EXAMINING THE EFFICACY OF ANTIBIOTICS AND THE PROTEOMIC RESPONSE IN THE TREATMENT OF *STAPHYLOCOCCUS AUREUS* BIOFILMS GROWN IN-VITRO.

By

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Thesis

Submitted to the Faculty of the

Graduate School of Vanderbilt University

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

in

Chemistry

December, 2011

Nashville, Tennessee

Approved:

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To my amazing mother, Diana, who has supported me in every decision I've made from start to finish and without whom I would have accomplished nothing. With love and adoration.

ACKNOWLEDGEMENTS

This work would not have been possible without the funding of the Vanderbilt University Chemistry Department and the constant guidance and support of my principal investigator Dr. Richard Caprioli, my collaborator Dr. Eric Skaar, and the members of the Mass Spectrometry Research Center. I am especially indebted to Dr. Richard Caprioli who has been incredibly supportive of my career goals and who worked with me to develop this work. Thank you for pushing me.

I am grateful to those members of the lab who allowed me to present them with ideas and who helped me to develop my research. Special thanks to Erik Todd, David Rizzo, Chad Chumbley, Patrick Rawhouser, Kerri Grove, and Jessica Moore Hooten for their encouragement, technical support, and for their friendship.

I would also like to thank Hayes McDonald for his assistance in performing the LC-MS/MS experiments included in this thesis. Thank you for your guidance and patience and for your enthusiasm.

I am grateful for the support of my family as well, especially my mother to whom this work is dedicated. I would like to thank my parents and grandparents for their guidance and love.

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CHAPTER I

INTRODUCTION

Bacterial Biofilms

Microbial biofilms form when microorganisms irreversibly adhere to a surface, producing extracellular polymers that facilitate adhesion. These microorganisms may attach to living or non-living material and are common in indwelling medical devices such as catheters (**Figure 1**) and artificial hearts as well as on surfaces in water treatment facilities and the food industry.[1]

Biofilms possess very different characteristics from freely suspended organisms or bacterial colonies grown on an agar surface[1]. They grow upwards from the surface in layers and are inherently antibiotic resistant. The layered structure of the bacteria means that depending on their location within the biofilm, the microorganisms are experiencing different environmental conditions which in turn produce physiological differences making it difficult to treat the entire film[2]. What may kill the bacteria in one region of the film may not have any effect on another region. As a result, infections resulting from biofilm introduction into the body are very difficult to eradicate.

Biofilms may be composed of Gram-positive or Gram-negative bacteria or yeasts. The majority of biofilms which develop on indwelling medical devices are composed of multiple species of bacteria. Species commonly isolated from these types of devices include the gram-positive *Staphylococcus aureus* and *Staphylococcus epidermidis* and

the Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa.* Bacteria capable of forming biofilms usually originate from the patient's skin microflora, exogenous microflora from medical personnel, or various other contaminated sources.[1]

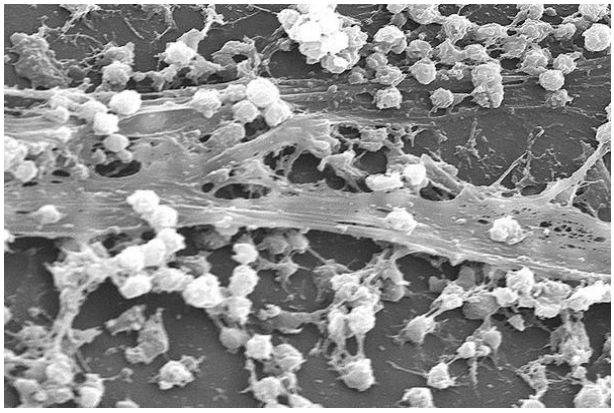

Figure 1. A *Staphylococcus aureus* biofilm on the surface of a medical catheter removed from a patient. Electron micrograph magnified 2363x.[2]

Biofilm Formation

Biofilm formation begins with freely suspended bacteria which attach to a surface via weak, Van der Waals forces. The cells can then anchor themselves more permanently using cell adhesion structures such as pili[2]. The bacteria undergo maturation and form extracellular polymeric substances (polysaccharides), which surround and encase the cells, linking them to one another in a sheet-like structure. These extracellular polysaccharides may also prevent penetration of antimicrobial

agents into the film and impair opsonization by the host[3]. Following maturation, the biofilm will release cells into the environment enabling the bacteria to spread and colonize other surfaces (**Figure 2**)[2].

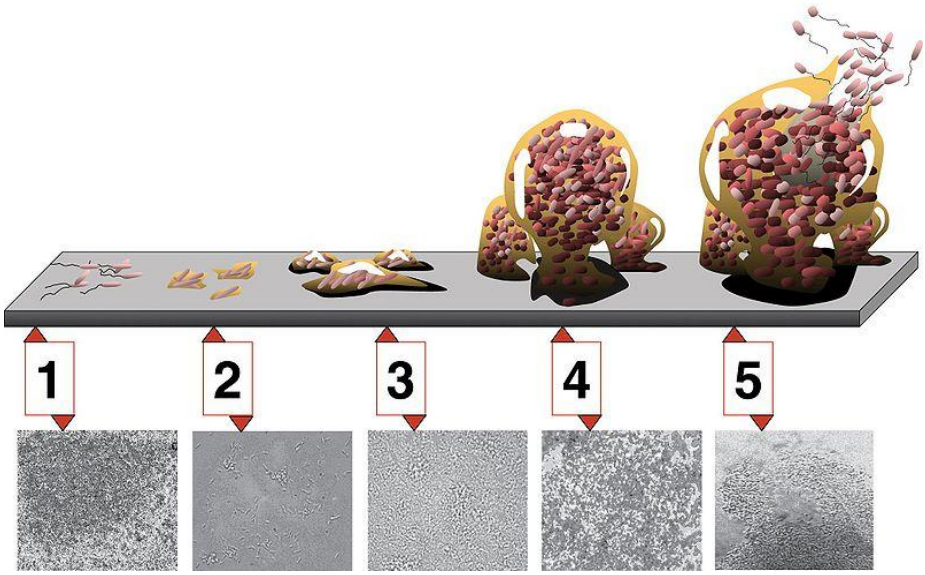

Figure 2. Schematic representation of the growth and development of a bacterial biofilm. Five steps in the development of a biofilm: (1) Initial Attachment, (2) Irreversible Attachment, (3) Maturation I, (4) Maturation II, (5) Dispersion. Each stage in the development of a biofilm is paired with a photomicrograph image of a developing *Pseudomonas aeruginosa* biofilm.[2]

The growth and attachment rates of biofilms are dependent on several factors including the type of fluid the device is exposed to, the flow rate of fluid through the device, and the physicochemical characteristics of the surface. Temperature, the presence/absence of an antimicrobial drug in the system, and the nutrient composition of the medium also play a role in biofilm development on surfaces and in the body.[1]

Staphylococcus aureus, Virulence Factors, and the Peptidoglycan

Staphylococcus aureus is a facultative, anaerobic, gram-positive, coccal bacteria typically found on the surface of the skin and inside the human nose[4]. It is the most common species of staphylococcus to cause staph infections and is one of the five most common causes of nosocomial infections, infections resulting from treatment in a hospital or health care center. *S. aureus* can cause a wide range of diseases and infections including boils, abscesses, pneumonia, endocarditis, toxic shock syndrome, and sepsis[5].

When grown on tryptic soy broth (TSB) agar plates the bacteria form large, round, golden colonies. They derive their color from the carotenoid pigment staphyloxanthin, which also acts to protect the bacteria from reactive oxygen species released by the immune system in response to infection[6,7]. Staphyloxanthin acts as a virulence factor, a gene product produced by a pathogen which enables it to colonize a host and enhances its potential to cause disease. Other virulence factors associated with this species of bacteria include fibronectin binding protein A (acts as a bridge between bacteria and the host cell), elastin-binding protein (aids in the colonization of host tissue and may play a role in the regulation of cell growth), and clumping factor A (binds to complement proteins released by the host in response to infection)[8].

S. aureus also feature a number of surface proteins, bound to the cell by Sortase A, which help them to survive inside a host organism. For example, Protein A, an IgG binding protein, acts as an immunological disguise and inhibits phagocytic engulfment.[9]

S. aureus also produces an enzyme called β-lactamase which breaks open the β-lactam ring of β-lactam antibiotics such as penicillin and ampicillin, deactivating their antibacterial properties[5,10]. This compound is responsible for many bacterial strains' resistance to β-lactam antibiotics.

Gram-positive bacteria such as *S. aureus* feature a layer within the cell wall known as the peptidoglycan. This cell wall layer is also present in gram-negative bacteria although it is significantly thinner. The peptidoglycan is composed of interlocking chains of identical peptidoglycan monomers consisting of two sugars: N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM). The peptidoglycan monomers are synthesized in the cytosol and transported to the membrane by a carrier molecule called bactoprenol (**Figure 3**).[11]

S. aureus bacteria reproduce asexually and therefore the peptide bridges must be broken (via the enzyme autolysin) in order to allow new peptidoglycan monomers to be inserted in the cell wall resulting in cell wall growth and eventually binary fission[11]. Transpeptidases or penicillin binding proteins (PBPs) are the bacterial enzymes responsible for cross-linking peptidoglycan monomers forming a rigid cell wall[12].

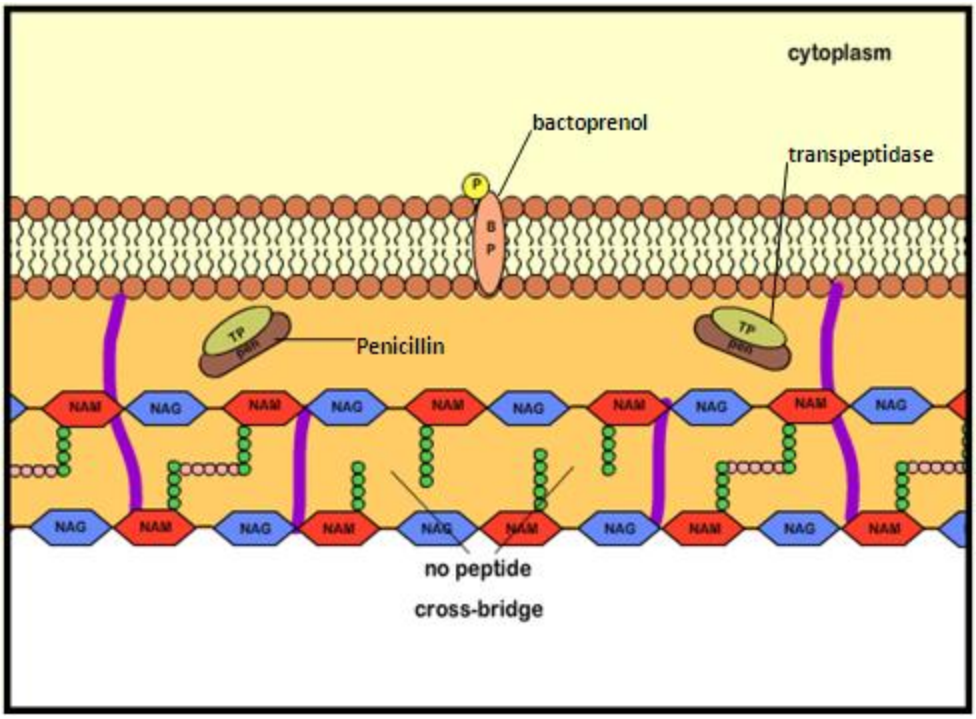

Figure 3. Structure of the Gram-positive peptidoglycan and cell wall. Note the penicillin bound transpeptidases which result in holes (no peptide cross-bridges) between the peptidoglycan monomers.[11]

Ampicillin

Ampicillin is a β-lactam antibiotic from the penicillin family with a mass of approximately 349.41 g/mol. Ampicillin differs from penicillin only by the presence of an amino group which helps the drug to penetrate the outer membrane of Gram-negative bacteria. It is also commonly used to treat infections caused by Gram-positive bacteria, those species featuring a cell wall rich in peptidoglycan[13]. Ampicillin irreversibly binds with transpeptidases in the cell wall by forming a highly stable penicilloyl-enzyme intermediate[11]. Autolysin continues to break the peptide bridges in order to allow cell wall expansion, but without unbound transpeptidases to fill the holes with new

peptidoglycan monomers, the cells undergo lysis due to osmotic pressure[11]. Ampicillin effectively inhibits the final stage of bacterial wall synthesis, binary fission, and results in cell death.

Alterations in the penicillin-binding proteins of bacterial strains are responsible in part for the antibiotic resistance demonstrated by some bacteria (E.g. Methicillin resistant *Staphylococcus aureus* or MRSA). These resistant strains will not respond to low levels of antibiotic and require a large dosage, in some cases larger than is clinically achievable.[13]

Summary

The treatment of infections caused by exposure to bacterial biofilms has become a topic of interest in recents years, especially with the increased usage of plastics in the medical field (prostetics, catheters, IVs, artificial hearts, etc.). Recent studies have resulted in the development of many theories to account for the antibiotic resistance of biofilms, but their proteomic response to drug treatments remains largely unstudied[3,14,15].

The development of in-vitro methods for determining proteomic response across a biofilm would provide a useful tool for the medical community in treating biofilm related infections. The identification of proteins involved in biofilm response to antimicrobial agents would not only enable medical professionals to prescribe more effective antibiotics, but it would also allow scientists to develop more efficient drug treatments. If the proteins involved in the defense mechanisms of bacterial biofilms

could be illucidated, scientists could develop drugs that specifically bind said proteins, expediting the irradication of infection.

CHAPTER II

TRACKING DRUG DIFFUSION AND PROTEIN CHANGES ACROSS A *STAPHYLOCOCCUS AUREUS* BIOFILM EXPOSED TO AMPICILLIN USING MALDI IMS

Introduction

MALDI Imaging Mass Spectrometry

Matrix-assisted laser desorption/ionization or MALDI is defined as the selective transduction of photon energy to the desorption of molecules and/or ions from the condensed phase resulting in gas phase ions. It features a laser, usually a nitrogen laser, which when fired at a sample with an applied matrix solution, produces a plume of ions which can then be detected by a mass spectrometer, typically a time-of-flight (TOF) instrument.[16]

The matrix consists of crystallized molecules dissolved in a solution of water and an organic solvent such as acetonitrile or ethanol. Common matrices include 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid or SA), α-cyano-4-hydroxycinnamic acid (CHCA), and 2,5-dihydroxybenzoic acid (DHB). Selection of a matrix is dependent on the analyte of interest. For instance, sinapinic acid is commonly used in the detection of proteins whereas DHB is used primarily for lipids. Matrices can either be pre-mixed with the sample, or may be spotted manually or mechanically over a sample or onto tissue[17]. The main roles of the matrix are to cocrystallize with the analyte, to absorb incident

photon energy from the laser in order to prevent destruction of the sample, and to aid in the ionization of the sample analytes[18].

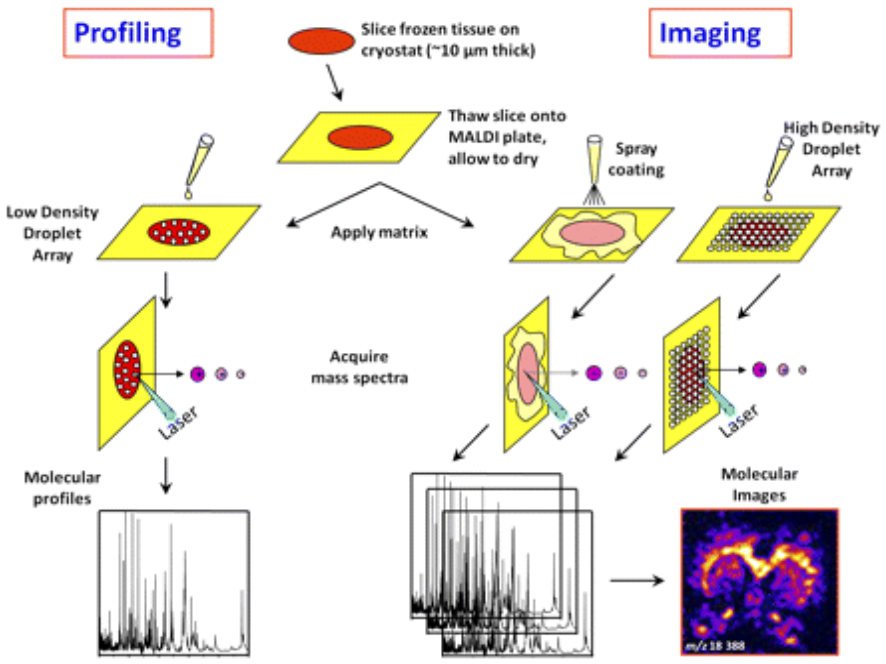

Figure 4. Schematic representation of the steps involved in profiling and IMS analysis of tissue samples.[19]

Sample preparation involves the sectioning of either fresh froxen or formalin-fixed parafin-embedded (FFPE) tissue (typically 5-10 µm in thickness) and mounting on a conductive MALDI surface such as a gold plate or ITO coated slide. The sample is washed to remove salts and lipids before recieving a coating of matrix and undergoing analysis on a mass spectrometer. Ionization of the sample is achieved by firing a laser in an ordered pattern across the surface of the sample, ablating material and creating an

average spectrum for each spot. Each spot or pixel contains many analyte signals, each of which can be individually displayed within the tissue when a false color scale is applied to the optical image displaying relative intensity.[20]

MALDI Imaging Mass Spectrometry has gained popularity in recent years due to its ability to detect the presence of various biological molecules, most notably proteins and drug molecules in tissue[21]. It has also been successfully utilized in the mapping of structural components of various organs and in the discernment of normal versus tumor tissue in mice[22]. Following preparation of a sample, IMS can be used to determine the relative abundance and location of proteins, drugs, and metabolites within tissue creating a protein map across different regions of the sample[23].

Imaging Bacterial Proteins

Analysis of bacterial biofilms is typically accomplished through visual observations made using electron and optical microscopies or via laser desorption postionization mass spectrometry (LDPI-MS). LDPI-MS has previously been used to detect peptides within a *Bacillis subtilis* biofilm as well as to detect the presence of rifampicin in a treated *Staphylococcus epidermidis* biofilm grown on an ITO slide[24,25]. However, this method provides no spatial information regarding the distribution of peptides or drug molecules within the sample.

