

4 February 2010

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## **Chem-Bio News – S&T Edition**

### **1. NEW CLASSES OF ORTHOPOXVIRUS VACCINE CANDIDATES BY FUNCTIONALLY**

**SCREENING A SYNTHETIC LIBRARY FOR PROTECTIVE ANTIGENS:** *"New vaccines might be developed from productive combinations of these new and existing antigens to confer potent, broadly efficacious protection and be contraindicated for none."*

**2. CENTRAL ORIGIN OF THE ANTINOCICEPTIVE ACTION OF BOTULINUM TOXIN TYPE A:** *"The results demonstrate the necessity of retrograde axonal transport and involvement of the central nervous system for the antinociceptive activity of BTX-A."*

**3. TRANSITION STATE ANALOGUES IN STRUCTURES OF RICIN AND SAPORIN RIBOSOME-INACTIVATING PROTEINS:** *"Catalytic forces originate primarily from leaving group activation evident in both RTA and SAP in complex with transition state analogues."*

### **4. DEVELOPMENT OF A PIG JEJUNAL EXPLANT CULTURE FOR STUDYING THE GASTROINTESTINAL TOXICITY OF THE MYCOTOXIN DEOXYNIVALENOL:**

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### **5. VIBRIO CHOLERAE O1 OGAWA DETOXIFIED LIPOPOLYSACCHARIDE STRUCTURES AS INDUCERS OF CYTOKINES AND OXIDATIVE SPECIES IN MACROPHAGES:**

*"The results revealed effective structure-immunomodulating relationships of dLPS-derived moieties that are desirable in subcellular anti-cholera vaccine design."*

### **6. SURFACE PLASMON RESONANCE BIOSENSOR FOR THE DETECTION OF OCHRATOXIN A IN CEREALS AND BEVERAGES:**

*"This approach with simple sample preparation provides a powerful tool for the rapid and sensitive quantitative determination of OTA in food matrices."*

### **7. CATHEPSIN B-MEDIATED AUTOPHAGY FLUX FACILITATES THE ANTHRAX TOXIN RECEPTOR 2-MEDIATED DELIVERY OF ANTHRAX LETHAL FACTOR INTO THE CYTOPLASM:**

*"These results suggested that the ANTXR2-mediated cytoplasmic delivery of LF was enhanced by CTSB-dependent autophagic flux."*

### **8. VIRULENCE REGULATOR APHB ENHANCES TOXR TRANSCRIPTION IN VIBRIO CHOLERA:**

*"Our data indicate that V. cholerae possesses an additional regulatory loop that use AphB to activate the expression of two virulence regulators, ToxR and TcpP, which together control the expression of the master virulence regulator ToxT."*

### **9. LETHAL FACTOR UNFOLDING IS THE MOST FORCE-DEPENDENT STEP OF ANTHRAX TOXIN TRANSLOCATION:**

*"We propose a broad molecular mechanism for translocation-coupled unfolding, which is applicable to both soluble and membrane-embedded unfolding machines."*

### **10. PROPAGATION AND BREAKUP OF LIQUID MENISCI AND AEROSOL GENERATION IN SMALL AIRWAYS:**

*"It was shown that menisci tend to diminish in size as the capillary number increases above the critical value, and a number of small droplets may be formed during normal breathing."*

### **11. PLASMONIC NANOBUBBLES COMBINE DIAGNOSIS AND TREATMENT IN ONE THERANOSTIC METHOD:**

*"Multifunctional nanoparticles are at the core of a growing field called theranostics that develops technologies physicians can use to diagnose and treat diseases in a single procedure. The major promise of theranostics is to bring together key stages of a medical treatment,*

*such as the diagnosis and therapy, and thus to make a treatment shorter, safer and more efficient."*

## **12. SPRAY-ON LIQUID GLASS IS ABOUT TO REVOLUTIONIZE ALMOST EVERYTHING:**

*"According to the manufacturers, liquid glass has a long-lasting antibacterial effect because microbes landing on the surface cannot divide or replicate easily."*

# CB Daily Report

## **Chem-Bio News**

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### **NEW CLASSES OF ORTHOPOXVIRUS VACCINE CANDIDATES BY FUNCTIONALLY SCREENING A SYNTHETIC LIBRARY FOR PROTECTIVE ANTIGENS**

Gene Therapy Weekly

January 28, 2010

"The licensed smallpox vaccine, comprised of infectious vaccinia, is no longer popular as it is associated with a variety of adverse events. Safer vaccines have been explored such as further attenuated viruses and component designs."

