

10 June 2010

This supplement has been prepared to present scientific and technical news items that may be of more interest to technical personnel at RDT&E activities and the labs, or the medics rather than the broader readership of the basic CB Daily. Due to the nature of the material, the articles, if available online, are usually only available through subscription services thus making specific links generally unavailable. Thus, usually only the bibliographic citation is available for use by an activity's technical library.

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Chem-Bio News – S&T Edition

1. STRUCTURAL EVIDENCE THAT HUMAN ACETYLCHOLINESTERASE INHIBITED BY TABUN AGES THROUGH O-DEALKYLATION:

"This first structure of hAChE inhibited by a nerve agent provides structural insight into the inhibition and aging mechanisms and a structural template for the design of molecules capable of reactivating aged hAChE."

2. COMPREHENSIVE PANEL OF REAL-TIME TAQMAN POLYMERASE CHAIN REACTION ASSAYS FOR DETECTION AND ABSOLUTE QUANTIFICATION OF FILOVIRUSES, ARENAVIRUSES, AND NEW WORLD HANTAVIRUSES:

"Although these real-time hemorrhagic fever virus assays are qualitative (presence of target), they are also quantitative (measure a single DNA/RNA target sequence in an unknown sample and express the final results as an absolute value (e.g., viral load, PFUs, or copies/mL) on the basis of concentration of standard samples and can be used in viral load, vaccine, and antiviral drug studies."

3. A NEW SILOXANE POLYMER FOR CHEMICAL VAPOR SENSOR: *"In addition, the sensitivity of the fabricated QCM sensor to DMMP was up to 23.07 Hz/ppm, and it is much higher than that of the other chemical vapors under the same circumstances, the detection limits (LODs) of this fabricated QCM sensor was obtained as low as 0.13 ppm."*

4. A SELF-REFERENCING GLUTAMATE BIOSENSOR FOR MEASURING REAL TIME NEURONAL GLUTAMATE FLUX:

"Quantification of neurotransmitter transport dynamics is hindered by a lack of sufficient tools to directly monitor bioactive flux under physiological conditions. Traditional techniques for studying neurotransmitter release/uptake require inferences from non-selective electrical recordings, are invasive/destructive, and/or suffer from poor temporal resolution."

5. DISTRIBUTION OF HOMOLOGOUS PROTEINS TO PUFFER FISH SAXITOXIN AND TETRODOTOXIN BINDING PROTEIN IN THE PLASMA OF PUFFER FISH AND AMONG THE TISSUES OF FUGU PARDALIS EXAMINED BY WESTERN BLOT ANALYSIS:

"These data suggest that puffer fish commonly possess glycoproteins homologous to PSTBP. but the sizes of N-glycan are specific to the species."

6. INTERACTION OF DIMETHYLMETHYLPHOSPHONATE WITH ZEOLITE Y: IMPEDANCE-BASED SENSOR FOR DETECTING NERVE AGENT SIMULANTS:

"Sensing data were obtained over a temperature range of 300-350 degrees C, with best results at 320 degrees C. This device exhibited minimal response to CO, NH3, methane, and propane, possible interferents in the ambient air."

7. NEW "TREE OF LIFE" ESTABLISHED FOR ONE OF THE LARGEST GROUPS OF BACTERIA:

"A new "tree of life" has been constructed by researchers at the Virginia Bioinformatics Institute (VBI) at Virginia Tech for the gamma-proteobacteria, a large group of medically and scientifically important bacteria that includes Escherichia coli, Salmonella typhimurium, and other disease-causing organisms."

8. DENATURED RICIN CAN BE DETECTED AS NATIVE RICIN BY IMMUNOLOGICAL METHODS, BUT NONTOXIC IN VIVO:

"Detection of native and denatured ricin is very difficult, and the investigating agencies, forensic scientists, and analysts should be very careful while interpreting the results."

9. 4TH NATIONAL CONFERENCE ON ENVIRONMENTAL SAMPLING AND DETECTION OF BIOTHREAT AGENTS:

"December 7-9, 2010 in New Orleans, LA."

10. A STAPHYLOCOCCUS AUREUS SMALL RNA IS REQUIRED FOR BACTERIAL VIRULENCE AND REGULATES THE EXPRESSION OF AN IMMUNE-EVASION MOLECULE:

"Here we show that one of

the small pathogenicity island RNAs, named SprD, contributes significantly to causing disease in an animal model of infection. We have identified one of the targets of SprD and our in vivo data demonstrate that SprD negatively regulates the expression of the Sbi immune-evasion molecule, impairing both the adaptive and innate host immune responses."