Imaging Mass Spectrometry has previously been used to track proteins associated with infection in mice infected with *S. aureus*. Corbin et al. used IMS to image calprotectin, a Ca^{2+} binding protein which inhibits the growth of bacterial pathogens in-

vivo[26]. Calprotectin is primarily found in the kidney where staphylococcal abscesses form and represents an innate immune response by the body to infection. Recent experiments have utilized the same technology to create co-registered three dimensional images of calprotectin in an infected mouse kidney.[27]

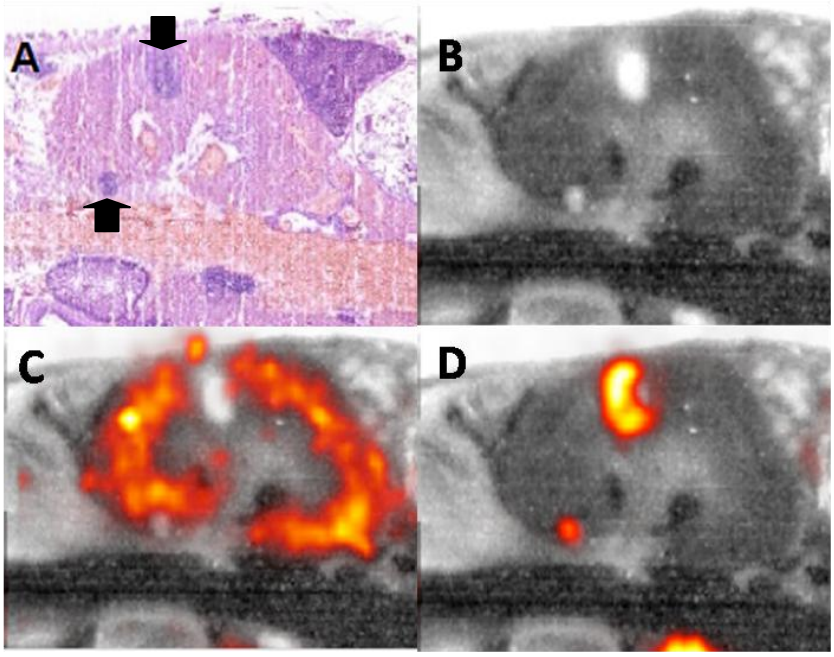

Figure 5. Co-registered IMS and MRI images displaying signals associated with calprotectin as well as a structural protein[27]. **A)** Hematoxylin and eosin stained image showing kidney abscesses (denoted by arrows) in an infected animal. **B)** Blockface image showing the same kidney abscesses. **C)** Coregistered IMS and MRI image of the protein mass at m/z 5,020 (cortex specific protein). **D)** Coregistered IMS and MRI image of the protein mass at m/z 10,165 (calprotectin).

Previous studies have also proven IMS to be a useful tool in the analysis of bacterial colonies. This technology has been used to image various proteins and

metabolic products of bacteria grown on MALDI targets and to track interactions between different bacterial colonies including metabolic exchange, the expression of cannabalistic factors, and colony-to-colony signaling[28].

Dorrestein et al. utilized IMS in the study of metabolic exchange between *Bacillus subtilis* and *Streptomyces coelicolor* and determined that *B. subtilis* inhibits the production of secondary metabolites in *S. coelicolor*. However, other metabolites, including prodiginines, were shown to be upregulated in *S. coelicolor* in the presence of *B. subtilis*.[28]

Additionally, IMS was utilized in the identification of cannabilistic factors in *B. subtilis*. A normal strain was allowed to interact with a mutant strain of the same bacteria on nutrient agar. The MALDI data revealed the presence of sporulation killing factor (Skf) and sporulation delaying protein (Sdp) in the normal strain, both of which were utilized by the bacteria in the cannabalistic killing of the mutant strain.[29]

Imaging Mass Spectrometry can also be applied to the study of biofilms. Following the application of a liquid matrix, a biofilm grown on a conductive surface can be imaged using MALDI to determine the molecular profile of the biological system. If the sample is treated with a drug, such as an antibiotic, tandem mass spectrometry can be used to establish distribution of the drug molecule across the film. These images can then be compared, in order to determine whether or not molecular descriptors are up or down-regulated in the film in response to the drug.

Methods and Materials

Materials

The MALDI matrices, sinapinic acid, 2,5-dihydroxybenzoic acid, and α-cyano-4-hydroxycinnamic acid as well as the hematoxylin were purchased from Sigma Chemical Co. (St. Louis, MO). Acetic acid, ethanol, Xylene, eosin, and ampicillin were purchased from Fisher Scientific (Suwanee, GA). Tin oxide coated slides (ITO slides) were purchased from Delta Technologies (Stillwater, MN).

Determining a Proper Matrix

Colonies of the Newman strain of *Staphylococcus aureus* were isolated on a TSB agar plate. The bacteria were streaked over the surface of an entire agar plate and incubated for 24 hours at 37°C to create a lawn of bacterial growth. Antibiotic (20µL) was applied to small round filter papers at concentrations of 100, 10, 1, 0.1, 0.01, and 0.001 µg/mL. The filter papers were applied to the streaked agar plates in an evenly spaced, circular pattern and the plates were placed in an incubator at 37°C overnight. Kanamycin, erythromycin, chloramphenocol, ampicillin, and Zyvox were tested.

The zone of clearing around the filter papers was observed and measured. Once an ideal concentration was determined, the antibiotic was analyzed using various matrices in order to determine which allowed for the formation of antibiotic specific fragment ions during MALDI MS/MS analysis.

Fresh samples of the antibiotic were prepared and 1 µL of the drug was mixed with 1 µL of a sinapinic acid matrix at a concentration of 20 mg/mL (in 50:50 acetonitrile

and water with 0.1% TFA added). DHB (30 mg/mL) and CHCA (20 mg/mL) were also tested. The mixture was spotted on a clean ITO slide for analysis. Pure matrix was spotted on the slide for comparison.

The spots were analyzed on a Thermo Scientific LTQ XL MALDI instrument (LTQ 20712) to produce both MS and MS/MS spectra. These spectra were compared with the results obtained from the pure matrix samples and it was determined that kanamycin and ampicillin produced the most intense fragment peaks when mixed with a sinapinic acid matrix (**Figure 6**).

Sample Preparation

Newman strain *Staphylococcus aureus* biofilms were grown on cut (25x25 mm) indium tin oxide (ITO) coated slides. The square slides were soaked in 100% ethanol for approximately 5 minutes then drained and exposed to UV light for 2 minutes. The slides were run through a flame for sterilization and placed into individual Petri dishes. Each slide was streaked with bacteria and 20 mL of TSB broth was added. The dishes were placed in an incubator at 37°C for 8 days. The TSB broth was drained and replenished daily.

Following the 8 day growth period, the TSB broth was drained and the edges of the slides were lightly dabbed with a Kim Wipe to wick away excess moisture. The biofilms received a small filter paper containing 20 µL of ampicillin at a concentration of 800 µg/mL in ddH_2O. Initial drug imaging experiments using the ideal concentration determined for ampicillin (10 µg/mL) failed to detect the antibiotic at that

concentration. It appears as though the layered structure of the biofilm may result in absorption of the drug deep into the film and so a much higher concentration was necessary for imaging of radial drug diffusion.

After application of the antibiotic, the biofilms were placed back in the incubator for 10 hours at 37°C. Following this period of drug exposure, the filter paper was removed and the underside of the biofilm cleansed with anhydrous ethyl alcohol, dried, and exposed to UV light in a UV Stratalinker 2400 four times at 4000 µJ x 100 of energy. The films were placed in a -80°C freezer for storage.

Samples were spotted on a Portrait 630 Acoustic Robotic Microspotter (Labcyte) with a 20 mg/mL sinapinic acid solution (in 50:50 water and acetonitrile solution with 0.1% TFA added). The samples received matrix in a block pattern (140 columns, 140 rows) consisting of five drops each pass for a total of six passes with 150 µm spacing. The spotted biofilms were scanned using an Epson Perfection 4990 Photo scanner at a resolution of 2400 dpi in 16-bit grayscale (**Figures 8B and 9B**).

Analysis

Drug imaging was performed on a Thermo LTQ XL MALDI instrument, monitoring for m/z 350.1 with a collision energy of 27.0 µJ over a mass window from m/z 95.00-450.00. The imaging was done in positive, profile mode at a laser energy of 15 µJ with 3 microscans per step, 5 shots per scan, and 10 scans per file. ImageQuest software was used to analyze the data (**Figures 8A and 9A**).

Proteins were imaged using a Bruker Autoflex II Mass Spectrometer over the mass window from 800-10,000 Da in 50 shot steps for a total of 400 shots per matrix spot with a raster of 150x150 µm. The instrument was run in positive, linear mode with the linear voltage set at 3.1 kV, a laser repetition rate of 1000 Hz, ion source voltages of 19.5 kV (1) and 18.3 kV (2), a delay time of 350 ns, and a lens voltage of 6 kV. FlexImaging software was used to analyze the data (**Figures 8C-D and 9C-D**).

Determining Biofilm Thickness

Samples were cultured using the previously described protocol. Three biofilms were taken from storage, thawed, and small portions were scraped away at various points using a razor blade. The films were placed on an Olympus BX-50 microscope. Using Image Pro-Plus 7.0 software, the etched positions on the film were put into focus using an Olympus 150x objective lens for a total magnification of approximately 3151x. The bottom or ITO slide surface was set as the Z-bottom. The top or biofilm surface was set as the Z-top and the software calculated the difference creating a measurement of biofilm thickness in micrometers. This was repeated for 25 individual points across each of the three biofilms and the values were averaged (**Figure 11**).

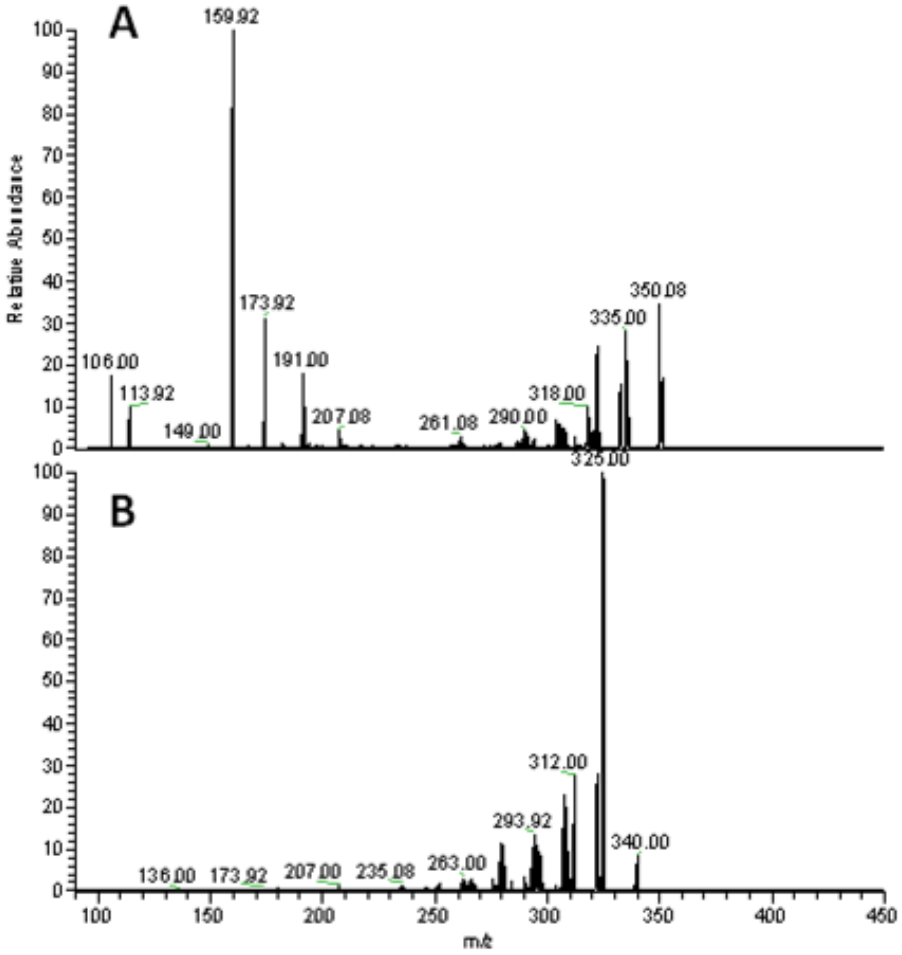

Figure 6. A) MALDI MS/MS spectra for m/z 350.1, ampicillin. Note fragment peaks at m/z 159.92, 173.92, and 191.0. This spectra represents the data collected for 1 µL of ampicillin (10 µg/mL in ddH_2O) mixed with 1µL of sinapinic acid (in 50:50 water:acetonitrile with 0.1% TFA added). Note: when imaging ampicillin on a biofilm the first two fragment peaks are shifted to m/z 160.08 and 174.08 respectively most likely due to the thickness of the biofilm which affects the conductivity of the ITO surface and results in peak shifting. **B)** MALDI MS/MS spectra for pure sinapinic acid matrix under the same conditions.

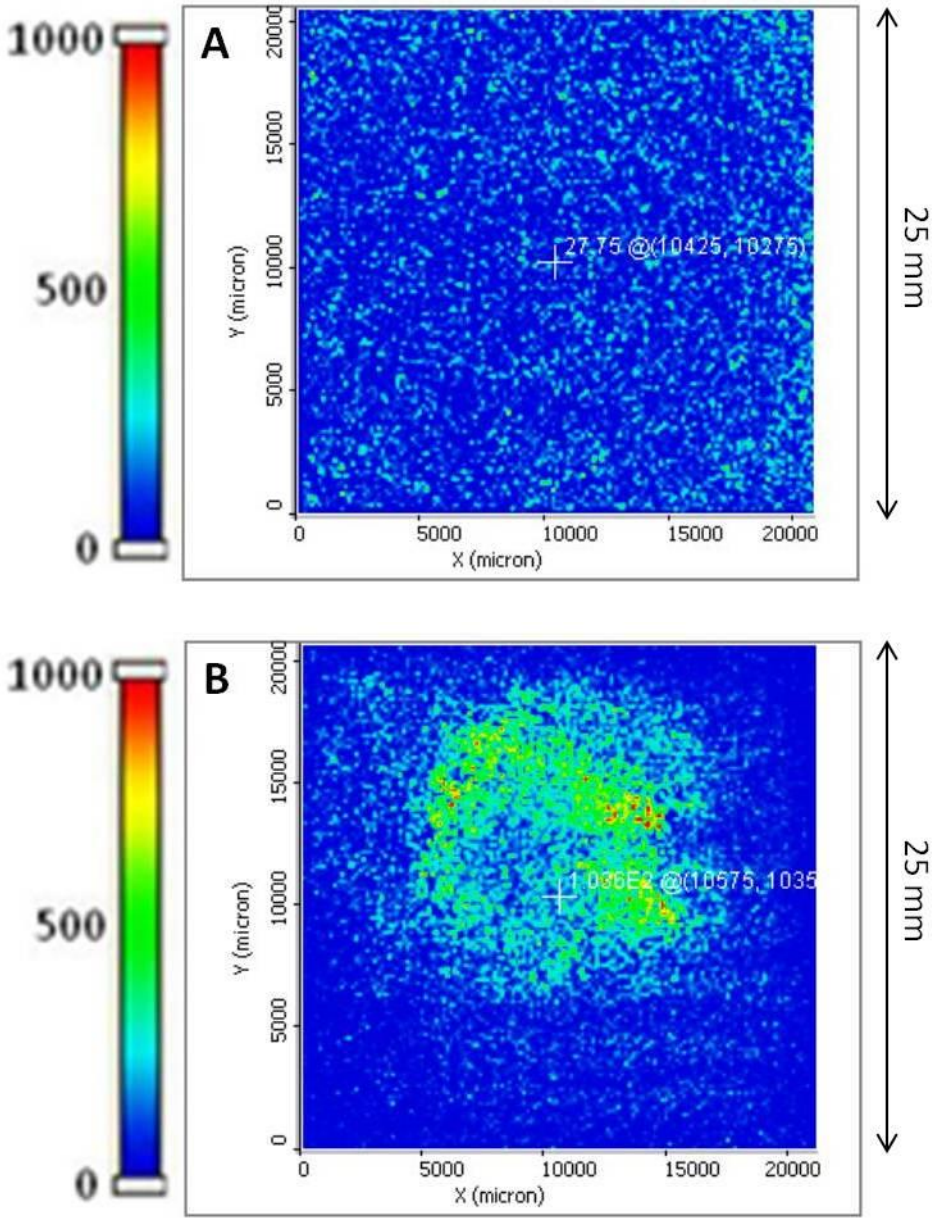

Figure 7. Ampicillin imaging for the mass range [m/z 159.58-160.58 plus m/z 173.58-174.58] of **A)** a control, *S. aureus* biofilm exposed to 20 µL of ddH_2O for 10 hours at 37°C and **B)** a *S. aureus* biofilm exposed to 20 µL of 800 µg/mL of ampicillin for 10 hours at 37°C.

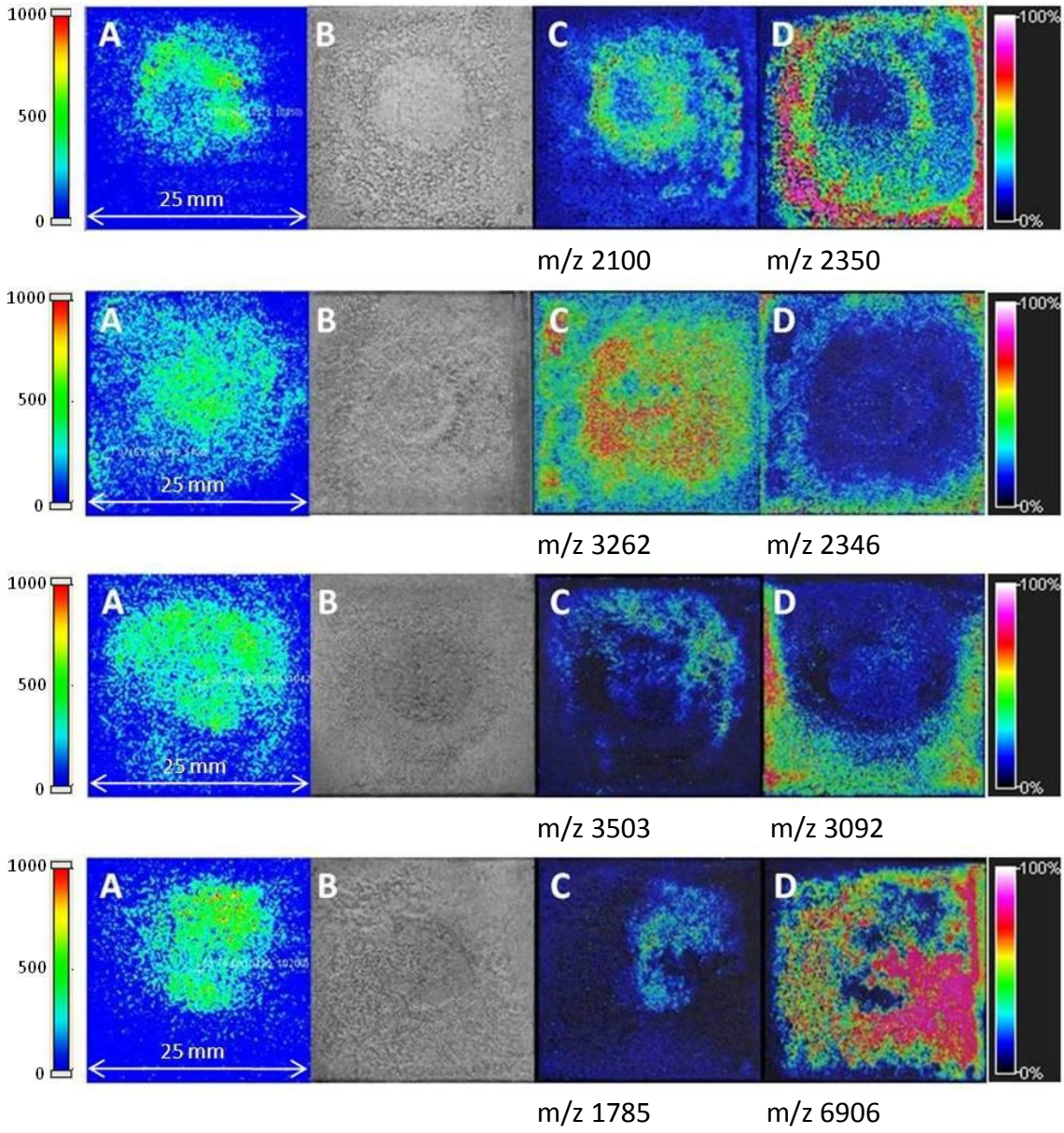

Figure 8. Examples of IMS revealing various proteins/peptides specific to exposure to ampicillin in a *S. aureus* biofilm. **A)** Drug imaging performed on a Thermo LTQ MALDI mass spectrometer over the mass range [m/z 159.58-160.58 plus m/z 173.58-174.58]. **B)** Scanned image of the biofilm following application of matrix. **C)** Protein signals, yet to be identified, which are antibiotic specific. **D)** Protein signals (yet to be identified) specific to the areas of the film which have not been exposed to the drug. The mass-to-charge ratios are given below and intensity scales beside the corresponding images.