"However, these alternatives typically provide compromised breadth and strength of protection. We conducted a genome-level screening of cowpox, the ancestral poxvirus, in the broadly immune-presenting C57BL/6 mouse as an approach to discovering novel components with protective capacities. Cowpox coding sequences were synthetically built and directly assayed by genetic immunization for open-reading frames that protect against lethal Pulmonary infection. Membrane and non-membrane antigens were identified that partially protect C57BL/6 mice against cowpox and vaccinia challenges without adjuvant or regimen optimization, whereas the 4-pox vaccine did not."

"New vaccines might be developed from productive combinations of these new and existing antigens to confer potent, broadly efficacious protection and be contraindicated for none."

The full article can be found at: (A. Borovkov, et. al., "New classes of orthopoxvirus vaccine candidates by functionally screening a synthetic library for protective antigens". Virology, 2009;395(1):97-113).

Link not available

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### **CENTRAL ORIGIN OF THE ANTINOCICEPTIVE ACTION OF BOTULINUM TOXIN TYPE A**

Drug Week

January 22, 2010

"Here we provide behavioural evidence for an axonal transport and the central origin of the antinociceptive effect of botulinum toxin type A (M-A). In rats we investigated the effectiveness of BTX-A on "mirror pain" induced by unilateral repeated intramuscular acidic saline injections (pH 4.0)."

"Since experimental evidence suggest that bilateral pain induced by acidic saline is of central origin, peripheral application of BTX-A should have no effect on this type of pain. However, here we demonstrated that the unilateral subcutaneous BTX-A (5 U/kg) application diminished pain on the ipsilateral, and on the contralateral side too. When injected into the proximal part of a distally cut sciatic nerve, BTX-A still reduced pain on the contralateral side. Colchicine, an axonal transport blocker, when injected into the ipsilateral sciatic nerve, prevented the effect of the peripheral BTX-A injection on both sides. Additionally, when BTX-A (1 U/kg) was applied intrathecally in the lumbar cerebrospinal fluid, the bilateral hyperalgesia was also reduced."

"The results demonstrate the necessity of retrograde axonal transport and involvement of the central

nervous system for the antinociceptive activity of BTX-A."

The full article can be found at: (L. Bachrojecky, et. al., "Central origin of the antinociceptive action of botulinum toxin type A". Pharmacology Biochemistry and Behavior, 2009;94(2):234-238). Link not available.

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## **TRANSITION STATE ANALOGUES IN STRUCTURES OF RICIN AND SAPORIN RIBOSOME-INACTIVATING PROTEINS**

Science Letter

January 26, 2010

"Ricin A-chain (RTA) and saporin-L1 (SAP) catalyze adenosine depurination of 28S rRNA to inhibit protein synthesis and cause cell death. We present the crystal structures of RTA and SAP in complex with transition state analogue inhibitors."

"These tight-binding inhibitors mimic the sarcin-ricin recognition loop of 28S rRNA and the dissociative ribocation transition state established for RTA catalysis. RTA and SAP share unique purine-binding geometry with quadruple pi-stacking interactions between adjacent adenine and guanine bases and 2 conserved tyrosines. An arginine at one end of the pi-stack provides cationic polarization and enhanced leaving group ability to the susceptible adenine. Common features of these ribosome-inactivating proteins include adenine leaving group activation, a remarkable lack of ribocation stabilization, and conserved glutamates as general bases for activation of the H<sub>2</sub>O nucleophile."