11. ANTIBACTERIAL NANOPARTICLES FROM BACTERIA: *"Scientists have found that silver nanoparticles made using bacteria have better antibacterial properties than their chemically synthesised counterparts."*

CB Daily Report

Chem-Bio News

STRUCTURAL EVIDENCE THAT HUMAN ACETYLCHOLINESTERASE INHIBITED BY TABUN AGES THROUGH O-DEALKYLATION

Life Science Weekly
June 8, 2010

"According to a study from Grenoble, France, "Tabun is a warfare agent that inhibits human acetylcholinesterase (hAChE) by rapid phosphorylation of the catalytic serine. A time-dependent reaction occurs on the tabun adduct, leading to an 'aged' enzyme, resistant to oxime reactivators."

"The aging reaction may proceed via either dealkylation or deamidation, depending on the stereochemistry of the phosphoramidyl adduct. We solved the X-ray structure of aged tabun-hAChE complexed with fasciculin II, and we show that aging proceeds through O-dealkylation, in agreement with the aging mechanism that we determined for tabun-inhibited human butyrylcholinesterase and mouse acetylcholinesterase. Noteworthy, aging and binding of fasciculin II lead to an improved thermostability, resulting from additional stabilizing interactions between the two subdomains that face each other across the active site gorge."

"This first structure of hAChE inhibited by a nerve agent provides structural insight into the inhibition and aging mechanisms and a structural template for the design of molecules capable of reactivating aged hAChE."

The full article can be found at: (E. Carletti, et. al., "Structural evidence that human acetylcholinesterase inhibited by tabun ages through O-dealkylation". Journal of Medicinal Chemistry, 2010;53(10):4002-8). Link not available.

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COMPREHENSIVE PANEL OF REAL-TIME TAQMAN POLYMERASE CHAIN REACTION ASSAYS FOR DETECTION AND ABSOLUTE QUANTIFICATION OF FILOVIRUSES, ARENAVIRUSES, AND NEW WORLD HANTAVIRUSES

Medical Letter on the CDC & FDA

May 30, 2010

"Viral hemorrhagic fever is caused by a diverse group of single-stranded, negative-sense or positive-sense RNA viruses belonging to the families Filoviridae (Ebola and Marburg), Arenaviridae (Lassa, Junin, Machupo, Sabia, and Guanarito), and Bunyaviridae (hantavirus). Disease characteristics in these families mark each with the potential to be used as a biological threat agent."

"Because other diseases have similar clinical symptoms, specific laboratory diagnostic tests are necessary to provide the differential diagnosis during outbreaks and for instituting acceptable

quarantine procedures. We designed 48 TaqMan-based polymerase chain reaction (PCR) assays for specific and absolute quantitative detection of multiple hemorrhagic fever viruses. Forty-six assays were determined to be virus-specific, and two were designated as pan assays for Marburg virus. The limit of detection for the assays ranged from 10 to 0.001 plaque-forming units (PFU)/PCR."

"Although these real-time hemorrhagic fever virus assays are qualitative (presence of target), they are also quantitative (measure a single DNA/RNA target sequence in an unknown sample and express the final results as an absolute value (e.g., viral load, PFUs, or copies/mL) on the basis of concentration of standard samples and can be used in viral load, vaccine, and antiviral drug studies."

The full article can be found at: (A.R. Trombley, et. al., "Comprehensive panel of real-time TaqMan polymerase chain reaction assays for detection and absolute quantification of filoviruses, arenaviruses, and new world hantaviruses". The American Journal of Tropical Medicine and Hygiene, 2010;82(5):954-60). Link not available.

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A NEW SILOXANE POLYMER FOR CHEMICAL VAPOR SENSOR

Journal of Technology & Science

May 30, 2010

"A new hexafluoroisopropanol groups functionalized hydrogen-bond acidic siloxane polymer for quartz crystal microbalance sensor (QCMs) application was synthesized via O-alkylation, Claisen rearrange, hydrosilylation and hexafluoroacetone groups (HFA) electrophilic addition reactions."

"This hexafluoroisopropanol-functionalized siloxane polymer was confirmed by FT-IR and H-1 NMR, and coated onto an AT-cut 8 MHz QCM sensor to investigate its gas sensitive responses to eight different typical organic volatile chemical vapors. The results indicated that this new siloxane polymer exhibited an excellent performance on the detection of the organophosphorus nerve agent stimulant dimethyl methylphosphonate (DMMP) vapor, and the frequency changes of this functionalized siloxane polymer based QCM sensors to the DMMP vapor were completely linear and with a regression coefficient of 0.9993 in the concentration range of 10-60 ppm."