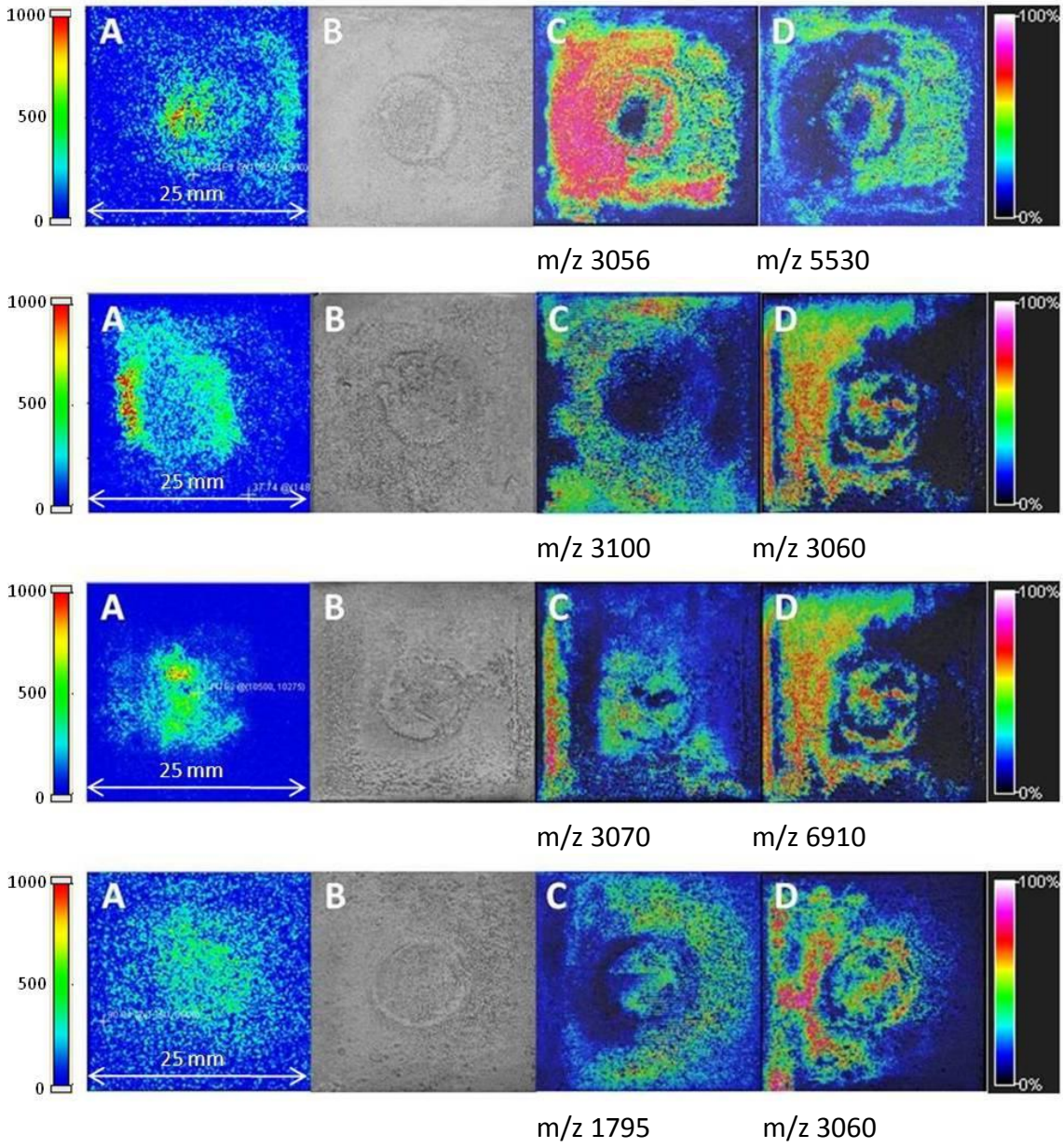

Figure 9. Examples of IMS revealing various proteins/peptides specific to structural components of the *S. aureus* biofilm. **A)** Drug imaging performed on a Thermo LTQ MALDI mass spectrometer over the mass range [m/z 159.58-160.58 plus m/z 173.58-174.58]. **B)** Scanned image of the biofilm following application of matrix. **C and D)** Unidentified proteins specific to structural components indicative of thicker/thinner regions of biofilm, differences in the amount of extracellular polysaccharides, etc. The mass-to-charge ratios are given below and the intensity scaled beside the corresponding images.

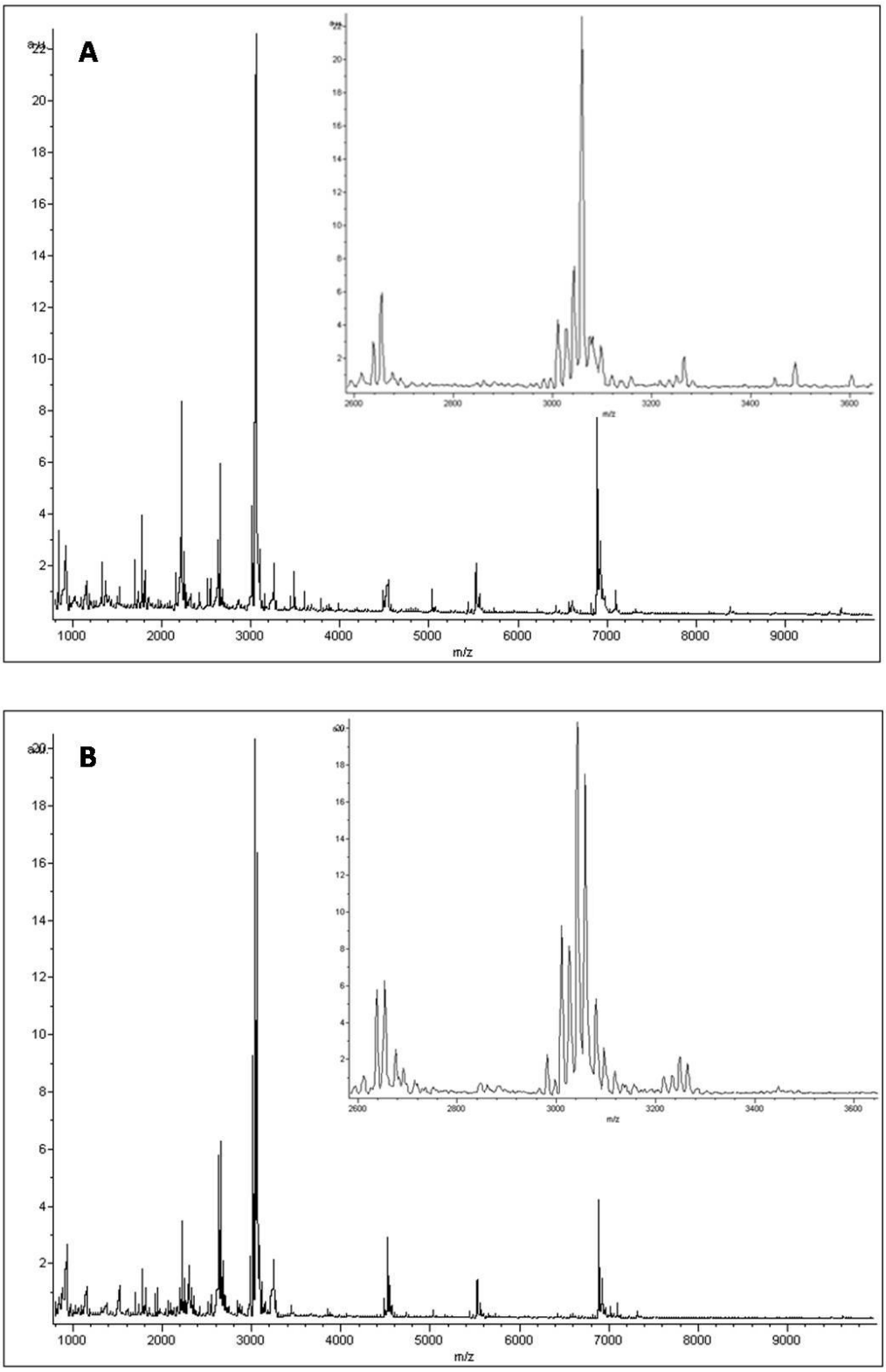

Figure 10. Example spectra from two *S. aureus* biofilms exposed to ampicillin. Zoom provided for m/z 2600-3600: proteins related to biofilm structure.

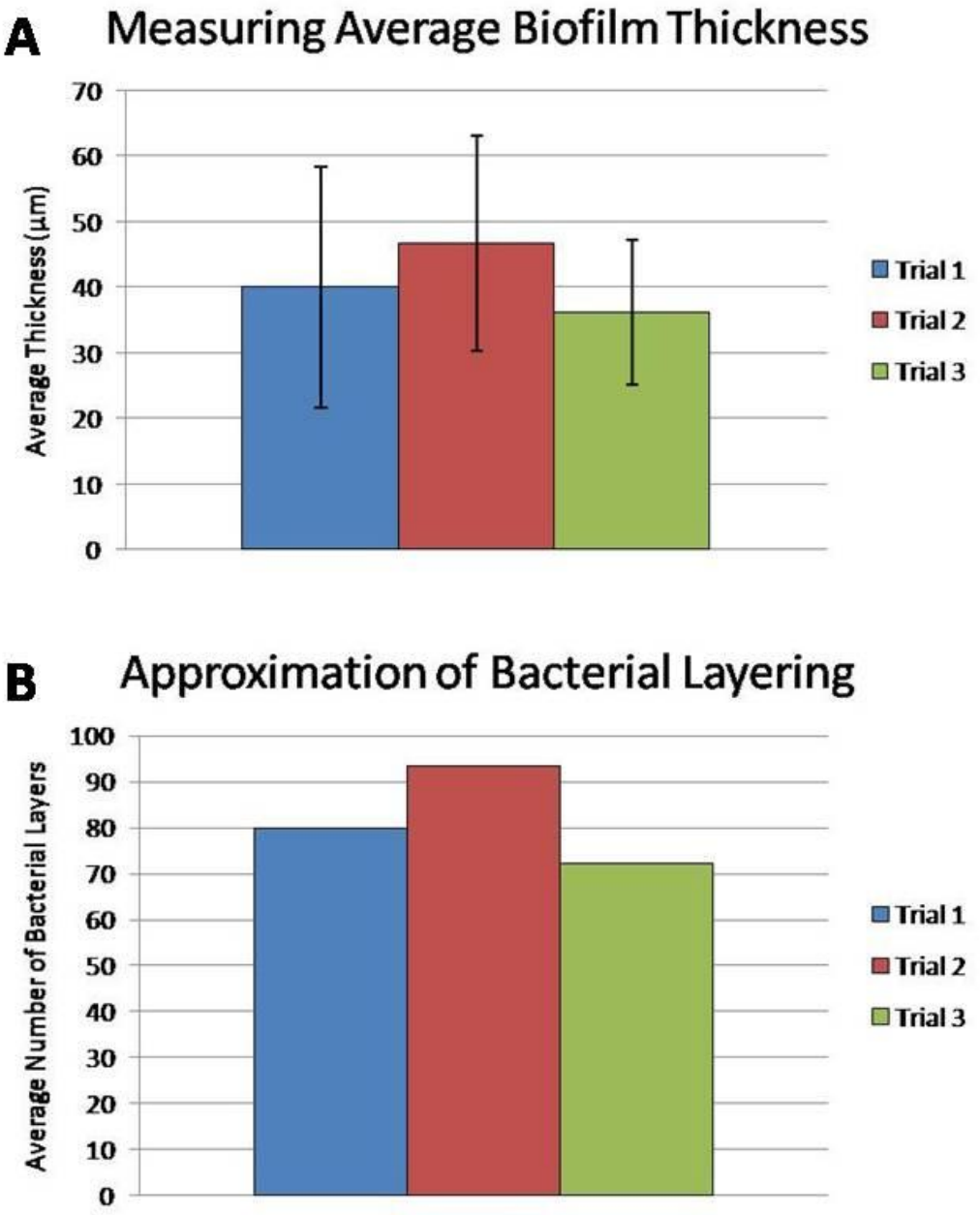

Figure 11. Determination of the average thickness and the average number of bacterial layers present in a *S. aureus* biofilm treated with ampicillin. **A)** Measurements made at 25 individual points for three separate bacterial biofilms resulted in the determination of average biofilm thickness. Results for trials 1, 2, and 3 were 40.02, 46.72, and 36.18 µm with standard deviations of ± 15.69, 18.33, and 10.98 µm respectively. **B)** Approximation of the number of bacterial layers for each trial based on the thickness of a single *S. aureus* bacterium (0.5 µm). Results for trials 1, 2, and 3 were approximately 80, 93, and 72 layers respectively.

Discussion

Utililizing IMS, the low mass (m/z 800-10,000) molecular profile of *Staphylococcus aureus* Newman Str. biofilms treated with ampicillin was examined and compared with drug imaging. Certain signals were more abundant in regions where the antibiotic was present (E.g. m/z 1785, 2100, etc.) while others were more abundant in areas where the drug was absent (E.g. m/z 2350, 6906, etc.). Several unidentified signals showed similar distributions to the drug (**Figure 8**) indicating that they were produced by the bacteria in response to the antibiotic. Molecular descriptors expressed only in drug free regions of the biofilm may have been triggered following cell-cell signaling from the bacteria that were exposed to the drug and may indicate a preemptive response to the oncoming drug treatment.

Especially intriguing were the signals that were present in the m/z 2600-3600 range. These signals were present in every sample analyzed and appear to correspond to structural features of the biofilm (**Figures 9 and 10**). Thicker regions of the biofilm contain more cells, resulting in areas that appear to "light up" when false color is applied to the image. If denser regions of biofilm could be identified they could be specifically targeted before they are able to disperse into the surrounding environment resulting in further colonization. This could prove useful in the targeted treatment of biofilms on exogenous surfaces such as those prevalent in water treatment facilities.

Results published by Bernardo, et. al using MALDI-TOF data collected from *Staphylococcus aureus* MRSA str. bacteria grown on Columbia blood agar and lysed in 50

mM sinapinic acid matrix (in acetonitrile/0.1% TFA 70:30 v/v) revealed the same general peak patterns observed here. Their spectra displayed the same groupings of signal reported here at approximately m/z 2500, 3000, 4500, and 5500 (**Figure 10**). They also reported unidentified signals at m/z 5526, 5567, 6890, and 6927 which were present in the samples analyzed in this study.[30]

Measurements of biofilm thickness revealed an average thickness of 40.97 μm indicating a biofilm composed of approximately 81 bacterial layers. Results across three different samples were relatively consistent (**Figure 11**) indicating that it is possible to grow *Staphylococcus aureus* biofilms in-vitro reproducibly. The use of a flow cell by Jones et. al resulted in a 69 μm thick *Staphylococcus aureus* MRSA str. biofilm after 2 days of growth in an artificial urine medium[31]. Measurements were made using a scanning electron microscope. Considering the sample preparation methods utilized here, the results reported seem reasonable as one would not expect biofilm adhesion and growth to occur as rapidly in a system without moving/flowing medium.

The imaging results produced in this experiment suggest that bacterial biofilms produce low molecular weight proteins/peptides in response to ampicillin and that the cells are likely communicating via cell to cell signaling resulting in the production of defensive proteins in areas awaiting antibiotic exposure. Additionally, bacteria exposed to the antibiotic treatment may cease production of proteins that are consistently being produced by healthy, unexposed bacteria. If we can *identify* which proteins are being produced by the bacteria in an attempt to protect themselves, we could develop

methods for blocking their production thereby allowing for more successful treatment. Analysis of the high mass proteins involved in the biofilms response would also be useful in determing the defensive mechanisms of the bacteria.

CHAPTER III

EVALUATING PROTEIN GRADIENTS ACROSS A TREATED *STAPHYLOCOCCUS AUREUS* BIOFILM USING LIQUID CHROMATROGRAPHY TANDEM MASS SPECTROMETRY

Introduction

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

Liquid Chromatography (LC) is a separation technique in which a liquid sample is pushed through a solid stationary phase in a packed column by a liquid mobile phase resulting in the separation of the chemical components of the sample. Chromatography can also be used to remove salts or contaminants as well as to concentrate samples prior to analysis.[32]

Different types of column packing materials include silica gels bound to various compounds such as alumina, size exclusion particles, and ion-exchange resins. The mobile phase used to elute the sample components off of the column will depend on the stationary phase being used as well as the polarity of the analyte but generally consists of methanol, acetonitrile, hexanes, or an organic-water mixture. The pH and ionic composition of the mobile phase will determine the analyte's retention time. Most common today are reversed phase (RP) columns which feature a hydrophobic, non-polar stationary phase, such as C-18, and a polar mobile phase. Reversed phase columns

are also commonly packed with silica particles covalently bound to non-polar alkyl chains for separations under alkaline conditions.[33]

The measurement of mass-to-charge ratios via mass spectrometry does not necessarily result in the unique identification of proteins so additional sample preparation and/or analyses are necessary[23]. Liquid chromatography is used to separate the chemical components of a sample *before* it is ionized and characterized by mass-to-charge ratios and relative abundance by tandem mass spectrometers[34]. This technique is common in the analysis of urine and blood for illicit drugs and their metabolites as well as in the determination of proteins and amino acids in all types of samples from homogenized tissue to serum to bacteria.

Strong Cation Exchange (SCX) Chromatography

Ion-exchange chromatography is a separation process in which charged analyte ions in flowing solution compete with mobile phase ions for sites with opposing charge on a solid stationary phase. Analyte ions that have a high affinity for the ion-exchange sites are retained longer than ions that do not compete well with the mobile phase ions resulting in varying retention times for the different components of the sample.[32]

The stationary phase may be a naturally occurring compound such as sodium aluminosilicate, a clay like montmorillonate, or a synthetic compound such as zirconium phosphate. More often, the stationary phase is composed of a resin prepared by copolymerization of styrene and divinylbenzene. The copolymerized resin is then covalently bound to ionic functional groups[35]. In the case of strong cation exchangers

(SCX), sulfonic acid functional groups are bound to the resin resulting in an anionic surface[36]. Therefore, samples must be acidified prior to separation on a SCX column.

