

**Date:** November 9, 2004

**To:** Microbiological Safety Committee

**From:** Tim Coughlin

**Subject:** Evaluation of the Protocol and Application (SA-04-002) Submitted by Dr. Liviu Movileanu, Professor of Physics, for use of a Biological Toxin.

Dr. Liviu Movileanu, Professor of Physics, has requested permission to possess and use a Biological Toxin. Dr. Movileanu has provided a Standard Operating Procedure (SOP) and submitted a Possession and Use Application. Please refer to Attachment A for Application SA-04-002 and Attachment B for the SOP. The review contained herein is performed for the purpose evaluating the proposed research protocols with the requirements of the Microbiological Safety Program. This document contains proprietary information and all project specific content is business confidential and may not be disclosed.

It was confirmed through conversations with CDC Representatives and Syracuse University Legal Council that Alpha-Hemolysin Toxin is **not** a "Select Agent" toxin, and is **not** included within the listing of Staphylococcal Enterotoxin under the Department of Health and Human Services, Possession, Use and Transfer of Select Agents and Toxins (CFR 42 Part 73).

However, this toxin falls under the purview of the Microbiological Safety Committee because of its biological origin and all use and storage of this material should meet the requirements set forth in the Microbiological Safety Program.

### **Research Overview**

This research focuses on polypeptide translocation and folding across a transmembrane protein pore. Staphylococcus Aureus Alpha-Hemolysin Toxin will be used to form a heptameric pore on lipid bilayers. A combination of single-molecule electrical techniques, membrane protein engineering and chemical modification will be used to examine the polypeptide translocation, folding dynamics and thermostability across the very stable transmembrane pore of Alpha-Hemolysin. The central idea is to thread a polypeptide chain through the transmembrane protein pore and to analyze the electrical fluctuations associated with this event. The research as proposed here is stated lead to a better understanding of (1) polypeptide translocation as a result of the balance between forces that drive the polypeptides into the pore lumen and those against this process; (2) conformational transitions of the polypeptide structures such as  $\alpha$ -helices,  $\beta$ -turns and loops, or small proteins that consists of a combination of these, probed at the single-molecule level; (3) alteration in thermostability of polypeptide folds (e.g. increased fold stability) in a confined nanocavity, since these conditions may better mimic the environment the proteins evolved to fold.

### **rDNA Assessment**

Mutant Alpha-Hemolysin Toxin will be produced through in vitro transcription/translation in room B105 Physics. The production process starts with a DNA plasmid provided by Texas A&M University (TAMU). Only the DNA plasmid will be provided by Texas A&M and at no time will/and or may biological cultures capable of producing Alpha-Hemolysin Toxin be possessed by Dr.

Movileanu. The plasmid DNA will be added to a Promega © E. Coli T7 S30 Extract System Kit. This Kit includes amino acids, enzymes and E. coli DNA plasmid.

Dr. Movileanu has stated that he will not be in possession of biological organisms at anytime. All Alpha-Hemolysin Toxin will be synthesized in vitro and all template plasmids sent from TAMU will be extracellular. Section III-F-1 of the *NIH Guidelines* states that rDNA molecules not in organisms or viruses are considered "Exempt". "Exempt Experiments", by definition, are exempt from the requirements of the NIH Guidelines. However, Exempt experiments must be conducted at Biosafety Level 1 and must meet the requirements of the SU Microbiological Safety Program.

Attachment C of this document contains Dr. Movileanu's in vitro transcription/translation protocol, a technical bulletin regarding the Promega © E. Coli T7 S30 Extract System Kit, and applicable excerpts from the *NIH Guidelines*.

The insertion of DNA encoding for the production Alpha-Hemolysin Toxin into any organism or virus is strictly prohibited without prior Microbiological Safety Committee approval.

### **Possession/Inventory Control Measures**

The protein expression kit used to produce the Alpha-Hemolysin Toxin contains enough reagents to produce 30, 50ul reactions. The concentration of Toxin for each reaction is estimated at 0.05 - 0.3ng/ml. Dr. Movileanu stated he will produce one (1) kit reaction at a time and anticipates that the amount of toxin produced may be of a sufficient quantity and quality to work with for several months. Dr. Movileanu has stated that at no time shall he be in possession of more than 5mg of Alpha-Hemolysin Toxin. Dr. Movileanu's inventory may not exceed more than 4.5 mg at any one time.

Electrophoresis will be conducted on the newly expressed toxin and the gel plate will be transported to the 7<sup>th</sup> floor of BRL for photo-radiography. After the production of the Toxin is confirmed through photo-radiography the protein will be removed from the Separation Gel and purified in B103, Physics building. The purified protein will be stored in a -80 freezer which must be labeled to identify the potential hazards of the material inside. A written log must be developed and implemented to track and document usage of the toxin. The inventory identifier (a number specific to a certain batch of toxin), time, date, quantity, storage location, individual removing inventory and the purpose for use must be logged for each use. Inventory records must be maintained for a period of no less than five years after toxin destruction.