"In addition, the sensitivity of the fabricated QCM sensor to DMMP was up to 23.07 Hz/ppm, and it is much higher than that of the other chemical vapors under the same circumstances, the detection limits (LODs) of this fabricated QCM sensor was obtained as low as 0.13 ppm."

The full article can be found at: (J. Huang, et. al., "A new siloxane polymer for chemical vapor sensor". Sensors and Actuators B - Chemical, 2010;146(1):388-394). Link not available.

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A SELF-REFERENCING GLUTAMATE BIOSENSOR FOR MEASURING REAL TIME NEURONAL GLUTAMATE FLUX

Biotech Week

May 26, 2010

"Quantification of neurotransmitter transport dynamics is hindered by a lack of sufficient tools to directly monitor bioactive flux under physiological conditions. Traditional techniques for studying neurotransmitter release/uptake require inferences from non-selective electrical recordings, are invasive/destructive, and/or suffer from poor temporal resolution."

"Recent advances in electrochemical biosensors have enhanced in vitro and in vivo detection of neurotransmitter concentration under physiological/pathophysiological conditions. The use of enzymatic biosensors with performance enhancing materials (e.g., carbon nanotubes) has been a major focus for many of these advances. However, these techniques are not used as mainstream neuroscience research tools, due to relatively low sensitivity, excessive drift/noise, low signal-to-noise ratio, and inability to

quantify rapid neurochemical kinetics during synaptic transmission. A sensing technique known as self-referencing overcomes many of these problems, and allows non-invasive quantification of biophysical transport. This work presents a self-referencing CNT modified glutamate oxidase biosensor for monitoring glutamate flux near neural/neuronal cells. Concentration of basal glutamate was similar to other in vivo and in vitro measurements. The biosensor was used in self-referencing (oscillating) mode to measure net glutamate flux near neural cells during electrical stimulation. Prior to stimulation, the average influx was $33.9 \pm 6.4 \text{ fmol cm}^{-2} \cdot \text{s}^{-1}$. Glutamate efflux took place immediately following stimulation, and was always followed by uptake in the $50\text{-}150 \text{ fmol cm}^{-2} \cdot \text{s}^{-1}$ range. Uptake was inhibited using threo-beta-benzyloxyaspartate, and average surface flux in replicate cells ($1.1 \pm 7.4 \text{ fmol cm}^{-2} \cdot \text{s}^{-1}$) was significantly lower than uninhibited cells."

The full article can be found at: (E.S. McLamore, et. al., "A self-referencing glutamate biosensor for measuring real time neuronal glutamate flux". Journal of Neuroscience Methods, 2010;189(1):14-22). Link not available.

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DISTRIBUTION OF HOMOLOGOUS PROTEINS TO PUFFER FISH SAXITOXIN AND TETRODOTOXIN BINDING PROTEIN IN THE PLASMA OF PUFFER FISH AND AMONG THE TISSUES OF FUGU PARDALIS EXAMINED BY WESTERN BLOT ANALYSIS

Energy & Ecology

June 11, 2010

"Puffer fish saxitoxin and tetrodotoxin binding protein (PSTBP) is a glycoprotein (200 kDa as a dimer) that we previously isolated from the plasma of *Fugu pardalis* (Yotsu-Yamashita et al., 2001). For the study on functions of PSTBP, here we examined distribution of homologous proteins to PSTBP in the plasma of seven species of puffer fish, and among the tissues of *E. pardalis* by Western blot analysis probed with a polyclonal IgG against unglycosylated PSTBP1 expressed in *E. coli* [sic: coli]."

"One or two major positive broad bands were detected at 105-140 kDa molecular weight range in the plasma (0.5 μg protein) of all species of puffer fish tested, while no band was detected in the plasma (5 μg protein) of fish other than puffer fish. Glycopeptidase F treated plasma of all species of puffer fish tested commonly showed the bands at approximately 42 kDa that was consistent to the molecular weight of unglycosylated PSTBP. These data suggest that puffer fish commonly possess glycoproteins homologous to PSTBP. but the sizes of N-glycan are specific to the species."

"Among soluble protein extracts (5 μg protein) from the tissues of *E. pardalis*, PSTBP was detected in all tissues examined, most prominently in heart, skin, and gall."