Ion-exchange resins are incorporated into high-performance liquid chromatography (HPLC) columns either as porous polymer beads or as porous silica particles coated in resin. The incorporation method is dependent upon whether the resin includes a strong or a weak ion-exchange site and on the extent of cross-linking between the divinylbenzene and polystyrene which determines the resin's permeability. Low levels of cross-linking result in larger pore size and more accessible ion-exchange sites but the resin will adsorb more water. Conversely, a resin with high levels of cross-linking will have smaller pores making the ion-exchange sites less accessible to analyte.[33]

Ion-exchange chromatography is common in organic chemistry as well as biochemistry. It is generally used to separate metallic ions as well as proteins and amino acids. It has also proven to be a useful tool in the structural elucidation of proteins from peptide sequences when used in conjunction with tandem mass spectrometry.[35]

MudPIT

Multidimensional Protein Identification Technology or MudPIT is a non-gel technique for the separation and identification of complex protein mixtures from peptides. MudPIT consists of a 2-dimensional chromatographic separation followed by electrospray/nanospray ionization and sample characterization by mass spectrometry. The first dimension is typically a strong cation exchange (SCX) column. The second is an

analytical reverse phase (RP) column which serves to separate the peptides in the sample and is compatible with electrospray ionization.[37]

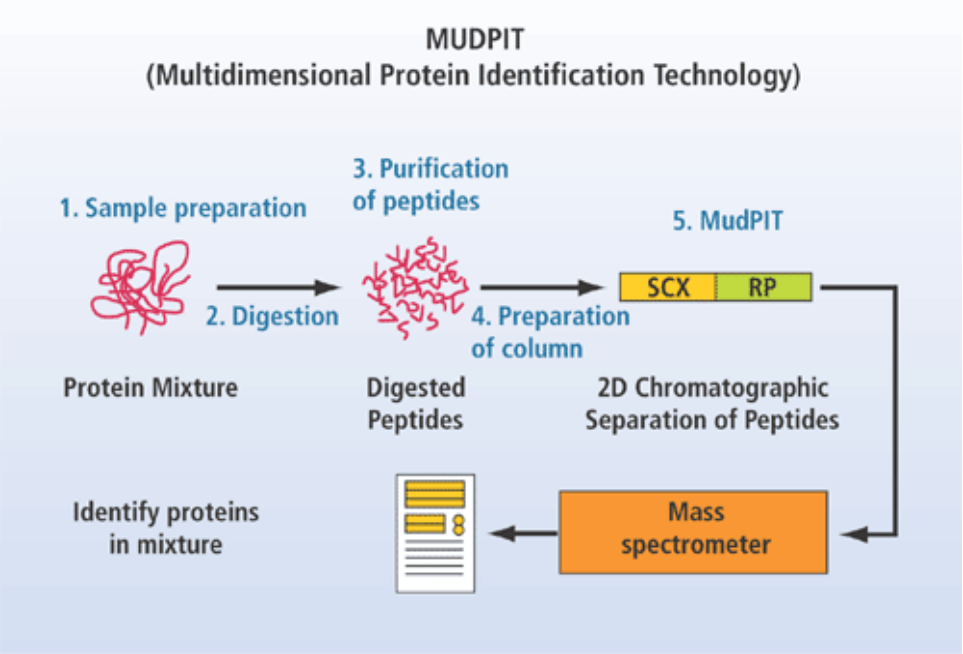

Figure 12. Schematic representation of the basic steps involved in the analysis of a complex protein mixture via MudPIT.[37]

Samples are first denatured and the proteins digested using a protease such as trypsin before being acidified and injected into the SCX column. Charged peptides bind to the ion-exchange sites while uncharged peptides pass through and are trapped on a RP trap column. The peptides are then eluted from the trap column onto an analytical RP column, separated, eluted, and detected by a mass spectrometer. Salt at a particular concentration is then pulsed through the SCX column displacing the bound peptides and driving them into the RP trap column. The salt is removed during a wash step and the peptides are separated on the analytical RP column for detection via MS. The most

intense peptide peaks are selected and fragmented during a second MS scan[37]. Peptide fragments can then be searched in a database such as SEQUEST to produce protein identifications.

Specific proteins involved in a bacterial biofilm's response to antibiotics can also be determined via liquid chromatography tandem mass spectrometry (LC-MS/MS) using MudPIT. Proteins can be collected from lysed *Staphylococcus aureus* biofilm cells, purified on a gel, digested using trypsin, and analyzed via MudPIT. Proteins can then be identified using a database and spectral counts for proteins involved in the bacteria's defensive mechanisms can be determined allowing for relative quantitation of said proteins. This method features a much higher dynamic range than what is possible in MALDI imaging and thus will be useful in identifying high mass proteins.

Methods and Materials

Materials

Acetic acid, ammonium acetate, dithiothreitol (DTT), iodoacetamide (IA), and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma Chemical Co. (St. Louis, MO). The methanol, Ambic, and ethanol were purchased from Fisher Scientific (Suwanee, GA). Laminator sheeting was purchased from 3M (St. Paul, MN). Trypsin was purchased from Promega (Madison, WI). All chromatography resins were purchased from Phenomenex (Torrance, CA). Fused silica columns were purchased from Polymicro

Technologies (Phoenix, AZ). Trifluoroacetic acid was purchased from Thermo Scientific (Asheville, NC). Formic acid and acetonitrile (with 0.1% formic acid added) were purchased from VWR International (Suwanee, GA). Phosphate buffered saline was purchased from Mediatech Inc. (Manassas, VA). Lysostaphin was purchased from AMBI Products LLC. (Lawrence, NY).

Counting Bacteria

LC-MS/MS analyses require a minimum of approximately $1x10^6$ cells for adequate detection of proteins so determining the average number of cells per mm^2 of *S. aureus* biofilms is imperative. This information was necessary in order to determine how large the sample sections needed to be in order to yield viable data.

S. aureus biofilms were grown and exposed to antibiotic using the method described below (See Sample Preparation). Upon removal from the -80°C freezer, samples were warmed to room temperature, and dried under a sterile fume hood. Three square portions of biofilm were cut from different regions of the sample. Each was placed in an Eppendorf tube and 200 µL of PBS (1x) buffer solution was added. The samples were sonicated in an ice bath for 60 minutes, vortexed, and the solution pipetted up and down to remove any remaining bacteria from the plastic surface.

The 200 µL samples were pipetted into the first three wells of a 100 well plate. The samples underwent a 10 fold dilution resulting in a total of 21 dilutions, 7 for each of the three squares. Each dilution was spotted (10 µL) onto a dry agar plate and the plates were incubated at 37°C overnight. Colony forming units (CFUs) were counted

within the highest dilution displaying bacterial growth. A second agar was spotted and analyzed using the same dilutions to confirm the results (**Table 1**).

Sample Preparation

Newman strain *Staphylococcus aureus* biofilms were grown on cut (25x25 mm) plastic laminator sheeting (Scotch). The square slides were soaked in 100% ethanol for approximately 5 minutes then drained and exposed to UV light for 1 hour. The slides were placed into individual Petri dishes. Each slide was streaked with bacteria and 20 mL of TSB broth was added and the dishes were placed in an incubator at 37°C for 8 days. The TSB broth was drained and replenished daily.

Following the 8 day growth period, the TSB broth was drained and the biofilms received a circular piece of filter paper containing 20 µL of ampicillin at a concentration of 800 µg/mL in ddH_2O. After application of the antibiotic, the biofilms were placed back in the incubator for 10 hours. Following this period of drug exposure, the filter paper was removed, the underside of the biofilm cleansed with anhydrous ethyl alcohol, and the films were placed in a -80°C freezer for storage.

The samples were thawed and dried in sterile a hood. A 4 mm wide strip was cut across the center of the film spanning the entire length of the sample. The strip was then cut into square sections approximately 4x4 mm in size and each added to a 1.5 mL Eppendorf tube. The tubes then received 150 µL of a stock solution composed of 500 µL of TM buffer, 7 µL of lysostaphin (an enzyme that effectively breaks the peptide bridges linking the NAG and NAM sugars of the peptidoglycan) and 7.5 µL of

phenylmethylsulfonyl fluoride (PMSF; a protease inhibitor). The samples were vortexed and incubated in a water bath at 37°C for 40 minutes. Occasional vortexing helped the bacteria to slough off of the plastic sheeting. At the end of the incubation period the plastic sheeting was removed and the samples were sonicated via probe for approximately 10 seconds to break open any protoplasts not destroyed by the lysostaphin treatment.

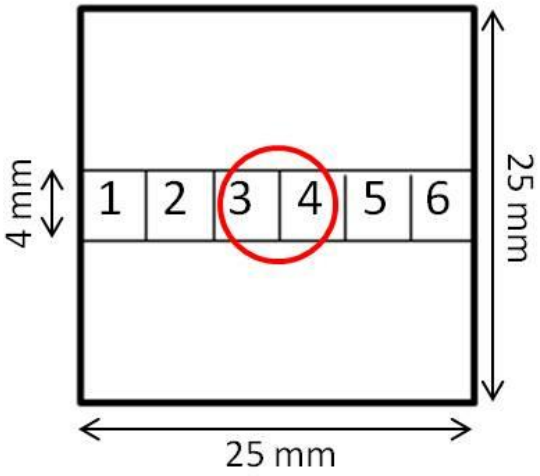

Figure 13. Schematic showing the general location of each 4x4 mm section cut from a *S. aureus* biofilm for analysis via LC-MS/MS. The red circle denotes the location of the antibiotic filter paper (7 mm in diameter). Note: Uneven diffusion of the drug favored section 4 over section 3 (**Figures 14-17**).

Short Stack

In order to digest the samples for LC-MS/MS analysis using a protease such as trypsin, the protease inhibitor (PMSF) used in sample preparation must first be removed. The simplest way to do this is to run a short stack.

The lysostaphin treated samples received 2.5 µL of NuPage LDS Sample Buffer (4x) for every 6.5 µL of sample material. To this solution, dithiothreitol, or DTT, (500 mM in ddH_2O) reducing agent was added in a 1:10 DTT to sample solution ratio. The samples were placed on a heat block at 55°C for 20 minutes. A short stack was run on a NuPage 10% Bis/Tris gel using MOPS (1x) running buffer, with 500 µL NuPage Antioxidant added to the upper (cathode) buffer chamber, at constant 200 V for 5 minutes. The gel was removed and placed on a plate shaker in a solution of 50 mL methanol, 10 mL acetic acid, and 40 mL Milli-Q water for 10 minutes. The fixing solution was removed and a staining solution composed of 55 mL Milli-Q water, 10 mL methanol, and 10 mL of Novex Stainer A was added to the sample on the plate shaker for 10 minutes. After 10 minutes, 5 mL of Novex Stainer B was added and the sample was left on the plate shaker for a minimum of 3 hours after which the staining solution was drained and 200 mL of Milli-Q water was added. The sample was allowed to destain overnight.

In-gel Digestion and Extraction

The short stacks were excised and added to 1.5 mL Eppendorf tubes. To the tubes, 100 mM Ambic solution was added, enough to cover the gels, and the samples were allowed to sit for 10 minutes. DTT reducing agent (45 mM) was added in a 1:10

DTT to Ambic ratio and the solution was incubated at 55°C for 20 minutes after which Iodoacetamide, or IA, (100 mM) was added in a 1:1 IA to DTT ratio. Due to the light sensitivity of IA, the sample was allowed to sit in the dark for 20 minutes. The Ambic, DTT, and IA solution was removed via pipette and the sample was treated with a solution of 50 mM Ambic with 50% acetonitrile (ACN) (enough to cover the gels) for 15 minutes to remove the stain. This process was repeated 2-3 times. The destaining solution was removed, enough ACN was added to cover the gels, and the samples were allowed to sit for 10 minutes. The ACN was removed and the gels were dried via speed vac for 5-10 minutes or until dry.

The dried samples received enough trypsin (0.01 mg/mL Promega Trypsin Gold in acetic acid and 25 mM Ambic) to rehydrate the gels. To the samples, 25 mM Ambic was added, enough to cover the rehydrated samples and the tubes were incubated overnight at 37°C. The Ambic solution was pipetted off of the gels and added to new vials. To the gels, a solution of 60% ACN with 0.1% trifluoroacetic acid (TFA) was added, enough to cover the samples. The solution was allowed to sit for 15 minutes and the ACN/TFA solution was removed and added to the secondary vials. This extraction was repeated a second time and the secondary vials were dried via speed vac for several hours. The resulting material was brought up in 50 µL of 0.1% formic acid and acidified for MudPIT analyses.

Analysis

Samples were loaded via pressure injection platform (New Objective, Woburn, MA) onto a 150 µm internal diameter, split phase MudPIT column consisting of 3-4 cm of Jupiter C-18 (5 µm, 300 Å pore size) and 3-4 cm of Luna SCX resin (5 µm, 100 Å pore size) fritted into an M520 filter union (IDEA, Oak Harbor, WA). After loading, the column was placed in line with an 18 cm Jupiter (3 µm, 300 Å pore size) 100 µm internal diameter, self-packed analytical column.

Peptides were resolved using an Eksigent 1D+ HPLC system through a 22 hour MudPIT separation with eleven 5 µL salt pulses (25, 50, 75, 100, 150, 200, 250, 300, 500, 700 mM, 1 M ammonium acetate) followed by an organic gradient (45% ACN with 0.1% TFA added; 95% ACN with 0.1% TFA added, on the last salt pulse only) at a flow rate of 500 nL/min to resolve each eluted set of peptides.

Tandem mass spectra were collected data-dependently using a Thermo Finnigan LTQ Mass Spectrometer, equipped with a nanoelectrospray source, with a collision energy of 35.0 µJ and a source voltage of 2.43 kV. The spectra were searched with SEQUEST against a *Staphylococcus aureus* Newman Str. database.

Table 1. Determination of the average number of colony forming units (CFUs) per square millimeter of bacterial biofilm. CFUs were counted at the highest dilution visible, back calculations resulted in the number of CFUs in 200 µL of the original stock solution, and the CFUs present per mm^2 of bacterial biofilm were determined. The average number of bacteria per square millimeter was calculated to be **5416895 CFU/mm^2 OR 5.42x10^6 CFU/mm^2**.

Sample Agar 1:	**CFU counted in lowest dilution:**	**CFU in 200 µL stock:**	**CFU/mm^2 of material:**
A (Corner)	1x10^9 CFU/mL	2x10^8 CFU	**11025966**
B (Center)	4x10^8 CFU/mL	8x10^7 CFU	**3103301**
C (Central edge)	1x10^8 CFU/mL	2x10^7 CFU	**897062**
Sample Agar 2:	**CFU counted in lowest dilution:**	**CFU in 200 µL stock:**	**CFU/mm^2 of material:**
A (Corner)	1x10^9 CFU/mL	2x10^8 CFU	**11025966**
B (Center)	6x10^8 CFU/mL	1.2x10^8 CFU	**4654951**
C (Central edge)	2x10^8 CFU/mL	4x10^7 CFU	**1794124**

Table 2. Summarized results for the defensive proteins identified which display trends across the biofilm. Data for the probability of correct protein identification, protein percentage of the total spectra, the number of peptides used to identify the protein, the number of unique peptides, the number of total spectra, the percentage of amino acids identified, and the molecular weight of the protein are given. Peptides for each protein and section are given in the appendix.

Penicillin-Binding Protein 1							
Section	**Prob**	**%Spec**	**#Pep**	**#Uniq**	**#Spec**	**%Cov**	**Weight**
1	100%	2.49E-05	6	6	6	0.13172042	83 kDa
2	100%	3.07E-05	7	7	7	0.14784946	83 kDa
3	100%	3.21E-05	5	5	7	0.088709675	83 kDa
4	100%	1.30E-05	3	3	3	0.10215054	83 kDa
5	100%	2.81E-05	6	6	6	0.12768817	83 kDa
6	100%	2.63E-05	4	4	6	0.1155914	83 kDa
Penicillin-Binding Protein 2							
Section	**Prob**	**%Spec**	**#Pep**	**#Uniq**	**#Spec**	**%Cov**	**Weight**
1	100%	9.13E-05	14	17	22	0.2957359	80 kDa
2	100%	6.14E-05	9	13	14	0.21595599	80 kDa
3	100%	8.70E-05	12	16	19	0.23796424	80 kDa
4	100%	9.54E-05	12	15	22	0.2696011	80 kDa
5	100%	1.13E-04	13	15	24	0.23933975	80 kDa
6	100%	6.57E-05	9	11	15	0.15543328	80 kDa
Penicillin-Binding Protein 3							
Section	**Prob**	**%Spec**	**#Pep**	**#Uniq**	**#Spec**	**%Cov**	**Weight**
1	100%	1.66E-05	4	4	4	0.11866859	77 kDa
2	100%	2.63E-05	5	5	6	0.083936326	77 kDa
3	100%	1.83E-05	4	4	4	0.11577424	77 kDa
4	100%	1.74E-05	4	4	4	0.14037627	77 kDa
5	100%	1.88E-05	3	3	4	0.060781475	77 kDa
6	100%	8.76E-06	2	2	2	0.05065123	77 kDa
Penicillin-Binding Protein 4							
Section	**Prob**	**%Spec**	**#Pep**	**#Uniq**	**#Spec**	**%Cov**	**Weight**
1	33%	0	0	0	0	0	48 kDa
2	100%	8.77E-06	2	2	2	0.07424594	48 kDa
3	100%	9.16E-06	2	2	2	0.10208817	48 kDa
4	100%	3.04E-05	5	5	7	0.14849187	48 kDa
5	100%	4.69E-06	1	1	1	0.04408353	48 kDa
6	100%	0	0	0	0	0	48 kDa

Table 2—cont.

Cell Division Protein FtsQ							
Section	**Prob**	**%Spec**	**#Pep**	**#Uniq**	**#Spec**	**%Cov**	**Weight**
1	99%	8.30E-06	2	2	2	0.075	50 kDa
2	100%	1.32E-05	3	3	3	0.06590909	50 kDa
3	100%	1.83E-05	3	4	4	0.15454546	50 kDa
4	100%	4.34E-06	1	1	1	0.027272727	50 kDa
5	100%	3.28E-05	3	4	7	0.08409091	50 kDa
6	100%	2.63E-05	3	4	6	0.10227273	50 kDa
General Stress Protein-Like Protein							
Section	**Prob**	**%Spec**	**#Pep**	**#Uniq**	**#Spec**	**%Cov**	**Weight**
1	100%	3.24E-04	12	19	78	0.68711656	18 kDa
2	100%	3.55E-04	9	15	81	0.5705522	18 kDa
3	100%	3.34E-04	11	13	73	0.68711656	18 kDa
4	100%	2.95E-04	12	17	68	0.70552146	18 kDa
5	100%	3.57E-04	12	18	76	0.68711656	18 kDa
6	100%	4.16E-04	13	17	95	0.70552146	18 kDa
Methicillin Resistance Expression Factor FemA							
Section	**Prob**	**%Spec**	**#Pep**	**#Uniq**	**#Spec**	**%Cov**	**Weight**
1	100%	2.08E-04	15	23	50	0.4047619	49 kDa
2	100%	2.41E-04	17	21	55	0.63095236	49 kDa
3	100%	1.65E-04	14	18	36	0.43571427	49 kDa
4	100%	2.86E-04	19	26	66	0.53571427	49 kDa
5	100%	2.72E-04	15	23	58	0.5285714	49 kDa
6	100%	2.10E-04	16	23	48	0.4952381	49 kDa

Table 3. LC-MS/MS MudPIT results for sections 1-6 taken from a *S. aureus* biofilm treated with ampicillin. The total number of spectral counts for each section as well as the number of proteins identified are reported (Min. # of peptides= 5, 99% protein, 95% peptide, 8.3% FDR). Normalized spectral counts for penicillin-binding proteins 1-4, cell division protein FtsQ, general stress protein-like protein, and methicillin resistance expression factor FemA.

