i.e., see upper unit cell of Fig. 4) to sense changes in the conformation (and associated surface charge) of the abstract "capture" molecule (i.e., also depicted in the upper unit cell of Fig. 4). Furthermore, if BMSs of the type discussed in references [17] and [18] are additionally functionalized to perform as aptamers (i.e., they will capture predefined molecular targets) then they can be used as switchable capture (or antibody) elements. Hence, these two molecular systems could be combined within a DNA cassette as shown in the upper part of Fig. 4 to define an "antibody" type smart material where the BMS is used to perform the "capture" of some predefined target (e.g., DNA strand) and the OMS "reports" the event through changes induced into the dielectric constant (i.e., measurable by spectral transmission and/or reflection measurements). Similarly, it is possible to envision more complex compound molecules (i.e., as represented in the lower portion of Fig. 5) that would be capable of executing "receptor" type functionality (i.e., both capture and report function) within a smart material system. The next two subsections will provide summary overviews of some molecular concepts that are under development by the program for future use in monitoring processes within antibody and receptor mimics that are defined within HBOF smart material systems.

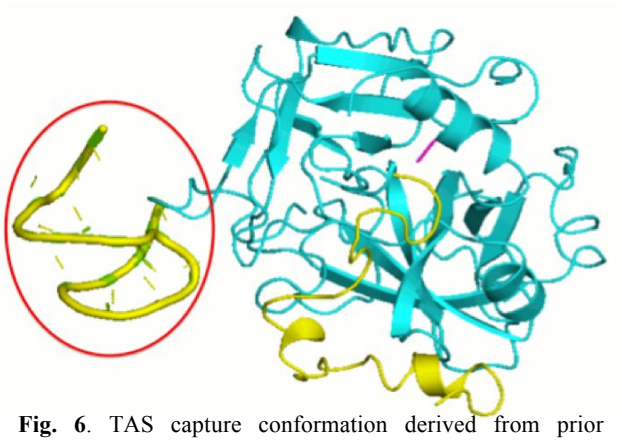

Fig. 6. TAS capture conformation derived from prior experimental and theoretical data. G15D molecule is yellow-tube inside circle.

3.2 Antibody Mimic Paradigm with OMS Reporting

An antibody mimic paradigm targeted for study under the program will employ a derivative of a well understood aptamer capture molecule (see Fig. 6), and the signaling of the associated capture processes will be performed by an optimized OMS (see Fig. 7). More specifically, the Thrombin DNA aptamer G15D, with sequence GGTTGGTGTGGTTGG, will be used as the "capture" element shown in the upper diagram of Fig. 11. This particular antibody mimic was chosen for the initial prototyping of the smart material because Thrombin (i.e., the target molecule) is nontoxic and affords safe development and testing, and DNA aptamers have been adapted to bio-toxins such as Abrin. The initial work will focus on understanding the capture functional of this Thrombin-aptamer system (TAS) both in its isolated form and when stapled to the cassette, and the later work will prescribe opportunities for realizing a switchable BMS form of the aptamer derivative to enable precise control of the capture process. The OMS research will seek to realize a design that allows for significant injection and collection of electrons, as this is of primary importance for achieving large dynamic range in the current switching. Hence, as illustrated in Fig. 7, a basic three-ring design which utilizes Benzoyl Chloride at the center

