Molecular Baskets and Claws Look to Detect and Grab Nerve Agents

Continuing work on molecular baskets could soon provide better protections against nerve agents for warfighters, first responders and civilians. The results of recent studies by DTRA CB/JSTO-funded researchers could aid in developing a novel methodology for the selective detection and acquisition that could lead to the ultimate goal of the rapid degradation of organophosphorus nerve agents (OPNA).

Researchers, led by Dr. Jovica Badjic from Ohio State University, are designing amphiphilic concave molecular hosts, which they refer to as “baskets,” to bind and degrade OPNA. In particular, each concave host is being computationally designed to comprise a hydrophilic rim and hydrophobic cavity for accommodating a particular organophosphonate molecule, akin in size and shape to a

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Important Milestone for Multi-threat Countermeasure

A medical countermeasure (MCM) showing promise against several different threats to U.S. warfighters met an important milestone and has been put on the “Fast Track” towards fulfilling the U.S. Food and Drug Administration (FDA) requirement of efficacy in “Animal Rule” studies. GSK944 is a chemical entity known as a Novel Bacterial Topoisomerase Inhibitor (NBTI) that targets replication machinery fundamental to many high priority threat pathogens. It was developed in a multi-year partnership between DTRA CB/JSTO and commercial drug manufacturer GlaxoSmithKline (GSK).

It received an FDA “head nod” of approval that the compound has shown efficacy within the rigorous statistical analysis on this highly powered study conducted at the Contract Research Organization Lovelace Respiratory Research Institute in New Mexico. The DTRA CB/JSTO and GSK team received a go-ahead to proceed with the second (required) efficacy and Pharmacokinetics (PK) study for treatment of highly lethal pneumonic plague. Had the first study failed, the program could have lost its opportunity to register the compound GSK944 for this threat pathogen. In addition, the compound in hand, GSK944, has rapidly progressed via “Fast Track” efforts under DTRA CB/JSTO sponsorship aiming to gain Pre-Emergency Use Authorization (EUA) status to fulfill the Multiple Drug Resistance (MDR), Broad Spectrum Anti-Threat Pathogens Antibiotics (BSABP) promise.

“Animal Rule” studies are experiments done in qualified animal models, recommended by the FDA in lieu of clinical studies that cannot be done with threat pathogens. “Animal Rule” studies require proof of efficacy, safety and tolerability, as well as drug exposure parameters that comply with FDA guidance aimed to secure safety margins in the absence of human clinical studies. The DTRA/GSK team has been asked to perform pivotal “Animal Rule” studies with GSK944 as one of the many requirements for registration for treatment of lethal plague infection. A second round of “Animal Rule” testing is expected to take place in the first quarter of 2014. If GSK944 passes those requirements, it could become the first novel antibacterial compound against threat agents that completed “Animal Rule” requirements prior to a large investment in late clinical studies (Phase II/III), which

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carry exponentially higher costs. In exercising this strategy, DTRA CB/ JSTO mitigated risks associated with advanced development investment in an unproven compound. It is also important to note that the program is by no means a “slam dunk”; much work is still ahead of the team.

The “Fast Track” method of developing drugs against emerging threats bacterial pathogens could significantly cut the costs and time it takes to get these compounds to warfighters. It represents what officials are calling a paradigm shift in how MCM discovery and testing is conducted. The protocol cuts the average cost by 70-80 percent and reduces by half the amount of time it takes to stockpile innovative drugs that could serve in emergency situation. This makes compounds available to the military years before the traditional drug development process that seeks a-priori regulatory licenses for civilian indication of the compound.

The GSK944 program meets Department of Defense goals of having one drug for multiple threat pathogens, known as BSABPs. In addition, DoD wants to develop novel compounds that are highly effective against MDR pathogens. This latter attribute is of significant importance in regards to “outpacing the threat” and gaining superior preparedness since many contemporary marketed antibiotics already succumb to MDR pathogens, including highly infectious threat pathogens.

Reengineered Bacterial Protein Could Serve as Broad-spectrum Nerve Agent Countermeasure

Warfighters could have better countermeasures to protect themselves from deadly VX nerve agents due to research funded by DTRA CB/JSTO at the Weizmann Institute of Science and the University of Washington.