Section:	**1**	**2**	**3**	**4**	**5**	**6**
Total Spectral Counts:	240892	227970	218301	230504	213151	228349
Number of Proteins Indentified:	1086	1087	1087	1088	1088	1086
Penicillin Binding Protein 1 (PBP1)	5.6422	6.9557	7.2638	2.9483	6.3765	5.9521
Penicillin Binding Protein 2 (PBP2)	20.6882	13.9114	19.7160	21.6205	25.5062	14.8804
Penicillin Binding Protein 3 (PBP3)	3.7615	5.9620	4.1507	3.9310	4.2510	1.9840
Penicillin Binding Protein 4 (PBP4)	0	1.9873	2.0754	6.8793	1.0628	0
Cell Division Protein FtsQ	1.8807	2.8211	3.7615	0.9404	6.5826	5.6422
General Stress Protein-like Protein	73.3489	76.1701	68.6471	63.9452	71.4682	89.3352
Methicillin Resistance Expression Factor FemA	47.0186	51.7204	33.8534	62.0645	54.5415	45.1378

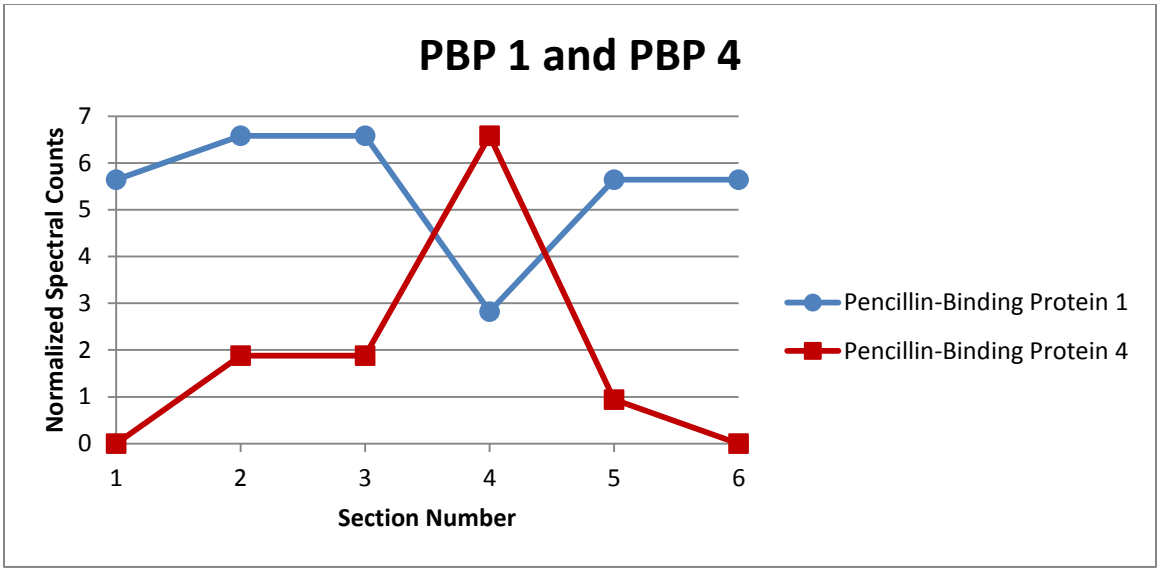

Figure 14. Plot displaying the normalized spectral counts for each section for penicillin-binding protein 1 and penicillin-binding protein 4. Note the opposing trends: down-regulation of penicillin-binding protein 1 in section 4 and up-regulation of penicillin-binding protein 4 in section 4. See **Figure 13** for section positions.

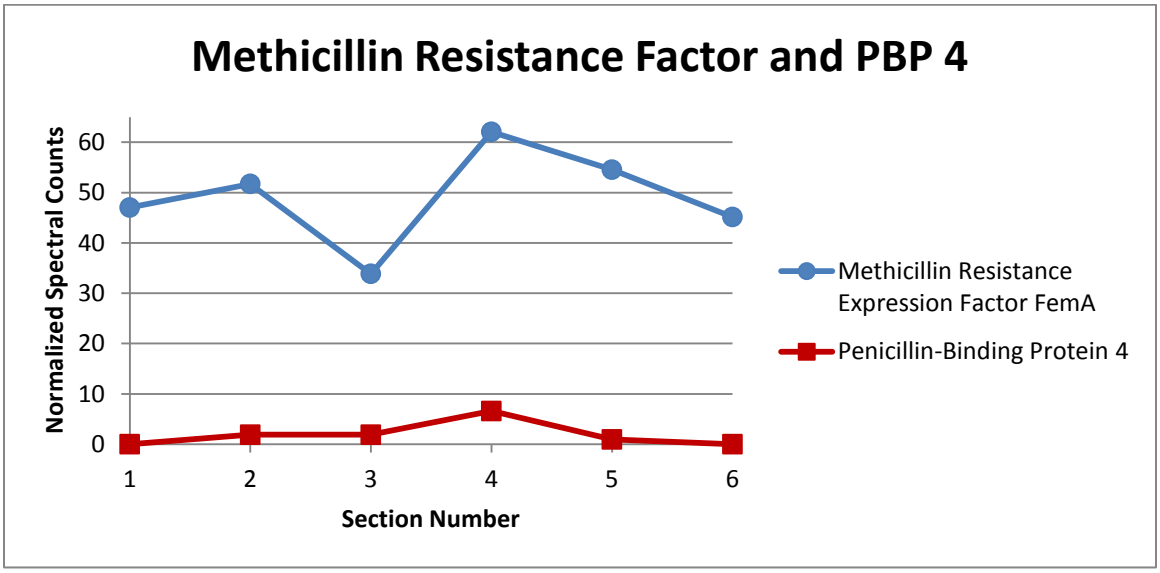

Figure 15. Plot displaying the normalized spectral counts for each section for methicillin resistance expression factor FemA and penicillin-binding protein 4. Note the same general trends: down-regulation in section 3 and up-regulation in section 4. See **Figure 13** for section positions.

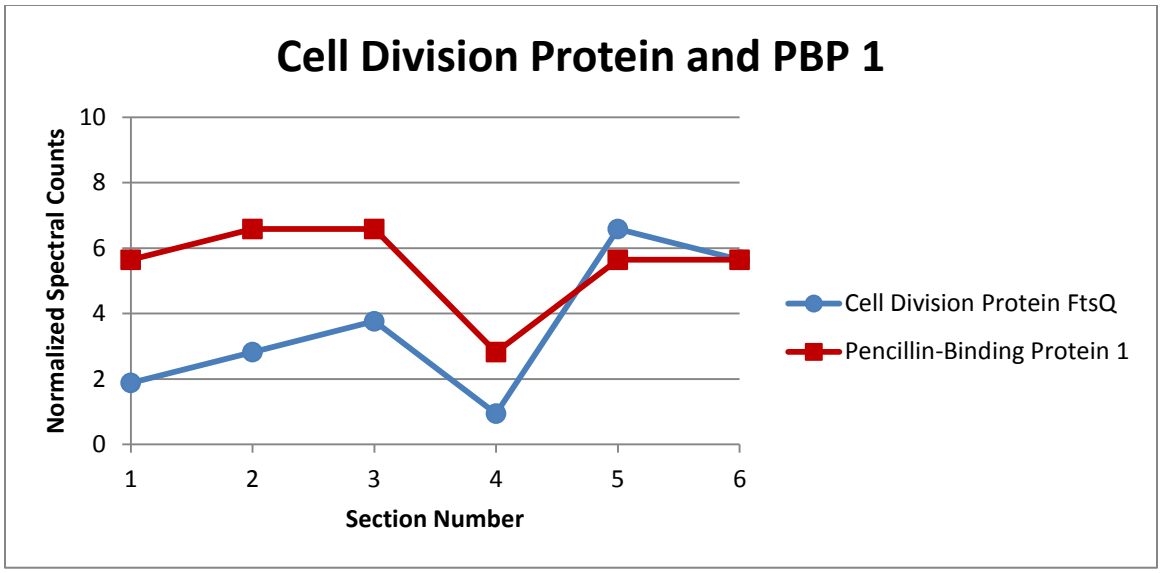

Figure 16. Plot displaying the normalized spectral counts for each section for cell division protein FtsQ and penicillin-binding protein 1. Note the same general trends: down-regulation in section 4 and up-regulation in the adjacent samples, 3 and 5. See **Figure 13** for section positions.

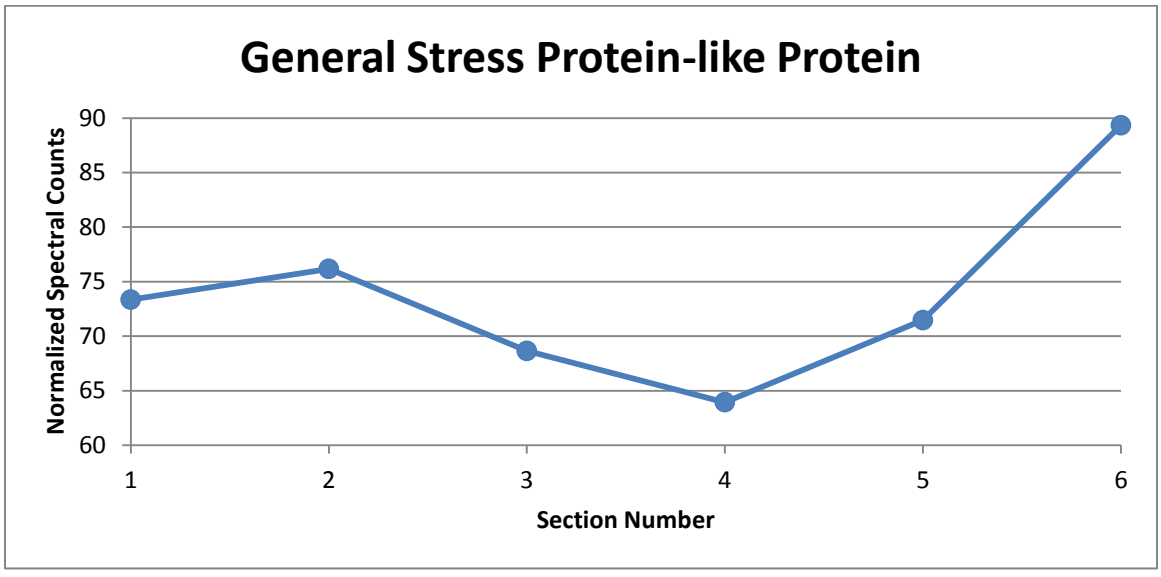

Figure 17. Plot displaying the normalized spectral counts for each section for general stress protein-like protein. Notice the down-regulation of this protein in section 4 and up-regulation in the other sections (6 especially). See **Figure 13** for section positions.

Discussion

Determination of the number of bacteria per mm^2 of bacterial biofilm revealed approximately $5.42x10^6$ CFU/mm^2 (**Table 1**). Previous studies involving *Pseudomonas aeruginosa* biofilms grown on a metal surface for 50 hours revealed $4.0x10^5$ CFU/mm^2 so the measurement presented in this study seems reasonable[3]. The number of bacteria counted in this experiment was sufficient for analyses via LC-MS/MS.

Analysis of gradient samples via MudPIT identified approximately 1087 proteins for each section of the biofilm including general stress proteins, immunoglobulins, MHC receptor proteins, cell division proteins, extracellular matrix and plasma proteins, ribosomal proteins, DNA gyrase proteins, and transpeptidases. All four of the transpeptidases or penicillin-binding proteins (PBPs) associated with *Staphylococcus aureus* Newman str. were detected.

There were also a number of stress related proteins present, consistent with what one would expect when bacteria are exposed to an antimicrobial drug. There were several proteins associated with virulence present as well, including protein A and a methicillin resistance factor.

Spectral counts of penicillin-binding proteins 1 and 4 revealed interesting trends across the biofilm. Penicillin-binding protein 1 was relatively consistent in sections 1-3 but was down-regulated in section 4 whereas penicillin-binding protein 4 was up-regulated in section 4 (**Figure 14**). These results are specific to the region of the film which received the antibiotic filter paper during sample preparation making these data

particularly interesting as they represent the biofilm's proteomic response to the drug treatment.

PBP 1 is the largest penicillin-binding protein of the four with a mass of 83 kDa while PBP 4 is the smallest with a mass of 48 kDa. Antibiotics that function by binding transpeptidases in the cell wall of *Staphylococcus aureus* bacteria specifically bind PBPs 1, 2, and 3, not PBP 4[38]. In response to the antibiotic treatment, the bacteria increase production of PBP 4 which binds ampicillin with very low affinity and whose transpeptidase activity can sustain cell growth in susceptible cells[38],[39]. Meanwhile PBP 1 production slows as it will be bound by the drug in the cell wall[38]. Note: Penicillin-binding proteins 2 and 3 were relatively consistent across all sections and didn't display any significant trends from section to section.

Intriguingly, there were several other proteins present in the samples which showed very similar trends to specific PBPs. For instance, methicillin resistance expression factor protein FemA displayed the same general trend as penicillin-binding protein 4. Both proteins were up-regulated in section 4 and down-regulated in the adjacent section, section 3 (**Figure 15**). As previously mentioned, antibiotic resistance in strains of *Staphylococcus aureus* in is part the result of alterations in the PBPs of the bacteria[13]. This may account for the heightened levels of methicillin resistance protein concurrent with up-regulation of penicillin-binding protein 4.

Cell division protein FtsQ and penicillin-binding protein 1 displayed the opposite trend. Both proteins were down-regulated in section 4 and up-regulated in the adjacent

sections, 3 and 5 (**Figure 16**). This trend may represent cell-to-cell signaling activities as the bacteria in section 4 may be warning the adjacent sections of the oncoming treatment. These sections then respond, attempting to bolster their immunity by increasing cellular density.

Additionally, methicillin resistance expression factor protein FemA and cell division protein FtsQ displayed opposing trends. Cell division protein FtsQ was down-regulated in section 4 while methicillin resistance expression factor protein FemA was up-regulated in 4. This may be a defense mechanism of the bacteria. When exposed to an antibiotic such as ampicillin they may put cell division activities aside in order to focus their energy on the production of resistance proteins in an attempt to defend themselves against the treatment.

Spectral counts for a general stress protein revealed down-regulation in section 4 with spikes in the protein at the outer edges of the film, section 6 for example (**Figure 17**). It may be that the bacteria in section 4 are dying and thus producing less of this protein. Meanwhile, cell-to-cell signaling may result in higher levels of stress protein in the unaffected areas of the film as they prepare to defend themselves against the oncoming treatment.

MudPIT analyses identified several proteins involved in the defensive response of a *S. aureus* Newman Str. biofilm to treatment with an antimicrobial drug. Cell division protein levels indicate that the bacteria attempt to increase cell density when alerted to the presence of an antibiotic. They also produce resistance proteins which may result in

the alteration of PBPs making them harder to bind in the cell wall. Some PBPs are up-regulated in response to the drug while others are down-regulated. Additionally, a few proteins show increased concentrations in the film concurrent with specific penicillin-binding proteins which may indicate symbiotic regulation of said proteins.

SYNOPSIS AND CONCLUSIONS

Imaging Mass Spectrometry allowed for the mapping of low mass proteins which were triggered in response to treatment with the antibiotic ampicillin. Images corresponding to individual mass-to-charge ratios indicated the presence of proteins which are up-regulated in response to the antibiotic, proteins triggered by cell-to-cell signaling, as well as proteins representative of structural components of the biofilm.

Identification of additional, higher mass proteins via liquid-chromatography tandem mass spectrometry revealed numerous proteins involved in the biofilm's defensive response to the treatment. These included stress proteins, resistance factors, transpeptidases or penicillin-binding proteins, as well as cell division proteins.

Cell division proteins were down-regulated in the section exposed to the largest dosage of antibiotic (section 4) and up-regulated in the surrounding sections indicating that the biofilm attempts to increase cell density when threatened with an antimicrobial drug. General stress proteins were also down-regulated in section 4, which may be indicative of cell death, but up-regulated in the surrounding regions. Both of these responses may be the result of cell-to-cell signaling.

Resistance factors were heightened in the affected section. This may represent the bacteria's last effort to survive the treatment. Penicillin-binding proteins 1 and 4 shared the same general trends as cell division protein FtsQ and methicillin resistance expression factor FemA respectively, which may point to a symbiotic relationship

between the proteins. Penicillin-binding proteins 2 and 3 levels were relatively consistent from section to section.

Biofilms grown in-vitro represent a better model for the study of infection in the human body as the biological system more closely resembles bacterial infections resulting from indwelling medical devices than do single layer colonies grown on an agar surface. Their unique structure, resulting in inherent antibiotic resistance, makes the treatment of infections resulting from bacterial biofilms very difficult. Further study of protein response and cell-to-cell signaling utilizing this method may shed light on how biofilms respond to various antimicrobial drugs and could help medical professionals develop more effective treatments.

Future work should involve the treatment and imaging of an infected animal treated with ampicillin to compare proteomic profiles in order to determine the extent to which these in-vitro models can be used in the study of biofilm related infections. Recent studies have already utilized Imaging Mass Spectrometry as a tool in the 3-dimensional mapping of infection and inflammatory response to infection across an entire animal[27].

Additional samples would help to confirm the LC-MS/MS results obtained here. Conducting these experiments using an antibiotic with a different mechanism of action such as kanamycin or Zyvox would also be very interesting as the proteomic response should involve a different set of defensive proteins.

Studies involving biofilms composed of several types of bacteria would be more informative as biofilm related infections are generally composed of multiple strains and/or species of bacteria. Furthermore, the use of a flow cell in these in-vitro models may allow us to further studies of biofilm growth, the development of antibiotic resistance, and to better mimic biological conditions inside the body.

