Write-up, July 2004

Qualifications and Contributions

1. In the food industry, attached bacteria demonstrate increased resistance to antimicrobial agents. Under favorable conditions these adhered cells reproduce and form colonies, which can lead to biofilm formation. Accumulation of biofilms can create a variety of problems for food processors, including increased fluid frictional resistance and decreased heat transfer efficiency. However, contamination of food products by bacterial pathogens is the most serious concern. During the doctoral thesis research, C. Bower explored a novel approach for controlling biofilms: coating food contact surfaces with a protein-based antimicrobial agent (nisin) to prevent the initial bacterial colonization. C Bower greatly enhanced the research plan that was initially provided by using image analysis, not merely as a tool to enumerate bacterial cells, but as a method for real-time documentation of the growth-inhibition experienced by *Listeria monocytogenes* cells when placed on nisin-coated surfaces. This "extra" study was independently conceived and successfully performed. Additionally, C. Bower adapted a tetrazolium dye-based assay to confirm the viability-loss of adhered cells. The time-lapse photographs that were captured during C. Bower's graduate school years are still being used in presentations today. C. Bower's protein adsorption techniques and image analysis protocols have been incorporated into several research theses and cited at least 54 times since 1995. EXHIBIT # 1.

2. Maintaining a specific molecular conformation is essential for the proper functioning of an enzyme. When a protein encounters an interface, it will attempt to minimize its free energy by undergoing some degree of conformational change. Adsorption of biologically active proteins, such as enzymes, presents a challenge since these molecules are organized to recognize a ligand or transition state and are not optimized for stability. As a post-doctoral research associate, C. Bower conducted a series of original research projects that expanded the range of applications that could benefit from the use of adsorbed antimicrobial proteins. As the lab shifted its focus from food-contact surfaces to medically-relevant materials, C. Bower explored the retention of nisin activity on catheters as a P.I. for a research grant awarded by Mallinckrodt Medical, Inc. At the same time, C. Bower was conducting studies with the antimicrobial enzyme, bacteriophage T4-lysozyme, using genetically engineered T4-lysozyme variants that differed by a single amino acid. As the only microbiologist in the Bioresource Engineering department, C. Bower was in charge of the *E. coli* stock cultures that carried the mutant T4-lysozyme genes, and was responsible for providing the correct variants and expertise to students and other researchers as needed. For spectrophotometric studies, adsorbed-proteins must not block the instrument's light path during data collection. C. Bower pioneered the use of different-sized silica nanoparticles to serve as soluble substrates for nisin and lysozyme. The novel nanoparticle techniques developed by C. Bower were used with circular dichroism to document changes in secondary structure during protein adsorption, as well as to spectrophotometrically follow activity losses for T4-lysozyme (EXHIBIT #2). This line of research was groundbreaking in demonstrating that less stable proteins bind more tightly and lose more of their secondary structure (thus losing more of their biological activity) than proteins with increased molecular stability. Based on these studies, C. Bower authored 7 peer-reviewed journal articles describing the adhesion characteristics of bacteria, the value of using antimicrobial proteins as barriers to adhesion, and the molecular basis for changes in protein function.

3. A novel approach to controlling unwanted microbial adhesion in clinical environments is to inhibit the initial attachment of bacteria, rather than trying to remove them once they have adhered. C. Bower evaluated the antimicrobial peptide nisin *in vitro* to determine

the most effective and practical conditions for use on biomedical implants, then tested nisin *in vivo* on actual medical implants (in blood vessels and in the upper airway) through a unique collaboration with Oregon State University's College of Veterinary Medicine. For this first-of-its-kind study, C. Bower conducted all of the initial *in vitro* experimentation (e.g., nisin film preparation and activity evaluation), as well as all of the microbiological challenges involving pathogenic bacteria. During the *in vivo* portion of the study, C. Bower prepared all antimicrobial and control solutions for the Veterinary school, then performed testing on the implantable devices after their removal. This study (EXHIBIT #3) was the first preclinical trial of nisin-treated implantable materials, and therefore represents an important first step for developing protein antimicrobial films on implantable medical devices. A major finding was that nisin-coated catheters appeared to have a protective effect on vascular endothelium. In a related study, C. Bower evaluated the antimicrobial peptide nisin for its ability to enhance cellular uptake of a common therapeutic drug, insulin, as well as for its safety when interacting with human cells during intranasal application. Human nasal mucosa was acquired from the Oregon Health Sciences University medical school, and was grown *in vitro* to serve as a model nasal interface. For this project, C. Bower developed all of the protocols and carried out all of the research. C. Bower found that these cell cultures produced tight junctions and synchronously beating cilia, suggesting that human cells can be reliably cultured for use in drug absorption studies. Nisin-stabilized microemulsions were also tested and were found to be highly resistant to bacterial contaminants such as *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Listeria monocytogenes*. This suggests that in addition to enhancing drug uptake, nisin might also have potential as a pharmaceutical biopreservative.