Researchers there are working on a way to reengineer a bacterial protein, Brevundimonas diminuta phosphotriesterase (PTE), to hydrolyze VX and its derivatives. This enzyme-based approach could lead to a medical countermeasure that would quickly degrade nerve agents in the blood before they can enter and damage the central nervous system. Since currently approved measures can only restore normal neural function and alleviate symptoms after nerve agent exposure, the technologies explored here could represent a first-in-kind solution to protect people before any damage is done.

In the journal ACS Chemical Biology article, “Engineering V-Type Nerve Agents Detoxifying Enzymes Using Computationally Focused Libraries,” the researchers detailed their computation and experimental efforts to engineer PTE variants with fast hydrolysis capability and high specificity for V-type nerve agents. The resulting enzyme variants can hydrolyze V and G nerve agents at sufficient speeds to warrant their testing as medical countermeasures. In fact, researchers used an integrated computational and experimental approach to increase the PTE detoxification rate of V-agents by 5,000-fold. More work is still necessary to develop these enzymes into a drug, but this research effort suggests that a broad-spectrum medical countermeasure for nerve agent exposure is within reach.

New Tool Gives Better Reachback, Collaborative Capabilities

A new bio-tool could give warfighters an improved way to identify and share threat information with scientists and commanders from around the globe. The DTRA/JTSO-funded program, known as Edge Bioinformatics, will provide enhanced analysis for High-Throughput Sequencing (HTS) data for advanced characterization of bio-threats. The program entails prototyping analysis pipelines for overseas military laboratories that are aligned to common use scenarios such as improving an assay or characterizing an unknown sample. The pipelines are developed within an environment that permits scientists back in the U.S. to remotely analyze data when overseas scientists request extra analytical support.

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The Defense Threat Reduction Agency’s (DTRA) Research and Development (J9) Directorate, Chemical and Biological (CB) Technologies Department, serves as the Joint Science and Technology Office for Chemical and Biological Defense. This newsletter highlights the organization’s accomplishments to protect warfighters and citizens through the innovative application of science and technology research.
In other work, the team discovered a way to produce "synthetic claws" that will selectively grab small chemical analytes in a claw-like motion.

In the *Journal of the American Chemical Society* article titled, "Assembly of Amphiphilic Baskets into Stimuli-Responsive Vesicles. Developing a Strategy for the Detection of Organophosphorus Chemical Nerve Agents," the researchers report their discovery that amphiphilic molecular baskets assembled into sizeable vesicles (see Figure 1) in water and then changed their appearance in the presence of organophosphorus (OP) compounds. Specifically, the baskets were found to aggregate into spherically shaped vesicles ("bubbles" of 350 nm in diameter) with the boundary bilayer comprising of tightly packed concave hosts (see Figure 1). The formation of vesicles was confirmed with an array of analytical techniques, including dynamic light scattering (DLS) measurements, transmission electron microscopy (TEM) and diffusion nuclear magnetic resonance (NMR) spectroscopy. Upon encountering in solution OPNA simulant, dimethyl phenylphosphonate (DMPP) that is similar in size to soman, (a nerve agent referred to as GD), the vesicles changed into nanoparticles of approximately 100 nm in diameter. With the assistance of computational methods and NMR spectroscopy, the authors deduced that DMPP guest slides into the cavity of molecular baskets comprising the vesicular boundary. This molecular event would change the basket’s shape and destabilize the packing of host molecules so that the formation of nanoparticles follows.

The stimuli-responsive nature of the basket-containing vesicles is rather unique and could be of great value for developing shape-changing materials capable of the selective detection and rapid degradation of nerve agents. The researchers believe that entrapping a degradation catalyst and/or chemical indicators in the reservoir of the responsive vesicles could, in the presence of nerve agents, release the cargo to signal the presence of toxic substances and initiate their degradation.