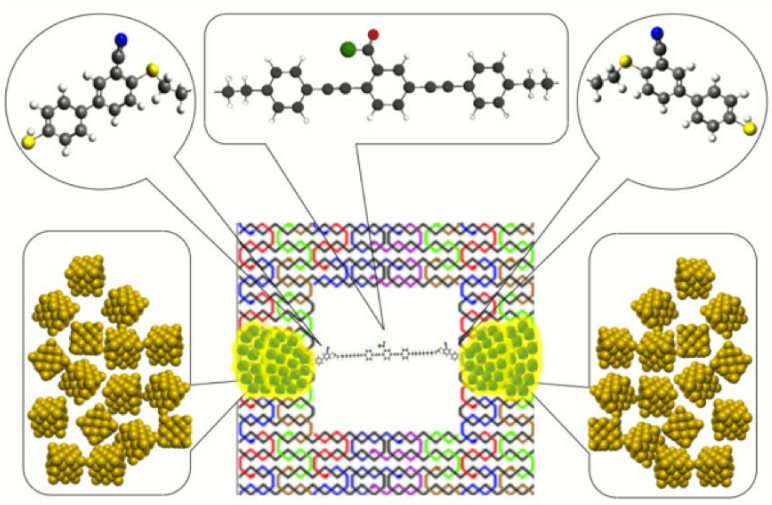

Fig. 7. OMS design with electron supply/sink of integrated gold super-cluster reservoirs (lower left & right) and OMS linker molecules (upper left & right) for tailoring the injection Fermi energy.

(see Ref. 17) will be combined with gold super-cluster reservoirs for providing adequate levels of electron injection and chemical variations to double ring molecule (4'-X-mercaptobiphenyl) have been shown previously (i.e., with X = CN & NH_2) to be effective in tailoring the relative work function (E) in molecular electronic systems will be investigated to raise the Fermi energy of the gold super-clusters relative to the OMS trapping state. The thiol groups of the base OMS will be bonded to modified versions of biphenylthiols, which in turn will be linked to the DNA cassette at points near the gold super-cluster as shown in Fig. 7.

3.3 Receptor Mimic Paradigm: Combined Capture/Reporter OMS System

The receptor mimic paradigm to be studied will utilize a combined capture/OMS molecular system where the capture process itself induces structural changes (conformation & electronic) that directly modulate the conductivity of the hybrid OMS reporter. This effort will utilize the well-understood phenylpyridyl capture system [19] to innovate a hybrid OMS reporter that is applicable to a number of highly toxic compounds (e.g., organophosphate gases (nerve agents) Sarin, Soman & Tabun and the model compounds of lesser toxicity disopropylfluorophosphate (DFP) and diethylchlorophosphate (DCP)) but that possesses a natural capture/report functionality that can be fluorescence tested by reaction with the less toxic compound thionyl chloride ($SOCl_2$). Furthermore, phenylpyridyl is a dual carbon-ring system (see Fig. 8) highly compatible for integration into the basic OMS paradigm, and with capture functionality that suggests opportunity for improved conductivity signaling. To explain, consider the capture reaction of an isolated phenylpyridyl functionalized to react to Sarin (i.e., Y=H in Fig. 8) as illustrated in Fig. 9. The initial state (configuration A) will display a planar structure for the two rings, and the conjugation of the dual ring system will not be broken by the reactions with the target phospho-species (in this case Sarin) leading to states B (fast transformation from A, < secs) and C (slow transformation from B, > minutes), or by the synthetic modifications that would be needed to incorporate the double-ring system as the combined capture/report element in the lower part of Fig. 9. These facts are very important for the design of the hybrid OMS reporting because, the regular planar alignment (on average) of the double-rings means that the HOMO-LUMO levels will remain essentially constant along most of the phenylpyridyl (i.e., from top to bottom in Fig 9), but the temporary bonding of the pendant group in state B creates a localized nonplanar feature which should significantly perturb a small portion of the energy ladder structure (i.e., create a defect) near the center of the double-ring. Hence, phenylpyridyl capture systems (PCSs) has potential as model reporters for binding captures of the type illustrated in Fig. 9. This might be accomplished by simple substitution of the basic three-ring OMS design in Fig. 7 by the PCS, but if not, it should be possible to realize effective energy ladder structures by adding functional groups to the PCS and/or by including it as a component in more complex compound design. These general design objectives will be pursued with the long-range goal being smart materials that can serve as receptor mimics for Sarin and possibly other toxic agents.

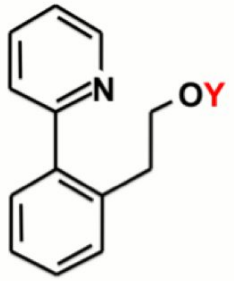

Fig. 8. Phenylpyridyl capture system, where Y influences the binding target.