The full article can be found at: (M. Yotsuyamashita, et. al., "Distribution of homologous proteins to puffer fish saxitoxin and tetrodotoxin binding protein in the plasma of puffer fish and among the tissues of *Fugu pardalis* examined by Western blot analysis". Toxicon, 2010;55(6):1119-1124). Link not available.

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INTERACTION OF DIMETHYLMETHYLPHOSPHONATE WITH ZEOLITE Y: IMPEDANCE-BASED SENSOR FOR DETECTING NERVE AGENT SIMULANTS

News of Science

June 6, 2010

"Dimethylmethylphosphonate (DMMP) is a simulant for the highly toxic organophosphate nerve agent Sarin (GB). The influence of DMMP on the ionic conductivity of zeolite Y is investigated by impedance spectroscopy."

"In the presence of 20-100 ppm of DMMP, the ionic conductivity of the sodium exchanged form of the zeolite showed an increase. The interaction between DMMP and the zeolite was elucidated by examining

different cation-exchanged zeolites as well as external surface modification with ceria to deactivate acidic groups. A mechanism involving the binding of the sodium cation with the phosphonate group of DMMP that results in facilitated inter cage motion of the cation is proposed. The change in impedance measured at a single frequency of 3000 Hz allows for the use of Na⁺-exchanged zeolite Y as a sensor for detecting ppm level of DMMP."

"Sensing data were obtained over a temperature range of 300-350 degrees C, with best results at 320 degrees C. This device exhibited minimal response to CO, NH₃, methane, and propane, possible interferences in the ambient air."

The full article can be found at: (X.G. Li, et. al., "Interaction of Dimethylmethylphosphonate with Zeolite Y: Impedance-Based Sensor for Detecting Nerve Agent Simulants". Journal of Physical Chemistry C, 2010;114(17):7986-7994). Link not available.

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NEW "TREE OF LIFE" ESTABLISHED FOR ONE OF THE LARGEST GROUPS OF BACTERIA

By Barry Whyte

Virginia Bioinformatics Institute of Virginia Tech

May 17, 2010

"A new "tree of life" has been constructed by researchers at the Virginia Bioinformatics Institute (VBI) at Virginia Tech for the gamma-proteobacteria, a large group of medically and scientifically important bacteria that includes Escherichia coli, Salmonella typhimurium, and other disease-causing organisms.* By building powerful phylogenetic trees, scientists are able to quickly identify similarities and differences between the make-up of many different organisms, crucial information in the search for treatments to fight anything from the bugs that cause food poisoning to the pathogens that cause life-threatening diseases such as cholera and the plague.

A "tree of life", or phylogenetic tree, is a way to visualize the evolutionary relationships among different biological species that have descended from a common ancestor. The gamma-proteobacteria tree developed by VBI researchers was reconstructed using powerful computers from as many as 30 million data points of bacterial sequence information."

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"The researchers selected 108 available genomes from the more than 200 complete and partial sequences available for the gamma-proteobacteria, placing the emphasis on the diversity of the bacterial species and quality of the original sequence data. Allan Dickerman, Assistant Professor at VBI, remarked: "The consensus tree that we have put together for the gamma-proteobacteria is a powerful tool that can be used to predict shared biology and analyze, for example, the novel ways that bacteria have adapted to their living environments. Phylogenomics provides for very accurate reconstructions of inheritance from common ancestors."

The researchers looked at a very large class of bacteria that lack a well-resolved phylogenetic tree. By placing emphasis on searches for single-copy genes, the scientists were able to radically improve the resolution of the evolutionary tree. Said Williams, "Some parts of our tree were still not fully resolved, but we believe that future work will improve our method further to handle these deficiencies."

The full article can be found at:

https://www.vbi.vt.edu/public_relations/press_releases/new_tree_of_life_established_for_gamma_prot_eobacteria

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DENATURED RICIN CAN BE DETECTED AS NATIVE RICIN BY IMMUNOLOGICAL METHODS, BUT NONTOXIC IN VIVO

Life Science Weekly
June 1, 2010

In the present study, we purified and denatured ricin in a boiling water bath for different time intervals. We further made an attempt to identify native and denatured ricin by immunobased detection systems. All the antigen/antibody-based assays identified native and denatured ricin. On SDS-PAGE, only native ricin was observed. In western blotting, ricin boiled for 3.75 min gave a strong band on X-ray film. On native polyacryl amide gel electrophoresis, native and denatured ricin gave ricin band in 60-kDa region. The denatured ricin did cause mortality up to 25 mg/kg, while 5 and 10 μ g/kg of native ricin caused 50% and 100% mortality, respectively."