In the other article in *Angewandte Chemie International Edition* titled, “A Molecular Claw: A Dynamic Cavitand Host,” they report a synthetic method of yet another type of cavitand obtaining, which they refer to as "molecular claws." These novel hosts, which could be prepared in large quantities within a short period of time, are internally functionalized to selectively "grab" small chemical analytes in a claw-like motion. In particular, the inner part of the claw is dynamic with alkyl chains being folded on the top of the large aromatic floor. Upon encountering a guest molecule in solution, the chains unfold to create a space for accommodating guest species (See Figure 2). Importantly, one can easily tailor the outer as well as the inner surface of these concave molecules to create a cavitand complementary to desired molecular targets. In addition, the photophysical characteristics of the large aromatic floor should alter in the presence of guests to report on the trapping event. As nerve agents could fit in the cavity of molecular claws, they are currently examining the possibility of employing these dynamics hosts for unambiguously reporting on their presence in the environment.

The articles build on previous DTRA CB/JSTO-funded work Badjic did on the subject. In his earlier works, he was able to contribute to the development of different classes of chemical constructs that bind specific classes of toxic chemical compounds. These discoveries are important in the effort to lead to the construction of molecules that promote selective detection and ultimate degradation of chemical warfare agents (CWAs) that could prove deadly to warfighters on the battlefield and citizens living in populous cities.

**Molecular Baskets ...** (continued from page 1)

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“Smart” Bacteria Sense and Defeat Infectious Agents

Significant progress has been made in DTRA CB/JSTO efforts to develop autonomous high-order functioning cells that sense and defeat infectious diseases and other maladies. This effort builds on previous work that focuses on understanding and manipulating biological signaling processes through acquiring and redesigning a trove of involved modular components, which can be rewired for controlling these processes for user-specified performance in a dynamic and heterogeneous environment. In this pursuit, the DTRA CB/JSTO-funded research team led by Dr. William E. Bentley from University of Maryland is developing “smart” bacteria that sense, track, pursue, fight and defeat infectious agents and other maladies afflicting the warfighter.

Very recently, the team published two articles. In the *Journal of the American Chemical Society* article titled, “Crystal Structures of the LsrR Proteins Complexed with Phospho-AI-2 and Two Signal-Interrupting Analogues Reveal Distinct Mechanisms for Ligand Recognition,” the scientists used detailed crystal structure determinations to reveal the specific mechanisms by which the binding of phosphorylated quorum sensing signal molecule in E. coli, auto-inducer-2 (AI-2-P), and its chemical analogues bind and actuate gene expression. Quorum sensing (QS) is a highly conserved mechanism of bacterial cell−to-cell communication. The researchers consider QS-based synthetic signal transduction pathways to represent a new generation of biotechnology toolbox members that they plan to exploit, especially since in an E. coli QS system network, the functional QS units can be parsed and rearranged to serve as synthetic genetic switches, oscillators, or biosensors. Specifically, the E. coli QS master regulator, LsrR, is uniquely positioned to actuate gene expression in response to a QS signal. This study revealed that the AI-2-P binding to the repressed state tetrameric LsrR destabilize tetramers’ repression of promoter sites by converting them into pairs of dimers, and the consequent release of these tetrameric assemblies from promoters enables gene expression.

The findings reported in the *ACS Synthetic Biology* article, “Evolved Quorum Sensing Regulator, LsrR, for Altered Switching Functions,” enabled the scientists to add two new tools to their toolbox. Specifically, through directed evolution, they synthesized two new LsrR molecules: an enhanced (eLsrR) and anti (aLsrR)
Antibodies Improving Ebola Survivability

New developments using monoclonal antibodies (mAbs) to block Ebola infection and even to treat after symptoms appear show promise in protecting warfighters and first responders. DTRA CB/JSTO-funded research by Mapp Biopharmaceutical, Inc. (San Diego, Calif.), working with the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID - Frederick, Md.), looked at the design, production, and testing of mAbs targeting the Ebola virus (EBOV) glycoprotein as a means to block the infection process.

Results from this work could ultimately give warfighters and first responders better protection and treatment against Ebola, an issue of increasing importance as human incursions into endemic regions occur more frequently.