Penicillin-Binding Protein 1						
Section	**Sequence**	**Prob**	**SEQUEST XCorr**	**deltaCn**	**NTT**	**Modifications**
1	(K)DLFAVVmDAKTGEILAYSQRPTFNPETGK(D)	95%	2.1823	0.2881	2	Oxidation (+16)
	(R)GKIYDRNGKVLAEDVERYK(L)	95%	2.1688	0.334	2	
	(K)GSGFVSHQSISK(G)	95%	1.7448	0.3966	2	
	(K)PRHVVDKKETAKK(L)	95%	2.0958	0.2289	1	
	(K)PRHVVDKKETAKKLSTVINMKPEEIEK(R)	95%	2.2605	0.3172	1	
	(K)YLVKNAQQPERGKIYDRNGKVLAEDVERYK(L)	95%	2.5173	0.2662	2	
2	(K)AFKPImENTLK(Y)	95%	1.69	0.3232	2	Oxidation (+16)
	(K)DLFAVVmDAKTGEILAYSQRPTFNPETGK(D)	95%	2.0552	0.3089	2	Oxidation (+16)
	(R)FYPNGNFASHLIGRAQK(N)	95%	2.5079	0.1692	2	
	(K)GPNPYFVSFMGDAPK(K)	95%	2.2454	0.317	2	
	(K)GSGFVSHQSISK(G)	95%	2.8141	0.5052	2	
	(K)LVAVIDKKASANSKKPRHVVDK(K)	95%	2.43	0.2214	2	
	(K)TGEILAYSQRPTFNPETGKDFGK(K)	95%	4.9078	0.5698	2	
3	(K)GPNPYFVSFMGDAPK(K)	95%	1.8428	0.3264	2	
	(K)GPNPYFVSFmGDAPK(K)	95%	3.1107	0.4181	2	Oxidation (+16)
	(K)GSGFVSHQSISK(G)	95%	2.6705	0.4364	2	
	(R)HVVDKKETAKKLSTVINMK(P)	95%	2.2636	0.3553	1	
	(R)HVVDKKETAKKLSTVINmKPEEIEK(R)	95%	2.026	0.2143	2	Oxidation (+16)
	(R)HVVDKKETAKKLSTVINmKPEEIEK(R)	95%	2.5926	0.386	2	Oxidation (+16)
	(R)YIHDIWGYIAPNTK(K)	95%	4.5403	0.5753	2	
4	(K)AIDNVSAKSLEPVTIGSGTQIK(A)	95%	2.3826	0.2046	2	
	(K)DLFAVVmDAKTGEILAYSQRPTFNPETGK(D)	95%	2.3871	0.4207	2	Oxidation (+16)
	(R)HVVDKKETAKKLSTVINmKPEEIEK(R)	95%	2.5638	0.2506	2	Oxidation (+16)

5	(K)GPNPYFVSFMGDAPK(K)	95%	2.9499	0.5744	2	
	(K)GSGFVSHQSISK(G)	95%	2.9704	0.4743	2	
	(K)IFDSYLSGSK(G)	95%	2.4501	0.2979	2	
	(K)IYDRNGKVLAEDVERYKLVAVIDKK(A)	95%	2.313	0.2036	2	
	(K)TGEILAYSQRPTFNPETGK(D)	95%	2.3256	0.3632	2	
	(R)YQPKDLFAVVMDAKTGEILAYSQR(P)	95%	2.4193	0.2646	1	
6	(K)ASANSKKPRHVVDKKETAKK(L)	95%	2.329	0.1973	2	
	(K)ASANSKKPRHVVDKKETAKK(L)	95%	1.9077	0.2592	2	
	(K)ASANSKKPRHVVDKKETAKK(L)	95%	2.2374	0.2622	2	
	(R)FYPNGNFASHLIGRAQK(N)	95%	2.5523	0.1649	2	
	(K)IDVEFSSENVDSNSTNNSDSNSDDKK(K)	95%	3.0831	0.1832	2	
	(K)TGEILAYSQRPTFNPETGKDFGK(K)	95%	4.2587	0.4493	2	

Penicillin-Binding Protein 2						
Section	**Sequence**	**Prob**	**SEQUEST XCorr**	**deltaCn**	**NTT**	**Modifications**
1	(K)AFKDENLGNVLQSGIK(I)	95%	3.9971	0.2397	2	
	(K)DAVLATEDNR(F)	95%	2.0345	0.3377	2	
	(R)DFKDVVNR(N)	95%	2.0174	0.327	2	
	(R)FYEHGALDYKR(L)	95%	2.4799	0.317	2	
	(R)KAQEAYLSYR(L)	95%	2.3743	0.3288	2	
	(R)KAQEAYLSYR(L)	95%	2.6786	0.3894	2	
	(R)LEQEYSKDDIFQVYLNK(I)	95%	4.976	0.2729	2	
	(R)LEQEYSKDDIFQVYLNK(I)	95%	4.3073	0.5397	2	
	(K)NLTGGFGSEGASTLTQQVVK(D)	95%	5.1489	0.5227	2	
	(K)NLTGGFGSEGASTLTQQVVK(D)	95%	4.3904	0.5888	2	
	(K)PFLAYGPAIENMK(W)	95%	3.3031	0.376	1	
	(R)QSFNIPALK(A)	95%	1.9132	0.2714	2	
	(K)QYGENSFVGHSQQEYPQFLYENVMSK(I)	95%	3.2361	0.3589	2	
	(K)RPSSVSGSIPSINVSGSQDNNTTNR(S)	95%	3.7415	0.297	2	
	(K)RPSSVSGSIPSINVSGSQDNNTTNR(S)	95%	3.0814	0.3918	2	
	(K)RPSSVSGSIPSINVSGSQDNNTTNR(S)	95%	4.0485	0.4092	2	
	(K)SHGTVSIYDALR(Q)	95%	3.0243	0.5103	2	
	(K)SHGTVSIYDALR(Q)	95%	3.0792	0.6966	2	
	(K)TGGLVAISGGR(D)	95%	2.4689	0.5055	2	
	(K)TGGLVAISGGR(D)	95%	2.7145	0.5464	2	
	(K)TLQNDVDNGSFYKNKDQQVGATILDSK(T)	95%	2.0425	0.2655	2	
	(K)TLQNDVDNGSFYKNKDQQVGATILDSK(T)	95%	2.6911	0.2436	2	
2	(K)AFKDENLGNVLQSGIK(I)	95%	2.89	0.4581	2	

2	(R)FYEHGALDYKR(L)	95%	2.7986	0.5002	2	
	(R)FYEHGALDYKR(L)	95%	3.2648	0.513	2	
	(R)FYEHGALDYKR(L)	95%	2.3663	0.5264	2	
	(R)HEHVNLK(D)	95%	1.8346	0.3456	2	
	(R)HEHVNLK(D)	95%	2.6965	0.2614	2	
	(R)LEQEYSKDDIFQVYLNK(I)	95%	2.52	0.1948	2	
	(-)MTENKGSSQPKKNGNNGGKSNSKKNR(N)	95%	2.2742	0.248	2	
	(K)NLTGGFGSEGASTLTQQVVK(D)	95%	4.9786	0.5382	2	
	(K)RPSSVSGSIPSINVSGSQDNNTTNR(S)	95%	4.4151	0.5772	2	
	(K)RPSSVSGSIPSINVSGSQDNNTTNR(S)	95%	5.1951	0.7026	2	
	(K)SHGTVSIYDALR(Q)	95%	3.0322	0.4375	2	
	(K)SHGTVSIYDALR(Q)	95%	3.5649	0.6387	2	
	(R)STHGGSDTSANSSGTAQSNNNTR(S)	95%	4.0098	0.3719	2	
3	(K)AFKDENLGNVLQSGIK(I)	95%	4.6048	0.2601	2	
	(K)AFKDENLGNVLQSGIK(I)	95%	4.3484	0.5594	2	
	(R)FYEHGALDYKR(L)	95%	2.5794	0.3948	2	
	(R)FYEHGALDYKR(L)	95%	2.666	0.5424	2	
	(R)HEHVNLK(D)	95%	2.0998	0.2841	2	
	(R)KAQEAYLSYR(L)	95%	2.7855	0.4024	2	
	(R)KAQEAYLSYR(L)	95%	2.377	0.5481	2	
	(K)KFAAK(L)	95%	1.2656	0.3277	2	
	(R)LEQEYSKDDIFQVYLNK(I)	95%	3.6555	0.1931	2	
	(K)NLTGGFGSEGASTLTQQVVK(D)	95%	5.3321	0.5767	2	
	(K)QYGENSFVGHSQQEYPQFLYENVMSK(I)	95%	3.7658	0.2981	2	
	(K)RPSSVSGSIPSINVSGSQDNNTTNR(S)	95%	2.3815	0.4074	2	
	(K)RPSSVSGSIPSINVSGSQDNNTTNR(S)	95%	3.7974	0.421	2	

3	(K)RPSSVSGSIPSINVSGSQDNNTTNR(S)	95%	4.66	0.49	2	
	(K)SHGTVSIYDALR(Q)	95%	3.1208	0.5218	2	
	(K)SHGTVSIYDALR(Q)	95%	2.8545	0.4602	2	
	(K)SHGTVSIYDALR(Q)	95%	3.2371	0.6995	2	
	(K)SMKDAVLATEDNRFYEHGALDYKR(L)	95%	2.1961	0.2641	2	
	(K)TGGLVAISGGR(D)	95%	2.782	0.5178	2	
4	(K)AFKDENLGNVLQSGIK(I)	95%	4.6313	0.5158	2	
	(K)AFKDENLGNVLQSGIK(I)	95%	4.5227	0.5248	2	
	(K)AMSDYTAYMLAEMLK(G)	95%	2.943	0.4744	2	
	(K)AWQSVKQNAGNDAPK(K)	95%	2.555	0.2519	2	
	(K)DAVLATEDNR(F)	95%	2.1279	0.4495	2	
	(R)DGETIEYDHTSHKAmSDYTAYmLAEmLKGTFK(P)	95%	2.4696	0.2272	1	Oxidation (+16), Oxidation (+16), Oxidation (+16)
	(R)HEHVNLK(D)	95%	2.2521	0.1987	2	
	(K)NLTGGFGSEGASTLTQQVVK(D)	95%	5.1864	0.5799	2	
	(K)RPSSVSGSIPSINVSGSQDNNTTNR(S)	95%	5.2425	0.6902	2	
	(K)RPSSVSGSIPSINVSGSQDNNTTNR(S)	95%	6.0911	0.6026	2	
	(K)RPSSVSGSIPSINVSGSQDNNTTNR(S)	95%	5.4526	0.6486	2	
	(K)SHGTVSIYDALR(Q)	95%	1.8799	0.2626	2	
	(K)SHGTVSIYDALR(Q)	95%	3.0028	0.5968	2	
	(K)SHGTVSIYDALR(Q)	95%	3.0474	0.6219	2	
	(K)SHGTVSIYDALR(Q)	95%	3.4992	0.4302	2	
	(K)TGGLVAISGGR(D)	95%	2.1907	0.3137	2	

4	(K)TGGLVAISGGR(D)	95%	2.3287	0.2756	2	
	(K)TGGLVAISGGR(D)	95%	2.4378	0.487	2	
	(K)TGGLVAISGGR(D)	95%	3.2734	0.5209	2	
	(K)TLQNDVDNGSFYKNKDQQVGATILDSK(T)	95%	2.06	0.2624	2	
	(K)TLQNDVDNGSFYKNKDQQVGATILDSK(T)	95%	2.8214	0.2873	2	
	(K)WATNHAIQDESSYQVDGSTFR(N)	95%	3.2166	0.4965	2	
5	(K)AFKDENLGNVLQSGIK(I)	95%	3.4165	0.2712	2	
	(K)AFKDENLGNVLQSGIK(I)	95%	4.3766	0.5348	2	
	(K)AFKDENLGNVLQSGIKIYTNMDKDVQK(T)	95%	2.6732	0.2625	2	
	(K)AQEAYLSYR(L)	95%	2.4816	0.4725	2	
	(R)DGETIEYDHTSHKAmSDYTAYmLAEmLKGTFK(P)	95%	2.2442	0.2588	1	Oxidation (+16), Oxidation (+16), Oxidation (+16)
	(R)FYEHGALDYKR(L)	95%	2.9056	0.6091	2	
	(R)HEHVNLK(D)	95%	1.8094	0.2957	2	
	(R)ITDKQWEDAK(K)	95%	3.3178	0.3955	2	
	(R)KAQEAYLSYR(L)	95%	1.5131	0.4106	2	
	(R)KAQEAYLSYR(L)	95%	2.2253	0.4214	2	
	(K)NLTGGFGSEGASTLTQQVVK(D)	95%	5.2712	0.5522	2	
	(K)NLTGGFGSEGASTLTQQVVK(D)	95%	4.8228	0.5795	2	
	(R)QSFNIPALK(A)	95%	2.0095	0.2518	2	
	(R)QSFNIPALK(A)	95%	1.8556	0.3261	2	
	(K)RPSSVSGSIPSINVSGSQDNNTTNR(S)	95%	4.1082	0.4471	2	
	(K)RPSSVSGSIPSINVSGSQDNNTTNR(S)	95%	4.23	0.6529	2	

5	(K)SHGTVSIYDALR(Q)	95%	2.6681	0.3092	2	
	(K)SHGTVSIYDALR(Q)	95%	3.1631	0.643	2	
	(K)SHGTVSIYDALR(Q)	95%	3.6949	0.6629	2	
	(K)SHGTVSIYDALR(Q)	95%	3.6203	0.6066	2	
	(K)TGGLVAISGGR(D)	95%	2.6429	0.3135	2	
	(K)TGGLVAISGGR(D)	95%	3.5654	0.6082	2	
	(K)TGGLVAISGGR(D)	95%	3.7845	0.5713	2	
	(K)TGGLVAISGGR(D)	95%	4.3807	0.6155	2	
6	(K)AFKDENLGNVLQSGIK(I)	95%	2.6575	0.6178	2	
	(K)AFKDENLGNVLQSGIK(I)	95%	3.9371	0.5585	2	
	(K)AFKDENLGNVLQSGIK(I)	95%	4.5466	0.5363	2	
	(R)FYEHGALDYK(R)	95%	2.0233	0.3007	2	
	(R)KAQEAYLSYR(L)	95%	2.1706	0.3414	2	
	(R)KAQEAYLSYR(L)	95%	2.2542	0.418	2	
	(K)KFAAK(L)	95%	1.3664	0.2856	2	
	(K)NKDQQVGATILDSK(T)	95%	2.3149	0.2453	2	
	(K)NKDQQVGATILDSK(T)	95%	2.8519	0.4297	2	
	(K)SHGTVSIYDALR(Q)	95%	1.975	0.2701	2	
	(K)SHGTVSIYDALR(Q)	95%	3.6895	0.6466	2	
	(K)TGGLVAISGGR(D)	95%	3.2119	0.6052	2	
	(K)TGGLVAISGGR(D)	95%	2.3836	0.3996	2	
	(K)TGTGTYGAETYSQYNLPDNAAK(D)	95%	3.4564	0.616	2	
	(K)TLQNDVDNGSFYKNKDQQVGATILDSK(T)	95%	2.0152	0.2612	2	

Penicillin-Binding Protein 3						
Section	**Sequence**	**Prob**	**SEQUEST XCorr**	**deltaCn**	**NTT**	**Modifications**
1	(K)KKEmKYTTDKSGKVTSSEVLNPGARGQDLK(L)	95%	2.5557	0.3309	2	Oxidation (+16)
	(K)LRSQGAKDMDNAMMVVQNPKNGDILALAGK(Q)	95%	2.3243	0.2	2	
	(K)NGHVTINDKQALmHSSNVYMFK(T)	95%	1.8657	0.2269	2	Oxidation (+16)
	(K)QALmHSSNVYmFK(T)	95%	2.0652	0.2649	2	Oxidation (+16), Oxidation (+16)
2	(K)DFWIQLHPK(K)	95%	2.5049	0.384	2	
	(K)DFWIQLHPK(K)	95%	2.3288	0.4462	2	
	(K)EVEALLDKQIKK(L)	95%	1.7484	0.2944	2	
	(K)EVEALLDKQIKKLR(S)	95%	2.1752	0.2947	2	
	(K)LPGVNTSMDWDR(K)	95%	2.1217	0.3959	2	
	(K)QDQYDKQLLSKIGKSQLDELSSK(D)	95%	2.3066	0.2499	2	
3	(R)GRILDRNGKVLVDNASKMAITYTR(G)	95%	2.953	0.2099	2	
	(R)GRKTTQSEmLDTAEKLSKLIKMDTK(K)	95%	2.4685	0.2707	2	Oxidation (+16)
	(K)LIKMDTKKITERDKKDFWIQLHPKK(A)	95%	2.322	0.2214	2	
	(K)QALMHSSNVYMFK(T)	95%	2.7335	0.4246	2	
4	(R)EmNAGTVLDPQMIKNEDVSEKEYAAVSQQLSK(L)	95%	2.673	0.1712	2	Oxidation (+16)
	(K)KKEmKYTTDKSGKVTSSEVLNPGARGQDLK(L)	95%	2.4367	0.3262	2	Oxidation (+16)
	(K)KLRSQGAKDMDNAMMVVQNPK(N)	95%	2.2067	0.3294	2	
	(R)SYFNKNGHVTINDK(Q)	95%	2.2733	0.2707	2	

5	(K)EVEALLDKQIKKLRSQGAKDmDNAmmVVQNPK(N)	95%	2.8804	0.3051	2	Oxidation (+16), Oxidation (+16), Oxidation (+16)
	(R)GLNQVGLGVK(T)	95%	2.4442	0.1575	2	
	(R)GLNQVGLGVK(T)	95%	2.713	0.465	2	
	(R)SQGAKDmDNAMMVVQNPK(N)	95%	2.7337	0.2561	2	Oxidation (+16)
6	(R)GIFGDVSTPAEGIPK(E)	95%	2.7251	0.576	2	
	(K)LRSQGAKDmDNAmmVVQNPK(N)	95%	2.1206	0.3419	2	Oxidation (+16), Oxidation (+16), Oxidation (+16)

Penicillin-Binding Protein 4						
Section	**Sequence**	**Prob**	**SEQUEST XCorr**	**deltaCn**	**NTT**	**Modifications**
1/6	N/A	N/A	N/A	N/A	N/A	N/A
2	(K)NLGGEKQRNmMGNALmER(S)	95%	2.0926	0.3035	2	Oxidation (+16), Oxidation (+16)
	(K)NTSDFVDLMNNKAK(A)	95%	2.0318	0.2849	2	
3	(K)QLAPTTHAVTYYTFNFSLEGAKmSLPGTDGLK(T)	95%	2.4255	0.2208	2	Oxidation (+16)
	(K)QRNMmGNALmER(S)	95%	1.4838	0.2685	2	Oxidation (+16), Oxidation (+16)
4	(K)FRINQVImGAGDYK(N)	95%	1.658	0.3189	2	Oxidation (+16)
	(K)FRINQVImGAGDYK(N)	95%	1.6015	0.3077	2	Oxidation (+16)
	(R)INQVIMGAGDYKNLGGEKQRNMmGNALMER(S)	95%	2.4228	0.2584	2	Oxidation (+16)
	(K)KDYKLVVEDGKVHADYPR(E)	95%	2.2314	0.262	2	
	(K)KDYKLVVEDGKVHADYPR(E)	95%	1.8306	0.3567	2	
	(K)NTHFVNPTGAENSR(L)	95%	3.4573	0.53	2	
	(K)QRNmmGNALmER(S)	95%	2.0245	0.2613	2	Oxidation (+16), Oxidation (+16), Oxidation (+16)
5	(K)NTSDFVDLmNNKAKAIGmK(N)	95%	2.5706	0.2365	2	Oxidation (+16), Oxidation (+16)

Cell Division Protein FtsQ						
Section	**Sequence**	**Prob**	**SEQUEST XCorr**	**deltaCn**	**NTT**	**Modifications**
1	(K)GTKEDDmIKALSEMTPEVRR(Y)	95%	2.4394	0.2263	2	Oxidation (+16)
	(R)YIAEVTYAPSKNK(Q)	95%	2.8196	0.1912	2	
2	(K)IAHVNINGNNHVSTSK(I)	95%	3.2144	0.4338	2	
	(K)VTQLKPLTLEEK(R)	95%	3.1207	0.4846	2	
	(K)VTQLKPLTLEEKR(K)	95%	2.7935	0.2427	2	
3	(R)GNTSSQSESDKNVTKSSQEENQAK(E)	95%	2.6702	0.2265	2	
	(-)mmDDKTKNDQQESNEDKDELELFTRNTSKKRR(Q)	95%	2.6108	0.2437	2	Oxidation (+16), Oxidation (+16)
	(K)VTQLKPLTLEEK(R)	95%	3.2762	0.4324	2	
	(K)VTQLKPLTLEEK(R)	95%	3.0908	0.5543	2	
4	(K)VTQLKPLTLEEK(R)	95%	3.5649	0.5112	2	
5	(K)NAINDLEENPLIK(S)	95%	2.6124	0.4154	2	
	(R)RYIAEVTYAPSK(N)	95%	2.2899	0.3547	2	
	(K)VTQLKPLTLEEK(R)	95%	2.4866	0.3546	2	
	(K)VTQLKPLTLEEK(R)	95%	3.3815	0.3854	2	
	(K)VTQLKPLTLEEK(R)	95%	3.1244	0.4071	2	
	(K)VTQLKPLTLEEK(R)	95%	2.2242	0.4142	2	
	(K)VTQLKPLTLEEK(R)	95%	3.5877	0.5939	2	
6	(K)GKYLPLLENGKLLK(G)	95%	3.8404	0.2848	2	
	(K)IAHVNINGNNHVSTSKINK(V)	95%	2.0412	0.3803	2	
	(K)VTQLKPLTLEEK(R)	95%	3.2794	0.3296	2	
	(K)VTQLKPLTLEEK(R)	95%	3.305	0.475	2	