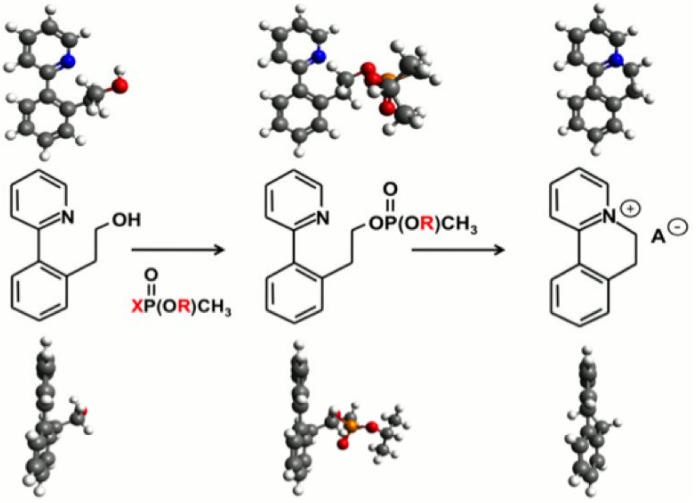

Fig. 9. The reaction steps of the phenylpyridyl system of Fig. 8 (i.e., with Y=H) upon exposure to the organophosphorus compound Sarin (i.e., X=F, R=Isopropyl) are shown along center. Two perpendicular views of the stable molecular conformations are given above and below, respectfully. State (A) is the natural conformation of the phenylpyridyl system. State (B) is the metaphesis product of the phenylpyridyl system due to bonding with pendant group $O=P(OR)CH_3$. State (C) illustrates the final products of a double-bonded phenylpyridyl and the isopropyl methylphosphonate anion (denoted as A $\ominus$) where a temporary electro-static binding occurs between the two.

4. FUTURE OPPORTUNITIES FOR MEDICAL APPLICATIONS

The evolving science based in DNA-based antibody and receptor mimics as discussed in the Section 3 has obvious relevance to advancing the state-of-the-art in synthetic vaccine development. For example, new vaccine strategies that utilize DNA origami with chemically defined structural epitopes (i.e., the part of an antigen that is recognized by the immune system) will offer advantages in their development, licensure, and production. Indeed, due to the fact that current methodologies for generating vaccines (attenuation of viruses, growth of less effective viruses in other species, etc.) do not necessarily lend much insight into their molecular-level properties, the U.S. Food and Drug Administration licenses vaccines under Biologics, as opposed to Drugs which now includes monoclonal antibodies, cytokines, growth factors, enzymes, immunomodulators, and recombinant proteins. The major difference between these two classifications is the ability of the manufacturer to define the purity, potency, and chemical structure of the product. As noted on the FDA website, "biological products are generally derived from living material--human, animal, or microorganism--are complex in structure, and thus are usually not fully characterized." Based upon these criteria and the defined chemical nature of a vaccine based upon DNA origami or other bio-molecular architectures, this product should be classified as a drug, which makes their licensure and production easier to obtain and maintain. For instance, an origami vaccine should be licensed under a New Drug Application as opposed to vaccines that are licensed under a Biologics License Application. Since the latter is specific to the manufacturer and facility, which limits the ability to increase production rapidly (surge) during a national emergency.

Another important advantage of a synthetic approach is reduced life cycle costs through reduced logistics. Currently, vaccine seed stocks (often biosafety level 3 (BSL3) materials which include indigenous or exotic agents which may cause serious or potentially lethal disease after inhalation) need to be stored and kept viable in cryogenic archives. To ensure a warm base, production capabilities also need to be maintained, even in the absence of need. Our nation's ability to produce the original small pox vaccine disappeared with the retirement of facilities and individuals at the Center for Disease Control (CDC) so the new methods are needed even for old threats. Hence, the new approaches being discussed here will contribute to the advancement of scientific knowledge in man-designed molecular structures and how they can be manipulated for both prophylaxis and treatment. More specifically, the long-term payoffs will be the ability to develop a pantry of synthetic reagents that can be readily formed into a vaccine for any organism. Vaccines and molecular recognition structures such as antibodies could be stored as CAD (computer aided design) files and synthesized when needed using reagents from the pantry. This envisioned pantry would include necessary compounds for rapid DNA origami construction. It would also include peptides and/or saccharides for the formation of the required epitopes, and the development of DNA origami as a substrate for proper epitope presentation. It might also be possible to define methods where the DNA origami structure acts as both substrate and epitope.