It is particularly compelling that the mAbs demonstrated effectiveness in infected primates, even after symptoms developed greater than four days after Ebola exposure (i.e., a realistic “trigger-to-treat” scenario). EBOV is a highly lethal disease that can quickly overwhelm its hosts’ immune system. To rigorously test the potential of their mAbs, scientists implemented a study protocol to mimic a disease treatment scenario, where the initial exposure to an infectious agent, such as Ebola, goes undetected. In this study, none of the infected subjects received the mAbs treatment until clinical signs (fever) and confirmation of the infectious agent (circulating virus particles) could be detected.

The work is detailed in the journal Science Translational Medicine article, “Therapeutic Intervention of Ebola Virus Infection in Rhesus Macaques with the MB-003 Monoclonal Antibody Cocktail,” where the researchers, in collaboration with USAMRIID, used sophisticated telemetry methods to monitor body temperature and a virus diagnostic protocol that detected Ebolavirus in the primates’ blood. The authors point out that, “the ability to treat at a later time point after initial exposure and to mitigate further morbidity and disease pathogenesis underscores the therapeutic potential of mAbs.”

Another benefit to Mapp’s mAbs is that, while originally derived from mice, Mapp has already “chimerized” the murine mAbs with human mAbs. This limits the human immune system from responding to mAbs treatment, thereby improving safety and likely prolonging their effectiveness in humans. With these advances, further study of the mAbs might one day support their advancement into human testing.

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“WE INVEST IN TRANSFORMATIONAL TECHNOLOGIES TO SAVE AND IMPROVE LIVES.”
Anthrax Genes Probed to Discover Better Countermeasures

Protections for warfighters and first responders against weaponized anthrax could soon be on the way. DTRA CB/JSTO-funded investigators from the U.S. Air Force Research Laboratory (AFRL) have found a way to turn off some genes in Bacillus anthracis that could lead to improved targeting of those genes by new antibiotics. Current antibiotics designed to protect against or fight anthrax infections, such as ciprofloxacin, doxycycline, and penicillin, do not always work, especially against genetically engineered strains.

In the journal BMC Biotechnology, the AFRL researchers published a paper titled, “Rapid targeted gene disruption in Bacillus anthracis,” which described how specific DNA sequences known as Group II introns were inserted into specific genes of B. anthracis, the causative agent of Anthrax and a known biowarfare agent. By inserting the introns into a specific gene, the AFRL group was able to shut off the gene and observe its effects on growing bacteria. This ability to directly select and inactivate a gene using just a very few manipulations, accelerates the ability to uncover new targets for vaccination strategies or useful targets for therapeutic intervention. Work is already underway at AFRL to translate these new targets into new antibiotics to protect warfighters and the general population against an anthrax bio-attack.

The demonstration included two examples of how an overseas scientist could analyze Next Generation Sequencing (NGS) data using the Edge Bioinformatics system. One of the examples was to identify a new strain of flu from a clinical sample and then quickly design an assay for that strain. Within this example, the team also demonstrated how overseas and U.S.-based scientists could work together through a remote connection to interpret results.

Also in attendance for the Edge Bioinformatics demonstration were representatives from Navy Medicine, Walter Reed Army Institute of Research (WRAIR), the Armed Forces Health Surveillance Center - Global Emerging Infections Surveillance and Response System, the United States Army Medical Research Institute for Infectious Diseases (USAMRIID), the Joint Program Executive Office (JPEO-CBD) the Department of Homeland Security (DHS) and DTRA. This is the program’s first year of funding. Success of the program will give warfighters and first responders better tools to gather and share threat information with experts who might not be on the front lines with them, resulting in better protections against those threats.

Bacterial Adhesion

Improved understanding of the interaction of microbes with functional surfaces is important in risk analysis and designing broad spectrum physical and medical countermeasures to chemical and biological-relevant pathogens. Colloidal models are frequently used to model the thermodynamics of bacterial attachment to surfaces. The inability to accurately model bacterial adhesion and biofilm formation inhibits the development of decontaminants that can more effectively clean environmental and protective surfaces contaminated by biotreats, such as tularemia and plague. The present discovery will lead to a more nuanced view of the bacterial cell surface and its relevance in attachment, which will improve predictive understanding of the interaction of proposed bacterial countermeasures with biofilms. This will enable the development, for example, of improved decontaminants and protective garments that will enable warfighters to effectively counter bacterial threats.

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