"Catalytic forces originate primarily from leaving group activation evident in both RTA and SAP in complex with transition state analogues."

The full article can be found at: (Meng-Chiao Ho, et. al., "Transition state analogues in structures of ricin and saporin ribosome-inactivating proteins". Proceedings of the National Academy of Sciences of the United States of America, 2009;106(48):20276-20281). Link not available.

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## **DEVELOPMENT OF A PIG JEJUNAL EXPLANT CULTURE FOR STUDYING THE GASTROINTESTINAL TOXICITY OF THE MYCOTOXIN DEOXYNIVALENOL: HISTOPATHOLOGICAL ANALYSIS**

Gastroenterology Week

January 25, 2010

"The digestive tract is a target for the mycotoxin deoxynivalenol (DON), a major cereals grain contaminant of public health concern in Europe and North America. Pig, the most sensitive species to DON toxicity, can be regarded as the most relevant animal model for studying the intestinal effects of DON."

"A pig jejunal explants culture was developed to assess short-term effects of DON. In a first step, jejunal explants from 9-13 week-old and from 4-5 week-old pigs were cultured in vitro for up to 8 h. Explants from younger animals were better preserved after 8 h, as assessed by morphological scores and by villi lengths. In a second step, DON dose-related alterations of the jejunal tissue were observed, including shortened and coalescent villi, lysis of enterocytes, oedema. After 4 In of DON exposure of explants from 4-5 week-old pigs, a no-effect concentration level of 1 mc M was estimated (corresponding to diet contaminated with 0.3 mg DON/kg) based on morphological scores, and of 0.2 mc M based on villi lengths."

"Our data indicate that pig intestinal explants represent a relevant and sensitive model to investigate the effects of food contaminants."

The full article can be found at: (M. Kolfclauw, et. al., "Development of a pig jejunal explant culture for studying the gastrointestinal toxicity of the mycotoxin deoxynivalenol: Histopathological analysis". *Toxicology in Vitro*, 2009;23(8 Sp. Iss.):1580-1584). Link not available.

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### **VIBRIO CHOLERAE O1 OGAWA DETOXIFIED LIPOPOLYSACCHARIDE STRUCTURES AS INDUCERS OF CYTOKINES AND OXIDATIVE SPECIES IN MACROPHAGES**

Drug Week

February 5, 2010

"Multidrug resistance in several strains of *Vibrio cholerae* has encouraged anti-cholera vaccine developmental attempts using various subcellular moieties. In order to examine the immunological efficacy of detoxified LPS (dLPS)-derived saccharide immunogens, ex vivo activation of mouse peritoneal macrophages (MPhis) was investigated."

"The immunomodulatory effect was evaluated via induction of the pro-inflammatory cytokines tumor necrosis factor-alpha, interleukin (IL)-1 alpha and IL-6 and acceleration of nitric oxide (NO) and reactive oxygen species (ROS). Immunologically active structures triggered mouse peritoneal MPhis to secrete cytokines and release NO/ROS, even at concentrations as low as 12.5 microg ml(-1). It was found that the O-specific polysaccharide moiety was more immunologically efficient than the glycolipid one, probably due to the position of 3-deoxy-D-manno-octulosonic acid."

"The results revealed effective structure-immunomodulating relationships of dLPS-derived moieties that are desirable in subcellular anti-cholera vaccine design."

The full article can be found at: (E. Paulovicova, et. al., "Vibrio cholerae O1 Ogawa detoxified lipopolysaccharide structures as inducers of cytokines and oxidative species in macrophages". *Journal of Medical Microbiology*, 2010;59(Pt 2):158-64). Link not available.

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### **SURFACE PLASMON RESONANCE BIOSENSOR FOR THE DETECTION OF OCHRATOXIN A IN CEREALS AND BEVERAGES**

Journal of Technology & Science

January 31, 2010

"Ochratoxins are a group of mycotoxins produced as secondary metabolites by fungi which contaminate a large variety of food and feed commodities. Due to their teratogenic and carcinogenic properties, ochratoxins present a serious hazard to human and animal health."