As discussed earlier, DNA origami based structures offer a capability for defining fine details over the conformation of the entire nanostructure, and constructing relatively large (100nm x 100nm x 2nm) closed-form DNA components. The relatively large surface area can be decorated with multiple precisely addressed haptens, antigens, or other molecular entities. For example, typical origami designs often have on the order of 250 short, "staple" or "helper" strands. Each of these strands can accommodate at least one pendant group, giving rise to the capability of presenting up to on the order of 250 different epitopes on one origami platform. An example of this type of capability a "DNA cassette" based display of a simple protein, Neutravidin, which was generated by the Norton Group at Marshall University, is shown in Figure 10. Neutravidin is widely used in protein biotechnology (not in vaccine work) because it has extraordinarily strong affinity for biotin. The entire design for the origami construct is represented schematically in Fig. 10 (A). Two staples, one on either side of a window feature in the origami, were biotinylated during synthesis. These staples were incorporated into the origami during the annealing process, along with all of the other staples. In a separate step, the Neutravidin was incubated with the pre-formed Origami constructs. Three Origami constructs are visible in the AFM image shown in Fig. 10 (B). The leftmost one is not modified and the other two have apparently been modified. Here, the Neutravidin is large enough to be readily visible as the two white spots on the two modified origami in Fig 10 (B). It may be noted that the AFM does not present objects with equal resolution and the in plane resolution is much worse than the out of plane (z) resolution, due to tip artifacts. Hence, the Neutravidins are actually less than 3 nm in diameter, representing a much smaller fraction of the origami surface than they appear to occupy. This type of demonstration is important because studies on haptenated polymers have shown that the immunogenicity of the polymers is controlled by

the molecular weight of the polymer and the number of attached haptens. The polymer can change from almost non-immunogenic to highly immunogenic when the molecular weight of the polymer is over 100k dalton and the number of

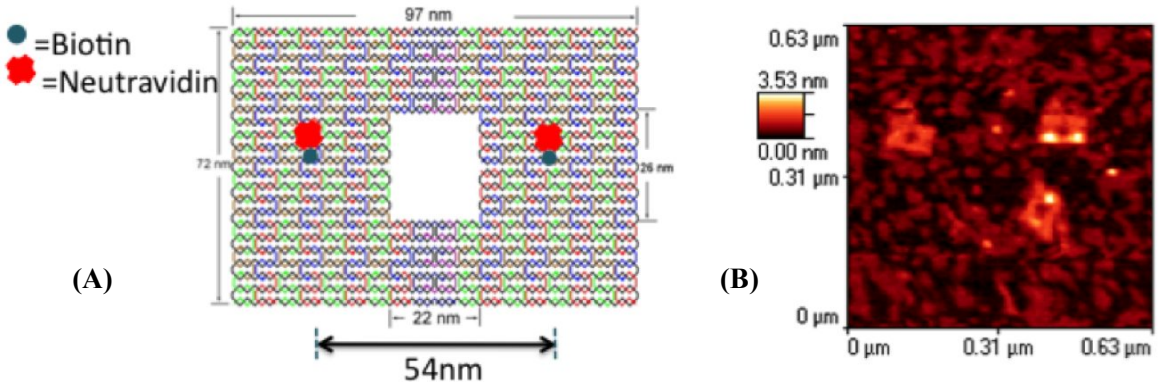

Fig. 3. (A) Design of a "DNA cassette" based display of the simple protein, Neutravidin, which is to be located on either side of the widow feature by biotinylation during synthesis. (B) AFM image of self-assembled design from (A). Note that the biotin is too small to be observed. These are unpublished result that was provided by the Norton Group at Marshall University.

attached haptens exceeds 20 [20]. The molecular weight of a typical DNA origami structure can be over 2M daltons, and, theoretically, each staple strand can attach to one antigen or hapten molecule, which will allow up to 250 attachments on the DNA origami. Therefore, DNA origami will be a powerful platform to present high numbers of varied immunogenic molecules, and this should facilitate new synthetic vaccine discoveries.