6	(K)VTQLKPLTLEEK(R)	95%	2.8442	0.5506	2	
	(K)VTQLKPLTLEEK(R)	95%	3.3587	0.5463	2	

General Stress Protein-Like Protein						
Section	**Sequence**	**Prob**	**SEQUEST XCorr**	**deltaCn**	**NTT**	**Modifications**
1	(K)ANNVATDANHSYTSR(V)	95%	1.6932	0.2969	2	
	(K)ANNVATDANHSYTSR(V)	95%	2.2438	0.4337	2	
	(K)ANNVATDANHSYTSR(V)	95%	3.1471	0.5257	2	
	(K)ANNVATDANHSYTSR(V)	95%	2.8133	0.6563	2	
	(K)ANNVATDANHSYTSR(V)	95%	4.8066	0.6056	2	
	(K)ANNVATDANHSYTSR(V)	95%	5.112	0.6512	2	
	(K)ANNVATDANHSYTSR(V)	95%	5.3561	0.7208	2	
	(R)ETTDLLHK(V)	95%	1.7845	0.3186	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	2.9893	0.2814	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	3.4548	0.5593	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	4.2424	0.5513	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	4.4229	0.5699	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	4.6063	0.6499	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.6843	0.0973	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.028	0.3282	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.5639	0.2789	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.3006	0.3989	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.531	0.3001	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.2944	0.3077	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.4266	0.4318	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.77	0.3045	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.5796	0.3381	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.9098	0.3839	2	

1	(K)ISQVVQWSNVAmEIADK(W)	95%	2.7885	0.4646	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.4968	0.3984	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.571	0.5122	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.8169	0.5713	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.497	0.5536	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.0526	0.578	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.4758	0.5077	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.6886	0.5998	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.1643	0.5985	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.7858	0.6019	2	
	(K)ISQVVQWSNVAmEIADK(W)	95%	4.5594	0.6213	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADK(W)	95%	5.402	0.6367	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	5.5813	0.6439	2	
	(K)ISQVVQWSNVAmEIADK(W)	95%	5.1295	0.6438	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADK(W)	95%	5.0359	0.6247	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADK(W)	95%	5.3584	0.6731	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	5.267	0.6679	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	2.6033	0.2593	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	2.6801	0.2143	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	2.6886	0.2851	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.8675	0.2829	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.9977	0.512	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.762	0.464	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	3.4759	0.6355	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	4.3385	0.5084	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	4.8308	0.6489	2	Oxidation (+16)

	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	4.5204	0.5966	2	
	(R)LNSVVDAVK(G)	95%	1.463	0.2285	2	
	(R)LNSVVDAVK(G)	95%	2.8091	0.3322	2	
	(R)LTEDIQGK(V)	95%	1.7342	0.27	2	
	(R)LTEDIQGK(V)	95%	2.3842	0.3608	2	
	(R)LTEDIQGK(V)	95%	1.9973	0.333	2	
	(K)NLDYVAK(T)	95%	1.6584	0.2949	2	
	(K)NLDYVAK(T)	95%	1.5706	0.3377	2	
	(K)NLDYVAKTLDGVEGQVQGITR(E)	95%	2.1871	0.2541	2	
	(K)TLDGVEGQVQGITR(E)	95%	3.218	0.3371	2	
	(K)TLDGVEGQVQGITR(E)	95%	3.2202	0.313	2	
	(K)TLDGVEGQVQGITR(E)	95%	3.0016	0.4789	2	
	(K)TLDGVEGQVQGITR(E)	95%	4.3721	0.4195	2	
1	(K)TLDGVEGQVQGITR(E)	95%	4.9002	0.3502	2	
	(R)VTNSITHNISQNEDK(I)	95%	2.399	0.2648	2	
	(R)VTNSITHNISQNEDK(I)	95%	2.4583	0.2715	2	
	(R)VTNSITHNISQNEDK(I)	95%	3.3814	0.4161	2	
	(R)VTNSITHNISQNEDK(I)	95%	3.7033	0.4525	2	
	(R)VTNSITHNISQNEDK(I)	95%	4.4687	0.4698	2	
	(R)VTNSITHNISQNEDK(I)	95%	4.5814	0.5742	2	
	(R)VTNSITHNISQNEDK(I)	95%	4.9	0.5962	2	
	(R)VTNSITHNISQNEDK(I)	95%	5.2939	0.5672	2	
	(R)VTNSITHNISQNEDK(I)	95%	4.3672	0.6541	2	
	(R)VTNSITHNISQNEDK(I)	95%	4.7476	0.6272	2	
	(R)VTNSITHNISQNEDK(I)	95%	4.9785	0.6255	2	
	(R)VTNSITHNISQNEDK(I)	95%	3.935	0.5513	2	

1	(R)VTNSITHNISQNEDKISQVVQWSNVAmEIADK(W)	95%	5.485	0.593	2	Oxidation (+16)
	(R)VTNSITHNISQNEDKISQVVQWSNVAmEIADK(W)	95%	5.8382	0.621	2	Oxidation (+16)
	(R)VTNSITHNISQNEDKISQVVQWSNVAmEIADK(W)	95%	6.4424	0.6404	2	Oxidation (+16)
2	(R)ETTDLLHK(V)	95%	1.7999	0.2777	2	
	(R)ETTDLLHK(V)	95%	1.8672	0.3071	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	4.0132	0.595	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	2.7324	0.4032	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	3.3507	0.5846	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	4.1832	0.6325	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.2218	0.3411	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.1388	0.2657	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.8137	0.2047	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.7191	0.2504	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.3537	0.3195	2	
	(K)ISQVVQWSNVAmEIADK(W)	95%	2.8217	0.4405	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.719	0.3835	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.2178	0.4061	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.7536	0.4031	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.9909	0.4531	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.4351	0.3576	2	
	(K)ISQVVQWSNVAmEIADK(W)	95%	2.8786	0.4988	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.8388	0.4416	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.8734	0.5054	2	

2	(K)ISQVVQWSNVAMEIADK(W)	95%	4.5161	0.3895	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.958	0.533	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.5346	0.5722	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.473	0.5589	2	
	(K)ISQVVQWSNVAmEIADK(W)	95%	4.4817	0.5681	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADK(W)	95%	4.0391	0.6285	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.525	0.6358	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.7674	0.6242	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	5.0968	0.6153	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.8132	0.6704	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	5.6263	0.6332	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	5.2612	0.6796	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	1.914	0.3627	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	2.9743	0.2143	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	2.981	0.248	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	2.4068	0.284	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	3.1216	0.2897	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	2.7257	0.3581	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.1655	0.3591	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	2.997	0.3682	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	3.5988	0.3718	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	2.8635	0.4198	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	3.4104	0.4003	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	2.9479	0.5046	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.2427	0.5314	2	Oxidation (+16)

2	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	2.8082	0.5933	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.365	0.4185	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	3.6589	0.4661	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	3.4383	0.4648	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	3.9034	0.464	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	5.1092	0.6231	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	4.4421	0.514	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	5.6259	0.6726	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	5.2273	0.6724	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	4.7841	0.6903	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	4.6441	0.5382	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	5.7308	0.6635	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	5.0592	0.5612	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	4.1874	0.5732	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	5.81	0.6997	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	4.8426	0.7335	2	
	(R)LTEDIQGK(V)	95%	2.1872	0.4047	2	
	(R)LTEDIQGK(V)	95%	2.5865	0.4184	2	
	(K)TLDGVEGQVQGITR(E)	95%	2.8351	0.323	2	
	(K)TLDGVEGQVQGITR(E)	95%	3.6433	0.3781	2	
	(K)TLDGVEGQVQGITR(E)	95%	3.9793	0.3916	2	
	(K)TLDGVEGQVQGITR(E)	95%	3.9664	0.4358	2	
	(K)TLDGVEGQVQGITR(E)	95%	4.8177	0.4443	2	
	(K)TLDGVEGQVQGITR(E)	95%	4.5955	0.4519	2	
	(K)VDRLNSVVDAVK(G)	95%	3.0741	0.4017	2	
	(K)VDRLNSVVDAVK(G)	95%	3.0426	0.3874	2	

2	(R)VTNSITHNISQNEDK(I)	95%	3.1493	0.6628	2	
	(R)VTNSITHNISQNEDK(I)	95%	4.0067	0.4975	2	
	(R)VTNSITHNISQNEDK(I)	95%	4.4107	0.5817	2	
	(R)VTNSITHNISQNEDK(I)	95%	4.7651	0.6294	2	
	(R)VTNSITHNISQNEDK(I)	95%	3.8745	0.5572	2	
	(R)VTNSITHNISQNEDKISQVVQWSNVAMEIADK(W)	95%	5.4695	0.4391	2	
	(R)VTNSITHNISQNEDKISQVVQWSNVAmEIADK(W)	95%	5.9555	0.6041	2	Oxidation (+16)
	(R)VTNSITHNISQNEDKISQVVQWSNVAmEIADK(W)	95%	5.6555	0.6192	2	Oxidation (+16)
	(R)VTNSITHNISQNEDKISQVVQWSNVAMEIADK(W)	95%	6.519	0.6129	2	
	(R)VTNSITHNISQNEDKISQVVQWSNVAmEIADK(W)	95%	6.4345	0.6465	2	Oxidation (+16)
3	(K)ANNVATDANHSYTSR(V)	95%	3.2158	0.4596	2	
	(K)ANNVATDANHSYTSR(V)	95%	3.6471	0.5122	2	
	(R)ETTDLLHK(V)	95%	1.7748	0.2356	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	2.3062	0.4486	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	3.2572	0.4913	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	4.6423	0.6442	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	3.9254	0.6463	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	4.9319	0.6277	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.4074	0.259	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.7994	0.0927	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.7811	0.1606	2	

3	(K)ISQVVQWSNVAMEIADK(W)	95%	2.6276	0.4383	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.1997	0.2828	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.2416	0.315	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.8047	0.3336	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.955	0.515	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.2818	0.49	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.5689	0.4653	2	
	(K)ISQVVQWSNVAmEIADK(W)	95%	3.6685	0.4211	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.3157	0.3899	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.0915	0.4382	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.9359	0.5073	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.9255	0.5396	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.8844	0.468	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.397	0.4458	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.824	0.5149	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.1702	0.6046	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.2907	0.5907	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.5076	0.5826	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.2733	0.6101	2	
	(K)ISQVVQWSNVAmEIADK(W)	95%	5.0497	0.5579	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADK(W)	95%	4.5947	0.6458	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.8919	0.6247	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	5.2527	0.6424	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	5.5708	0.6516	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.7721	0.656	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	5.57	0.6615	2	

3	(K)ISQVVQWSNVAmEIADK(W)	95%	5.3686	0.6467	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	2.1178	0.2438	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	2.6819	0.2528	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	2.4689	0.4141	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.1986	0.3838	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.4168	0.3827	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	4.6367	0.4504	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	4.0325	0.542	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.5084	0.4878	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.9163	0.5329	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	5.2621	0.6534	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	3.7158	0.5475	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	5.4664	0.6637	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	4.4086	0.5792	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	5.1878	0.7127	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.8072	0.6051	2	Oxidation (+16)
	(R)LNSVVDAVK(G)	95%	1.4176	0.2351	2	
	(R)LNSVVDAVK(G)	95%	3.0781	0.3585	2	
	(R)LNSVVDAVK(G)	95%	3.1226	0.4116	2	
	(R)LTEDIQGK(V)	95%	2.0358	0.3829	2	
	(K)NLDYVAK(T)	95%	1.7945	0.3272	2	
	(K)TLDGVEGQVQGITR(E)	95%	2.3239	0.2809	2	
	(K)TLDGVEGQVQGITR(E)	95%	2.76	0.364	2	
	(K)TLDGVEGQVQGITR(E)	95%	3.0162	0.3375	2	
	(K)TLDGVEGQVQGITR(E)	95%	4.1329	0.3889	2	
	(K)TLDGVEGQVQGITR(E)	95%	4.0754	0.3866	2	

3	(K)TLDGVEGQVQGITR(E)	95%	4.2171	0.4365	2	
	(R)VTNSITHNISQNEDK(I)	95%	3.5123	0.4319	2	
	(R)VTNSITHNISQNEDK(I)	95%	4.3083	0.5172	2	
	(R)VTNSITHNISQNEDK(I)	95%	4.6416	0.5788	2	
	(R)VTNSITHNISQNEDKISQVVQWSNVAMEIADK(W)	95%	3.7585	0.3579	2	
	(R)VTNSITHNISQNEDKISQVVQWSNVAMEIADK(W)	95%	4.2254	0.3434	2	
	(R)VTNSITHNISQNEDKISQVVQWSNVAMEIADK(W)	95%	5.693	0.4311	2	
	(R)VTNSITHNISQNEDKISQVVQWSNVAmEIADK(W)	95%	4.7312	0.558	2	Oxidation (+16)
	(R)VTNSITHNISQNEDKISQVVQWSNVAmEIADK(W)	95%	5.8973	0.6146	2	Oxidation (+16)
	(R)VTNSITHNISQNEDKISQVVQWSNVAMEIADK(W)	95%	6.4812	0.6585	2	
4	(K)ANNVATDANHSYTSR(V)	95%	3.382	0.4443	2	
	(K)ANNVATDANHSYTSR(V)	95%	4.8366	0.6263	2	
	(K)ANNVATDANHSYTSR(V)	95%	4.8074	0.7403	2	
	(K)ANNVATDANHSYTSR(V)	95%	5.2736	0.7187	2	
	(R)ETTDLLHK(V)	95%	1.7917	0.3304	2	
	(R)ETTDLLHK(V)	95%	1.829	0.2869	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	3.108	0.3062	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	3.237	0.5983	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	2.8698	0.4718	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	4.5826	0.6451	2	

4	(K)GIGDSVQTLNSSVDR(V)	95%	4.6022	0.6495	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.4822	0.2717	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.5831	0.3923	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.262	0.2926	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.9915	0.416	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.7823	0.4668	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.2595	0.4321	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.3505	0.4896	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.5072	0.4661	2	
	(K)ISQVVQWSNVAmEIADK(W)	95%	3.6559	0.5637	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.6143	0.633	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.7814	0.6041	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.8274	0.6237	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.4941	0.635	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.4447	0.634	2	
	(K)ISQVVQWSNVAmEIADK(W)	95%	4.8135	0.6213	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.9137	0.6538	2	
	(K)ISQVVQWSNVAmEIADK(W)	95%	5.4404	0.664	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADK(W)	95%	5.3777	0.6562	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.6637	0.728	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	2.5378	0.2628	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	2.5074	0.3755	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.1378	0.3527	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.2972	0.3512	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	3.262	0.3538	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.1151	0.4645	2	Oxidation (+16)

4	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	3.7536	0.4239	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	3.3454	0.4669	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	5.4876	0.6236	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	5.3769	0.6447	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	5.1133	0.6625	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	4.9527	0.6458	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	4.0814	0.7271	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	5.0776	0.6902	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	5.0793	0.7204	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	4.2089	0.6237	2	Oxidation (+16)
	(R)LTEDIQGK(V)	95%	2.2871	0.3396	2	
	(K)NLDYVAK(T)	95%	1.6865	0.2676	2	
	(K)NLDYVAKTLDGVEGQVQGITR(E)	95%	2.3553	0.4175	2	
	(K)TLDGVEGQVQGITR(E)	95%	3.0946	0.2913	2	
	(K)TLDGVEGQVQGITR(E)	95%	3.1264	0.2755	2	
	(K)TLDGVEGQVQGITR(E)	95%	2.9898	0.401	2	
	(K)TLDGVEGQVQGITR(E)	95%	4.6766	0.3901	2	
	(K)TLDGVEGQVQGITR(E)	95%	4.7364	0.4256	2	
	(K)VDRLNSVVDAVK(G)	95%	2.791	0.3353	2	
	(R)VTNSITHNISQNEDK(I)	95%	1.6031	0.2792	2	
	(R)VTNSITHNISQNEDK(I)	95%	0.9332	0.4837	2	
	(R)VTNSITHNISQNEDK(I)	95%	4.3597	0.4998	2	
	(R)VTNSITHNISQNEDK(I)	95%	3.3289	0.3616	2	
	(R)VTNSITHNISQNEDK(I)	95%	5.3487	0.6017	2	
	(R)VTNSITHNISQNEDK(I)	95%	5.2158	0.5753	2	
	(R)VTNSITHNISQNEDK(I)	95%	4.7465	0.6064	2	

4	(R)VTNSITHNISQNEDK(I)	95%	4.8085	0.6002	2	
	(R)VTNSITHNISQNEDK(I)	95%	3.6594	0.528	2	
	(R)VTNSITHNISQNEDKISQVVQWSNVAMEIADK(W)	95%	3.1344	0.3202	2	
	(R)VTNSITHNISQNEDKISQVVQWSNVAMEIADK(W)	95%	5.4021	0.5301	2	
	(R)VTNSITHNISQNEDKISQVVQWSNVAmEIADK(W)	95%	5.1745	0.5581	2	Oxidation (+16)
	(R)VTNSITHNISQNEDKISQVVQWSNVAmEIADK(W)	95%	6.2906	0.623	2	Oxidation (+16)
5	(K)ANNVATDANHSYTSR(V)	95%	1.7882	0.544	2	
	(K)ANNVATDANHSYTSR(V)	95%	3.3959	0.4327	2	
	(R)ETTDLLHK(V)	95%	1.7645	0.3172	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	2.3134	0.3745	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	3.9132	0.5229	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	1.6587	0.5144	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	3.9835	0.5392	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	4.6744	0.635	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	4.1418	0.6137	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.8223	0.1251	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.4691	0.3783	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.4625	0.3191	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.0183	0.3369	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.6431	0.2696	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.3894	0.5051	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.7622	0.2982	2	

5	(K)ISQVVQWSNVAMEIADK(W)	95%	2.6389	0.496	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.2104	0.3745	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.5982	0.3991	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.6853	0.3656	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.6329	0.4368	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.9834	0.357	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.9096	0.441	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.0278	0.4588	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.5568	0.5089	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.9144	0.4651	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.8401	0.5152	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.4131	0.5434	2	
	(K)ISQVVQWSNVAmEIADK(W)	95%	4.281	0.5622	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.1281	0.5287	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.3751	0.506	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.5828	0.5392	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.5049	0.5421	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.9612	0.646	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.3237	0.6272	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	5.0741	0.6502	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.9663	0.6914	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	5.4998	0.6615	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	5.4174	0.6619	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	5.861	0.6752	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	2.8815	0.2557	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.2472	0.3415	2	Oxidation (+16)