"Detection of native and denatured ricin is very difficult, and the investigating agencies, forensic scientists, and analysts should be very careful while interpreting the results."

The full article can be found at: (O. Kumar, et. al., "Denatured Ricin Can Be Detected as Native Ricin by Immunological Methods, but Nontoxic In Vivo". Journal of Forensic Sciences, 2010;55(3):801-807). Link not available

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4TH NATIONAL CONFERENCE ON ENVIRONMENTAL SAMPLING AND DETECTION OF BIO-THREAT AGENTS

CBRNIAC E-mail
June 04, 2010

"This year with Special Focus Sessions on Bio-Surveillance and Microbial Forensics

A leading forum for dialogue between government, industry, academia, and first responders to address critical issues in sampling and detection.

Save the date: December 7-9, 2010 in New Orleans, LA.

For additional information call 410-297-2300 or email info@sampling-conference.com."

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A STAPHYLOCOCCUS AUREUS SMALL RNA IS REQUIRED FOR BACTERIAL VIRULENCE AND REGULATES THE EXPRESSION OF AN IMMUNE-EVASION MOLECULE

By Svetlana Chabelskaya, Olivier Gaillot, Brice Felden
PLoS Pathogens
June 03, 2010

"Abstract

Staphylococcus aureus, a pathogen responsible for hospital and community-acquired infections, expresses many virulence factors under the control of numerous regulatory systems. Here we show that one of the small pathogenicity island RNAs, named SprD, contributes significantly to causing disease in an animal model of infection. We have identified one of the targets of SprD and our in vivo data demonstrate that SprD negatively regulates the expression of the Sbi immune-evasion molecule, impairing both the adaptive and innate host immune responses. SprD interacts with the 5' part of the sbi mRNA and structural mapping of SprD, its mRNA target, and the 'SprD-mRNA' duplex, in combination with mutational analysis, reveals the molecular details of the regulation. It demonstrates that the accessible SprD central region interacts with the sbi mRNA translational start site. We show by toeprint experiments that SprD prevents translation initiation of sbi mRNA by an antisense mechanism. SprD is a small regulatory RNA required for S. aureus pathogenicity with an identified function, although the mechanism of virulence control by the RNA is yet to be elucidated."

Author Summary

Bacteria possess numerous and diverse means of gene regulation using RNA molecules, including small RNAs (sRNAs). Here we show that one sRNA is essential for a major human bacterial pathogen, *Staphylococcus aureus*, to cause a disease in an animal model of infection. Our study provides evidence that this RNA regulates the expression of an immune evasion molecule secreted by the bacterium to impair the host immune responses, and we have solved the mechanism of the RNA-based regulation at molecular level. So far, the mechanism of bacterial virulence controlled by SprD is unrevealed, but that small RNA has a huge impact in the course of a bacterial infection. It implies possible new strategies in fighting against that major human and animal bacterial pathogen in preventing the expression of this regulatory RNA.”

The full article can be found at: <http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1000927;jsessionid=0E924AC80B5D6A1150C0C4B34EFB3F46.ambra02>
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ANTIBACTERIAL NANOPARTICLES FROM BACTERIA

By Manisha Laloo
Chemistry World
June 02, 2010

“Scientists have found that silver nanoparticles made using bacteria have better antibacterial properties than their chemically synthesised counterparts.

Mitchel Doktycz and colleagues at Oak Ridge National Laboratory and the University of Tennessee in the US incubated *Shewanella oneidensis* bacteria with silver nitrate solution to produce monodispersed silver nanoparticles.

Biological synthesis [of these particles] is really just an offshoot of a response mechanism of bacteria from metal stress,' says Doktycz. The team thinks that, when exposed to toxic Ag⁺, the bacteria converts it to stable Ag(0) as a defence. Such biological syntheses have advantages over chemical reduction routes to nanoparticles, as they give consistent sizes and material properties at room temperature and pressure, unlike the chemical route equivalents.

The team compared the toxicity of their biogenic nanoparticles to two types of chemically synthesised nanoparticles - bare silver nanoparticles (colloidal-Ag) and fatty acid coated silver nanoparticles (oleate-Ag). Assays carried out on *Escherichia coli*, *Bacillus subtilis* and *S. oneidensis* bacteria showed that for all three strains the biogenic nanoparticles had the highest toxicity levels. Colloidal-Ag was less potent, while oleate-Ag was not toxic to any of the bacteria.”

The full article can be found at: <http://www.rsc.org/chemistryworld/News/2010/June/04061001.asp>
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Steve Tesko: Steve.Tesko@anser.org

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