Standard immunology textbooks [e.g., Coico and Sunshine 2009] list four criteria for inducing immunogenicity, 1) Foreignness, 2) High Molecular Weight, 3) Chemical Complexity, and 4) Degradability. A high molecular weight DNA origami structure that is decorated with haptens can be designed to meet all of these criteria. Our initial work will focus on two species from the genus Burkholderia, ie. *B. mallei* and *B. pseudomallei.* An artificial vaccine must appear foreign to the immune system. The immune system determines foreignness using various mechanisms. One important mechanism is the use of Toll-like receptors (TLR). TLR's look for pathogen associated molecular patterns (PAMP). Activation of TLR's facilitates the initiation of the immune system by the production of pro-inflammatory cytokines by the activated cells. The TLR9 receptor is a TLR that specifically recognizes double sided DNA. The DNA can be made to look more foreign by the introduction of unmethylated CpG sites [CpG site wiki] into the DNA structure. DNA with an excess of unmethylated CpG sites is generally recognized by TRL9 as being of bacterial or viral origin. The placement of haptens can also enhance foreignness. The placement of the O-Antigen [21] of Burkholderia on the DNA structure will make the structure look like a Gram-negative bacteria. In particular the O-Antigen will interact with the Toll-like receptor TLR4. Activation of TRL4 and TRL9 will significantly increase the likelihood that the artificial vaccine will be recognized as foreign and processed by the immune system.

A good vaccine will induce both T-Cell independent and T-Cell dependent responses. A T-Cell independent response can be achieved by the direct interaction of the immunogen with B-cells. When several epitopes on the immunogen react with IgM molecules on the surface of a B-cell, the B-cell undergoes clonal proliferation and also begins to produce antibodies. However, other parts of the immune system do not engage unless there is also a T-Cell dependent response. In particular, memory cells are not formed without a T-Cell depended response. Without a T-Cell dependent response, any immunity is transient and disappears after a short time. For a T-Cell dependent response to occur the immunogen must be phagocytized by an antigen presented cell (APC). The immunogen is then partially digested by the APC in the form of enzymatic degradation. Fragments of the immunogen are then presented to the T-Cell via MHC molecules on the surface of the APC. In general carbohydrates and nucleic acids do not exhibit a strong T-Cell dependent response. Proteins are required for this step. For the Burholderia studies, adhesion molecules [22] will be used on the surface of *B. mallei* and *B. pseudomallei*. In order for an immunogen to elicit a T-Cell dependent response, the immunogen must be

degradable. The immunogen must be sufficiently stable to reach the site of interaction with B Cells, T Cells, APC's. However, it must be susceptible to partial enzymatic degradation by the APC's. The result of the enzymatic degradation must produce small peptides (8-12 amino acids) that are specific to the immunogen. The peptides will then be presented to the T-Cell to induce a T-Cell dependent response.

A DNA origami-based artificial vaccine can be designed to meet the four criteria of a good immunogen. 1) The structure can be made to appear foreign to the host, 2) The structure has a large molecule weight, 3) The structure is chemically complex and can be designed to induce T-Cell independent and T-Cell dependent responses, and 4) The structure can be design to be stable enough to reach the interaction site with B Cells, T Cells, APC's, but also susceptible to enzymatic degradation to produce unique peptides that are 8-12 amino acid long. The O-antigen (a polysaccharide) and the Burkholderia adhesins are readily available in sufficient quantities from a number of universities. If the long-term goals of the program are successful, it should motivate additional production sources for these haptens. The application goal is to develop memory cells that are sensitive to the adhesins and/or O-antigen. *B. mallei* and *B. pseudomallei* express these haptens on their surface. Thus, there is a reasonable probability that immunity will be formed against Burkholderia.

5. CONCLUSION

This paper has presented an overview of a U.S. Army and U.S. DTRA supported program that seeks to innovate a completely new class of functionalized DNA materials for use in threat-agent sensing and medical applications. Here, the major scientific components and the long-range goals of the program were first discussed, and the importance of DNA nanoscaffolds (e.g., DNA Origami) was illustrated. The paper also provided insights into some novel molecular elements for realizing hybrid biological-organic functionalized (HBOF) smart material for biological (and chemical) sensing in the context of antibody and receptor mimics, and extrapolations of these same basic techniques were provided to show their potential for developing synthetic vaccines in the future.

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