"There is an increasing need to establish a simple sensitive method to detect these toxins. Here we report a rapid and highly sensitive surface plasmon resonance (SPR) assay of ochratoxin A (OTA) using Au nanoparticles for signal enhancement on a mixed self-assembled monolayer (mSAM) surface. A competitive immunoassay format was used for the development of the OTA immunoassay, which is based on the immobilization of target OTA through its ovalbumin (OVA) conjugate with a polyethylene glycol (PEG) linker. The new OTA conjugate (OTA-PEG-OVA) showed remarkably enhanced performance characteristics compared with those based on the immobilization of a commercial bovine serum albumin BSA-OTA conjugate without a PEG linker. Although OTA concentrations as low as 1.5 ng mL(-1) could be directly detected on this surface, the limit of detection (LOD) can be dramatically improved to 0.042 ng mL(-1) for OTA by applying large gold nanoparticles (40 nm) for signal enhancement. Various chemical conditions to minimize the influence of the food matrix on assay performance were also investigated. Grain samples were simply extracted with 50% methanol and liquid samples treated with poly(vinylpyrrolidone) (PVP) (3 or 5%), without any sample clean-up or pre-concentration step prior to analysis. The LODs for OTA in oats and corn were 0.3 and 0.5 ng g(-1), respectively, while in wine and other beverages, LODs ranged from 0.058 to 0.4 ng mL(-1). No cross-reactivity was observed with three other common mycotoxins. In addition, the mSAM/OTAPEG-OVA Surface exhibited high stability

with over 600 binding/regeneration cycles.”

"This approach with simple sample preparation provides a powerful tool for the rapid and sensitive quantitative determination of OTA in food matrices."

The full article can be found at: (J. Yuan, et. al., "Surface plasmon resonance biosensor for the detection of ochratoxin A in cereals and beverages". *Analytica Chimica Acta*, 2009;656(1-2):63-71). Link not available

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## **CATHEPSIN B-MEDIATED AUTOPHAGY FLUX FACILITATES THE ANTHRAX TOXIN RECEPTOR 2-MEDIATED DELIVERY OF ANTHRAX LETHAL FACTOR INTO THE CYTOPLASM**

Biotech Week

February 3, 2010

"Anthrax lethal toxin (LeTx) is a virulence factor secreted by *Bacillus anthracis* and has direct cytotoxic effects on most cells once released into the cytoplasm. The cytoplasmic delivery of the proteolytically active component of LeTx, lethal factor (LF), is carried out by the transporter component, protective antigen, which interacts with either of two known surface receptors known as anthrax toxin receptor (ANTXR) 1 and 2."

"We found that the cytoplasmic delivery of LF by ANTXR2 was mediated by cathepsin B (CTSB) and required lysosomal fusion with LeTx-containing endosomes. Also, binding of protective antigen to ANXTR1 or -2 triggered autophagy, which facilitated the cytoplasmic delivery of ANTXR2-associated LF. We found that whereas cells treated with the membrane-permeable CTSB inhibitor CA074-Me-or CTSB-deficient cells had no defect in fusion of LC3-containing autophagic vacuoles with lysosomes, autophagic flux was significantly delayed."

"These results suggested that the ANTXR2-mediated cytoplasmic delivery of LF was enhanced by CTSB-dependent autophagic flux."

The full article can be found at: (S.D. Ha, et. al., "Cathepsin B-mediated autophagy flux facilitates the anthrax toxin receptor 2-mediated delivery of anthrax lethal factor into the cytoplasm". *Journal of Biological Chemistry*, 2010;285(3):2120-9). Link not available.