### **Personal Protective Equipment**

Eye/Face – Eye protection in the form of ANSI approved safety glasses with side shields must be worn at all times when handling Alpha-Hemolysin Toxin.

Clothing – Lab coats, closed toed shoes, long pants, must be worn when handling Alpha Hemolysin Toxin. Potentially contaminated lab coats and gloves shall not be worn outside room B103 or B105 or be stored in such a manner as to cross-contaminate individuals or clean clothing.

Gloves – Gloves that offer protection from Alpha Hemolysin Toxin must be worn at all times and changed when contaminated.

### **Safety Equipment**

Eyewash – Located in B105 (passed inspection on 10/20/04)

Safety Shower – Located in B105 (passed inspection on 10/20/04)

### **Security**

Dr. Movileanu is responsible for ensuring that Alpha-Hemolysin storage areas are secured in manner to prevent any unauthorized access, or removal of the toxin. Access to the refrigerator containing the toxin must be restricted to only authorized personnel whose work assignments require access and those who have been specifically trained in the hazards associated with Alpha-Hemolysin Toxin and the requirements of this evaluation (currently Liviu Movileanu and Apiping Zhu).

### **Disposal/Deactivation Procedures**

The Alpha-Hemolysin samples must be transferred from the appropriate containers (i.e. chambers, plastic centrifuge tube, etc.) using a set of pipettors and filter tips dedicated to work with Alpha-Hemolysin Toxin to a labeled glass beaker containing 2.5% Sodium Hypochlorite. (Note: 2.5% NaOCl solutions can be made by mixing water and bleach at a 2:1 ratio. The disinfectant solution shall be made fresh each time.) Sodium hypochlorite at a 2.5% concentration has been shown by the US Department of Defense to be effective in deactivating Staphylococcus Toxins. After a 30 minute incubation, the sample/2.5% NaOCl must be pH neutralized between the pH range of 5.5 – 10.5. Neutralized samples must be treated as radiological waste. The date and method of toxin deactivation must be entered on the inventory sheet.

### **Emergency/Spill Response**

The Emergency Assistance Directory and Chemical Spill SOP must be posted in the laboratory. Dr. Movileanu must ensure that all laboratory workers, working in room B103 and B105 receive documented training in emergency and chemical spill procedures.

### **Work Area**

Work with Alpha-Hemolysin Toxin must be limited to rooms B103 and B105 located in the Physics building. Alpha-Hemolysin Toxin will be handled, used and stored in a posted designated area. Where feasible, Alpha Hemolysin Toxin will be manipulated over plastic-backed disposable paper work surfaces. All disposable work surfaces must be treated in a manner as to adequately deactivate Alpha Hemolysin prior to disposal or unrestricted use. All surfaces and equipment (i.e. pipets) must be cleaned with 2.5% bleach after each use.

### **Training**

All individuals under the supervision of Dr. Movileanu that engage in the use of hazardous chemicals must attend Chemical Hygiene Program Training (CHP) provided by the Environmental Health Office (EHO).

All workers that will have access to Alpha-Hemolysin Toxin must be trained by Dr. Movileanu. All training must be documented and adequate proof of training must be maintained on-site. At a minimum, training must include the following specific topics:

- Storage and security requirements
- Inventory control method
- Specific hazards of all lab procedures
- Toxicology of agent
- Identification and use of required PPE
- Spill or leak procedures
- Accidental exposure procedures
- Incident reporting requirements
- Waste disposal procedures
- Disinfection, autoclave, and/or inactivation procedures
- Safety equipment use, and care

### **Transfers**

Transferring Alpha Hemolysin Toxin to any location or persons not specifically approved by the Microbiological Safety Committee as described in this document is prohibited. The transfer of biological material (toxin, cultures or plasmids) containing the DNA of Staphylococcus Aureus or containing the genes necessary for the production of Alpha Hemolysin-Toxin onto Syracuse University property is prohibited without Microbiological Safety Committee approval.

### **Recommendation**

Dr. Movileanu's request to use Alpha-Hemolysin Toxin as described in the Standard Operating Procedures and Possession and Use Application (04-002) is recommended for approval under the terms described in this evaluation. This recommendation is conditional upon maintaining compliance with requirements of the Microbiological Safety Program

**Reviewed by** (please print): Tim Coughlin

**Designation:** Industrial Hygiene Manager

**Signature:** \_\_\_\_\_ **Date:** \_\_\_\_\_

# **ATTACHMENT A**

## **ATTACHMENT B**

## **ATTACHMENT C**