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## **VIRULENCE REGULATOR APHB ENHANCES TOXR TRANSCRIPTION IN VIBRIO CHOLERA**

Health & Medicine Week

February 1, 2010

"In this study, we investigated the expression of the key virulence regulator ToxR under different conditions. We found that compared to that of wild type grown to stationary phase, the toxR expression was lower in an aphB mutant strain. AphB has been previously shown to be a key virulence regulator that is required to activate the expression of tcpP. When expressed constitutively, AphB is able to activate the toxR promoter. Furthermore, gel shift analysis indicates that AphB binds toxR promoter region directly. We also characterize the effect of AphB on the levels of the outer membrane porins OmpT and OmpU, which are known to be regulated by ToxR."

"Our data indicate that *V. cholerae* possesses an additional regulatory loop that use AphB to activate the expression of two virulence regulators, ToxR and TcpP, which together control the expression of the master virulence regulator ToxT."

The full article can be found at: (X.Xu, et. al., "Virulence regulator AphB enhances toxR transcription in *Vibrio cholera*". *BMC Microbiology*, 2010;10():3). Link not available.

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## **LETHAL FACTOR UNFOLDING IS THE MOST FORCE-DEPENDENT STEP OF ANTHRAX TOXIN TRANSLOCATION**

Medical Letter on the CDC & FDA  
February 7, 2010

"Cellular compartmentalization requires machinery capable of translocating polypeptides across membranes. In many cases, transported proteins must first be unfolded by means of the proton motive force and/or ATP hydrolysis."

"Anthrax toxin, which is composed of a channel-forming protein and two substrate proteins, is an attractive model system to study translocation-coupled unfolding, because the applied driving force can be externally controlled and translocation can be monitored directly by using electrophysiology. By controlling the driving force and introducing destabilizing point mutations in the substrate, we identified the barriers in the transport pathway, determined which barrier corresponds to protein unfolding, and mapped how the substrate protein unfolds during translocation. In contrast to previous studies, we find that the protein's structure next to the signal tag is not rate-limiting to unfolding. Instead, a more extensive part of the structure, the amino-terminal beta-sheet subdomain, must disassemble to cross the unfolding barrier. We also find that unfolding is catalyzed by the channel's phenylalanine-clamp active site."

"We propose a broad molecular mechanism for translocation-coupled unfolding, which is applicable to both soluble and membrane-embedded unfolding machines."

The full article can be found at: (K.L. Thoren, et. al., "Lethal factor unfolding is the most force-dependent step of anthrax toxin translocation". Proceedings of the National Academy of Sciences of the United States of America, 2009;106(51):21555-60). Link not available.

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## **PROPAGATION AND BREAKUP OF LIQUID MENISCI AND AEROSOL GENERATION IN SMALL AIRWAYS**

Drug Week  
February 5, 2010

"Droplets exhaled during normal breathing and not associated with coughing may pose hazardous agents to infective diseases dissemination. The objective is to explore the physical mechanism, which may lead to droplets formation."

"We hypothesize that liquid menisci occlusions, which may form inside small airways, travel along the airway, may lose mass and finally disintegrate into small droplets. This hypothesis was numerically investigated applying physiologically plausible values of the phenomenological coefficients and geometrical conformations. We show that three important dimensionless parameters control the motion and disintegration of menisci: the dimensionless mucus layer thickness, the dimensionless menisci initial thickness (all scaled by the airway radius), and the capillary number. Menisci traveling within airways may either remain at equilibrium or diminish or increase in size. Menisci that diminish in size may collapse into the mucus layer; form a large droplet that contains most of the menisci mass before disintegration; or form a larger number of small droplets (we show the forming of three or four droplets in a single occluded airway). A critical capillary number for menisci at equilibrium could be defined."

"It was shown that menisci tend to diminish in size as the capillary number increases above the critical value, and a number of small droplets may be formed during normal breathing."

The full article can be found at: (A. Malashenko, et. al., "Propagation and Breakup of Liquid Menisci and Aerosol Generation in Small Airways". Journal of Aerosol Medicine and Pulmonary Drug Delivery, UNKNOWN DATE;22(4):341-353). Link not available.

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## **PLASMONIC NANOBUBBLES COMBINE DIAGNOSIS AND TREATMENT IN ONE THERANOSTIC METHOD**

By Michael Berger  
Nanowerk.com  
February 01, 2010

“Multifunctional nanoparticles are at the core of a growing field called theranostics that develops technologies physicians can use to diagnose and treat diseases in a single procedure. The major promise of theranostics is to bring together key stages of a medical treatment, such as the diagnosis and therapy, and thus to make a treatment shorter, safer and more efficient.”

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“Lapotko and his colleagues at the Joint US-Belarusian lab for fundamental and biomedical nanophotonics at Rice University, in collaboration with researchers at the A.V.Lykov Heat and Mass Transfer Institute (Belarus) and M.D.Anderson Cancer Center (Houston, TX), have developed a novel method based on gold nanoparticle-generated transient photothermal vapor nanobubbles, a structure they refer to as plasmonic nanobubbles (PNB). These dynamically tuned intracellular plasmonic nanobubbles are well suited for cell theranostics since they combine diagnosis (through optical scattering), therapy (through mechanical, nonthermal and selective damage of target cells) and optical guidance of the therapy into one fast process.

In a paper in the January 25, 2010 online issue of Nanotechnology ("Tunable plasmonic nanobubbles for cell theranostics"), Lapotko and his team have now presented the first and laboratory stage proof of the principle for theranostics with plasmonic nanobubbles.

"We hypothesized that a combination of the photothermal properties of plasmonic nanoparticles with those of transient vapor bubbles may be a key solution of some of the above-mentioned problems with theranostic approaches," says Lapotko. "We achieved this through the development of a tunable nanoscale theranostic probe that is not a nanoparticle but a nanoparticle-generated event – the plasmonic nanobubble, which combines high optical brightness with localized mechanical impact. The PNB is a system that results from the interaction of optical radiation with a nanoparticle and its environment."

The full article can be found at: <http://www.nanowerk.com/spotlight/spotid=14603.php>  
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## **SPRAY-ON LIQUID GLASS IS ABOUT TO REVOLUTIONIZE ALMOST EVERYTHING**

By Lin Edwards  
Physorg.com  
February 02, 2010

“Spray-on liquid glass is transparent, non-toxic, and can protect virtually any surface against almost any damage from hazards such as water, UV radiation, dirt, heat, and bacterial infections. The coating is also flexible and breathable, which makes it suitable for use on an enormous array of products.

The liquid glass spray (technically termed “SiO<sub>2</sub> ultra-thin layering”) consists of almost pure silicon dioxide (silica, the normal compound in glass) extracted from quartz sand. Water or ethanol is added, depending on the type of surface to be coated. There are no additives, and the nano-scale glass coating bonds to the surface because of the quantum forces involved. According to the manufacturers, liquid glass has a long-lasting antibacterial effect because microbes landing on the surface cannot divide or replicate easily.

Liquid glass was invented in Turkey and the patent is held by Nanopool, a family-owned German

company. Research on the product was carried out at the Saarbrücken Institute for New Materials. Nanopool is already in negotiations in the UK with a number of companies and with the National Health Service, with a view to its widespread adoption.

The liquid glass spray produces a water-resistant coating only around 100 nanometers (15-30 molecules) thick. On this nanoscale the glass is highly flexible and breathable. The coating is environmentally harmless and non-toxic, and easy to clean using only water or a simple wipe with a damp cloth. It repels bacteria, water and dirt, and resists heat, UV light and even acids. UK project manager with Nanopool, Neil McClelland, said soon almost every product you purchase will be coated with liquid glass.

Food processing companies in Germany have already carried out trials of the spray, and found sterile surfaces that usually needed to be cleaned with strong bleach to keep them sterile needed only a hot water rinse if they were coated with liquid glass. The levels of sterility were higher for the glass-coated surfaces, and the surfaces remained sterile for months.”

The full article can be found at: <http://www.physorg.com/news184310039.html>

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