5	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.3524	0.3753	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	2.6084	0.5647	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	3.0665	0.4094	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	4.7059	0.49	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	4.2007	0.5281	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	3.8011	0.544	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	5.1683	0.6808	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	5.2941	0.7156	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	5.5624	0.7068	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	5.7129	0.7061	2	
	(R)LNSVVDAVK(G)	95%	2.9592	0.3224	2	
	(R)LTEDIQGK(V)	95%	2.1741	0.3804	2	
	(R)LTEDIQGK(V)	95%	1.9536	0.3625	2	
	(R)RGSANYKANNVATDANHSYTSR(V)	95%	2.4992	0.2334	2	
	(K)TLDGVEGQVQGITR(E)	95%	2.6033	0.4153	2	
	(K)TLDGVEGQVQGITR(E)	95%	1.2389	0.4146	2	
	(K)TLDGVEGQVQGITR(E)	95%	2.9704	0.2989	2	
	(K)TLDGVEGQVQGITR(E)	95%	3.4425	0.3435	2	
	(K)TLDGVEGQVQGITR(E)	95%	3.8166	0.3147	2	
	(K)TLDGVEGQVQGITR(E)	95%	2.9686	0.4048	2	
	(K)TLDGVEGQVQGITR(E)	95%	4.5739	0.3278	2	
	(K)TLDGVEGQVQGITR(E)	95%	3.5888	0.4237	2	
	(K)TLDGVEGQVQGITR(E)	95%	4.6791	0.4013	2	
	(K)TLDGVEGQVQGITR(E)	95%	4.0725	0.4952	2	
	(R)VTNSITHNISQNEDK(I)	95%	2.7091	0.3534	2	
	(R)VTNSITHNISQNEDK(I)	95%	2.1205	0.3624	2	

5	(R)VTNSITHNISQNEDK(I)	95%	4.9047	0.619	2	
	(R)VTNSITHNISQNEDKISQVVQWSNVAMEIADK(W)	95%	4.3503	0.409	2	
	(R)VTNSITHNISQNEDKISQVVQWSNVAmEIADK(W)	95%	5.9493	0.5822	2	Oxidation (+16)
	(R)VTNSITHNISQNEDKISQVVQWSNVAmEIADK(W)	95%	5.9375	0.5792	2	Oxidation (+16)
	(R)VTNSITHNISQNEDKISQVVQWSNVAmEIADK(W)	95%	5.6929	0.6263	2	Oxidation (+16)
	(R)VTNSITHNISQNEDKISQVVQWSNVAmEIADK(W)	95%	5.5903	0.6404	2	Oxidation (+16)
	(R)VTNSITHNISQNEDKISQVVQWSNVAmEIADK(W)	95%	6.2527	0.6843	2	Oxidation (+16)
	(R)VTNSITHNISQNEDKISQVVQWSNVAMEIADKWQNR(H)	95%	4.8587	0.3337	2	
6	(K)ANNVATDANHSYTSR(V)	95%	2.3282	0.2516	2	
	(K)ANNVATDANHSYTSR(V)	95%	2.4581	0.4267	2	
	(K)ANNVATDANHSYTSR(V)	95%	3.5413	0.4065	2	
	(K)ANNVATDANHSYTSR(V)	95%	2.9564	0.5339	2	
	(K)ANNVATDANHSYTSR(V)	95%	5.0252	0.6155	2	
	(K)ANNVATDANHSYTSR(V)	95%	4.9875	0.6517	2	
	(K)ANNVATDANHSYTSR(V)	95%	5.033	0.6783	2	
	(K)ANNVATDANHSYTSR(V)	95%	5.1954	0.6763	2	
	(K)ANNVATDANHSYTSR(V)	95%	5.1219	0.6961	2	

6	(R)ETTDLLHK(V)	95%	1.7994	0.3248	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	2.3883	0.3556	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	2.4136	0.4182	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	3.6829	0.5949	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	4.3851	0.5838	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	5.0123	0.6221	2	
	(K)GIGDSVQTLNSSVDR(V)	95%	4.3	0.6579	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.1789	0.3148	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.5591	0.2964	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	2.2417	0.3293	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.0719	0.3314	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.1617	0.2523	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.3958	0.342	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.8619	0.3177	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.3407	0.3112	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.075	0.4422	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.3542	0.4607	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.2696	0.4526	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.4493	0.5162	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.6872	0.5006	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.3452	0.4747	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.6218	0.4822	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.1884	0.5092	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	3.8183	0.5598	2	
	(K)ISQVVQWSNVAmEIADK(W)	95%	3.5529	0.6072	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADK(W)	95%	4.8325	0.6194	2	Oxidation (+16)

6	(K)ISQVVQWSNVAmEIADK(W)	95%	5.3473	0.6241	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADK(W)	95%	5.0518	0.6491	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	4.3126	0.7017	2	
	(K)ISQVVQWSNVAMEIADK(W)	95%	5.5007	0.7203	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	2.8616	0.1853	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	2.4745	0.2503	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	2.5623	0.2432	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	2.4695	0.277	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.0691	0.2668	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	2.7196	0.3269	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	3.1886	0.3155	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	2.5921	0.3552	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.2431	0.3778	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.2713	0.376	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.3895	0.4206	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.3045	0.4809	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	4.0761	0.455	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	3.6765	0.4955	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	4.0985	0.4854	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	3.9723	0.5439	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	5.1293	0.6655	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	5.0301	0.6439	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	4.3167	0.6866	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	5.3057	0.6681	2	Oxidation (+16)
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	5.0894	0.6721	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	4.9284	0.6956	2	

6	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	5.187	0.7033	2	
	(K)ISQVVQWSNVAmEIADKWQNR(H)	95%	4.0283	0.564	2	Oxidation (+16)
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	5.4924	0.6723	2	
	(K)ISQVVQWSNVAMEIADKWQNR(H)	95%	5.0728	0.6806	2	
	(R)LNSVVDAVK(G)	95%	1.4542	0.2739	2	
	(R)LTEDIQGK(V)	95%	1.8689	0.2602	2	
	(R)LTEDIQGK(V)	95%	1.8955	0.2929	2	
	(R)LTEDIQGK(V)	95%	2.1145	0.4086	2	
	(K)NLDYVAK(T)	95%	1.4611	0.2695	2	
	(K)NLDYVAK(T)	95%	1.5472	0.3792	2	
	(K)NLDYVAKTLDGVEGQVQGITR(E)	95%	2.3276	0.4596	2	
	(K)TLDGVEGQVQGITR(E)	95%	1.0217	0.4273	2	
	(K)TLDGVEGQVQGITR(E)	95%	4.3081	0.4198	2	
	(K)TLDGVEGQVQGITR(E)	95%	4.3726	0.4093	2	
	(K)TLDGVEGQVQGITR(E)	95%	4.023	0.4127	2	
	(K)TLDGVEGQVQGITR(E)	95%	4.6807	0.4383	2	
	(K)TLDGVEGQVQGITR(E)	95%	4.4344	0.4325	2	
	(K)VDRLNSVVDAVK(G)	95%	2.2623	0.3173	2	
	(R)VTNSITHNISQNEDK(I)	95%	4.6148	0.5565	2	
	(R)VTNSITHNISQNEDK(I)	95%	3.363	0.4248	2	
	(R)VTNSITHNISQNEDK(I)	95%	3.4746	0.5581	2	
	(R)VTNSITHNISQNEDK(I)	95%	4.8608	0.581	2	
	(R)VTNSITHNISQNEDK(I)	95%	5.2243	0.5777	2	
	(R)VTNSITHNISQNEDK(I)	95%	4.8595	0.6028	2	
	(R)VTNSITHNISQNEDK(I)	95%	5.0252	0.601	2	
	(R)VTNSITHNISQNEDK(I)	95%	4.9771	0.608	2	

6	(R)VTNSITHNISQNEDK(I)	95%	5.0668	0.6178	2	
	(R)VTNSITHNISQNEDKISQVVQWSNVAMEIADK(W)	95%	3.7601	0.1931	2	
	(R)VTNSITHNISQNEDKISQVVQWSNVAMEIADK(W)	95%	4.8302	0.5104	2	
	(R)VTNSITHNISQNEDKISQVVQWSNVAmEIADK(W)	95%	5.3874	0.5721	2	Oxidation (+16)
	(R)VTNSITHNISQNEDKISQVVQWSNVAMEIADK(W)	95%	6.1001	0.5826	2	
	(R)VTNSITHNISQNEDKISQVVQWSNVAMEIADK(W)	95%	6.5811	0.6141	2	
	(R)VTNSITHNISQNEDKISQVVQWSNVAMEIADK(W)	95%	7.2682	0.6208	2	
	(R)VTNSITHNISQNEDKISQVVQWSNVAmEIADK(W)	95%	6.0701	0.6217	2	Oxidation (+16)

Methicillin Resistance Expression Factor FemA						
Section	**Sequence**	**Prob**	**SEQUEST XCorr**	**deltaCn**	**NTT**	**Modifications**
1	(K)AFADRDDKFYYNR(L)	95%	3.6801	0.4099	2	
	(K)AFADRDDKFYYNR(L)	95%	2.9211	0.4972	2	
	(R)FLSEEELPIFR(S)	95%	2.5821	0.2967	2	
	(R)FLSEEELPIFR(S)	95%	2.457	0.4214	2	
	(K)FTEDAEDAGVVK(F)	95%	2.0653	0.4072	2	
	(K)FTEDAEDAGVVK(F)	95%	3.366	0.576	2	
	(K)FTNLTAK(E)	95%	1.4527	0.2425	2	
	(K)FTNLTAK(E)	95%	1.7554	0.3917	2	
	(K)FTNLTAK(E)	95%	1.4743	0.3357	2	
	(K)GFDPVLQIR(Y)	95%	1.4639	0.3015	2	
	(K)GFDPVLQIR(Y)	95%	2.6719	0.4474	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	2.5002	0.369	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.9416	0.5127	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.6544	0.4333	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.3533	0.4895	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.7493	0.4192	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.7009	0.4668	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.9556	0.4678	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.1744	0.6204	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.4204	0.588	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.4029	0.6074	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	5.011	0.6427	2	
	(K)GYNAEIIEYVGDFIK(P)	95%	2.3717	0.3942	1	

1	(K)LAEGYETHLVGIK(N)	95%	2.1942	0.3274	2	
	(K)LAEGYETHLVGIK(N)	95%	2.5798	0.3497	2	
	(K)LAEGYETHLVGIK(N)	95%	2.8663	0.4825	2	
	(K)LAEGYETHLVGIK(N)	95%	3.1862	0.5564	2	
	(K)LAEGYETHLVGIK(N)	95%	3.9769	0.607	2	
	(K)NNNNEVIAAcLLTAVPVmK(V)	95%	2.7783	0.444	2	Carbamidomethyl (+57), Oxidation (+16)
	(K)NNNNEVIAAcLLTAVPVMK(V)	95%	4.4751	0.6618	2	Carbamidomethyl (+57)
	(K)NNNNEVIAAcLLTAVPVMK(V)	95%	5.198	0.6663	2	Carbamidomethyl (+57)
	(R)SFMEDTSESK(A)	95%	2.98	0.526	2	
	(R)VLVPLAYINFDEYIK(E)	95%	2.1637	0.4058	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.7607	0.5432	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.4194	0.5681	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.1299	0.584	2	
	(R)VLVPLAYINFDEYIK(E)	95%	4.4665	0.5973	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.7071	0.6168	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.9496	0.6376	2	
	(R)VLVPLAYINFDEYIK(E)	95%	5.6657	0.5093	2	
	(K)YFYSNR(G)	95%	1.7188	0.2977	2	
	(R)YHSVLDLK(D)	95%	2.7725	0.3038	2	
	(R)YHSVLDLK(D)	95%	2.1593	0.3874	2	
	(R)YHSVLDLK(D)	95%	2.6836	0.339	2	
	(R)YHSVLDLK(D)	95%	2.6355	0.3715	2	

1	(R)YHSVLDLK(D)	95%	2.6481	0.4437	2	
	(R)YHSVLDLKDK(T)	95%	2.9712	0.2809	2	
	(R)YHSVLDLKDK(T)	95%	2.8534	0.3419	2	
	(R)YNFYGVSGK(F)	95%	2.1597	0.3348	2	
	(R)YNFYGVSGK(F)	95%	2.579	0.2915	2	
2	(R)cLYLHIDPYLPYQYLNHDGEITGNAGNDWFFDK(M)	95%	4.9604	0.4881	2	Carbamidomethyl (+57)
	(R)cLYLHIDPYLPYQYLNHDGEITGNAGNDWFFDK(M)	95%	5.6683	0.5716	2	Carbamidomethyl (+57)
	(K)DKTADDIIKNmDGLR(K)	95%	1.486	0.2648	2	Oxidation (+16)
	(K)DKTADDIIKNmDGLR(K)	95%	2.0558	0.2306	2	Oxidation (+16)
	(R)DNLQQQLDANEQK(I)	95%	4.8525	0.5674	2	
	(K)EFGAFTDSMPYSHFTQTVGHYELK(L)	95%	2.1348	0.1951	2	
	(K)EFGAFTDSmPYSHFTQTVGHYELK(L)	95%	3.4357	0.3462	2	Oxidation (+16)
	(K)EFGAFTDSMPYSHFTQTVGHYELK(L)	95%	3.1482	0.4419	2	
	(K)EFGAFTDSMPYSHFTQTVGHYELK(L)	95%	3.6986	0.5801	2	
	(K)EFGAFTDSmPYSHFTQTVGHYELK(L)	95%	3.6818	0.466	2	Oxidation (+16)
	(K)EFGAFTDSMPYSHFTQTVGHYELK(L)	95%	3.2711	0.6005	2	
	(K)EFGAFTDSmPYSHFTQTVGHYELK(L)	95%	3.066	0.6581	2	Oxidation (+16)
	(K)EFGAFTDSmPYSHFTQTVGHYELK(L)	95%	3.3541	0.6738	2	Oxidation (+16)
	(K)EFGAFTDSmPYSHFTQTVGHYELK(L)	95%	3.6779	0.6655	2	Oxidation (+16)
	(K)EFGAFTDSMPYSHFTQTVGHYELK(L)	95%	3.941	0.644	2	
	(R)FLSEEELPIFR(S)	95%	3.3434	0.4481	2	
	(K)FTEDAEDAGVVK(F)	95%	3.6556	0.5552	2	
	(K)GFDPVLQIR(Y)	95%	2.1558	0.3908	2	
	(K)GFDPVLQIR(Y)	95%	2.6538	0.4621	2	

2	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	2.8833	0.3578	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	2.5109	0.4298	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.0011	0.4059	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.3627	0.4296	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.8485	0.5275	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.0203	0.6072	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.9642	0.569	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.823	0.6109	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.1175	0.6153	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.7188	0.6453	2	
	(K)GYNAEIIEYVGDFIKPINKPVYAAYTALK(K)	95%	2.6584	0.2757	2	
	(K)GYNAEIIEYVGDFIKPINKPVYAAYTALK(K)	95%	3.1766	0.2956	2	
	(K)GYNAEIIEYVGDFIKPINKPVYAAYTALK(K)	95%	3.0553	0.3406	2	
	(K)GYNAEIIEYVGDFIKPINKPVYAAYTALK(K)	95%	3.3628	0.3698	2	
	(R)LQEEHGNELPISAGFFFINPFEVVYYAGGTSNAFR(H)	95%	5.1463	0.6439	2	
	(R)LQEEHGNELPISAGFFFINPFEVVYYAGGTSNAFR(H)	95%	5.4601	0.6627	2	
	(R)LQEEHGNELPISAGFFFINPFEVVYYAGGTSNAFR(H)	95%	5.8179	0.6656	2	
	(K)NNNNEVIAAcLLTAVPVMK(V)	95%	5.1904	0.6139	2	Carbamidomethyl (+57)
	(K)NNNNEVIAAcLLTAVPVmK(V)	95%	5.3714	0.6972	2	Carbamidomethyl (+57), Oxidation (+16)
	(K)RPENKKAHNKR(D)	95%	2.3491	0.2663	2	

2	(K)RPENKKAHNKR(D)	95%	2.4444	0.3147	2	
	(R)SFMEDTSESK(A)	95%	2.9611	0.4574	2	
	(K)TADDIIK(N)	95%	1.6403	0.1862	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.2386	0.449	2	
	(R)VLVPLAYINFDEYIK(E)	95%	2.5745	0.5102	2	
	(R)VLVPLAYINFDEYIK(E)	95%	2.7703	0.5246	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.0678	0.537	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.0654	0.5516	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.8311	0.5749	2	
	(R)VLVPLAYINFDEYIK(E)	95%	4.1383	0.6544	2	
	(R)YHSVLDLK(D)	95%	2.7586	0.3014	2	
	(R)YHSVLDLK(D)	95%	2.1599	0.3408	2	
	(R)YHSVLDLK(D)	95%	2.594	0.3888	2	
	(R)YHSVLDLKDK(T)	95%	3.1783	0.2026	2	
	(R)YHSVLDLKDK(T)	95%	3.175	0.1957	2	
	(R)YHSVLDLKDK(T)	95%	3.2204	0.334	2	
3	(K)AFADRDDKFYYNR(L)	95%	2.8198	0.3435	2	
	(R)DILNKDLNKALKDIEKRPENK(K)	95%	2.6758	0.2435	2	
	(K)EFGAFTDSMPYSHFTQTVGHYELK(L)	95%	3.5262	0.6173	2	
	(K)FTEDAEDAGVVK(F)	95%	3.3968	0.1995	2	
	(K)FTEDAEDAGVVK(F)	95%	3.4317	0.4834	2	
	(K)GFDPVLQIR(Y)	95%	2.3464	0.3602	2	
	(K)GFDPVLQIR(Y)	95%	2.4475	0.4496	2	
	(K)GFDPVLQIR(Y)	95%	2.4586	0.4676	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.4057	0.4343	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.1382	0.5473	2	

3	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.9973	0.5943	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.9595	0.5722	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.361	0.6052	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.8252	0.585	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.7963	0.6287	2	
	(K)LAEGYETHLVGIK(N)	95%	4.0566	0.5588	2	
	(K)LAEGYETHLVGIK(N)	95%	3.6034	0.5628	2	
	(K)NNNNEVIAAcLLTAVPVmK(V)	95%	3.1047	0.4449	2	Carbamidomethyl (+57), Oxidation (+16)
	(K)NNNNEVIAAcLLTAVPVMK(V)	95%	5.6852	0.6614	2	Carbamidomethyl (+57)
	(R)SFMEDTSESK(A)	95%	3.2099	0.435	2	
	(K)TADDIIK(N)	95%	1.8398	0.2487	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.1494	0.492	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.2263	0.61	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.5314	0.5801	2	
	(R)VLVPLAYINFDEYIK(E)	95%	4.1606	0.5975	2	
	(R)VLVPLAYINFDEYIK(E)	95%	4.2587	0.5777	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.999	0.6302	2	
	(R)YHSVLDLK(D)	95%	2.8819	0.3296	2	
	(R)YHSVLDLK(D)	95%	2.7474	0.259	2	
	(R)YHSVLDLK(D)	95%	2.3374	0.3272	2	
	(R)YHSVLDLK(D)	95%	2.608	0.44	2	
	(R)YHSVLDLK(D)	95%	2.4222	0.4121	2	
	(R)YHSVLDLKDK(T)	95%	2.8193	0.1289	2	

3	(R)YHSVLDLKDK(T)	95%	2.6737	0.3742	2	
	(R)YNFYGVSGK(F)	95%	2.3811	0.4708	2	
	(R)YNFYGVSGK(F)	95%	2.4428	0.4147	2	
4	(K)AFADRDDKFYYNR(L)	95%	2.6609	0.3324	2	
	(K)AFADRDDKFYYNR(L)	95%	3.2954	0.3728	2	
	(K)DKTADDIIK(N)	95%	1.9979	0.367	2	
	(K)EFGAFTDSmPYSHFTQTVGHYELK(L)	95%	2.883	0.2016	2	Oxidation (+16)
	(K)EFGAFTDSMPYSHFTQTVGHYELK(L)	95%	2.6429	0.2106	2	
	(K)EFGAFTDSmPYSHFTQTVGHYELK(L)	95%	2.6328	0.2342	2	Oxidation (+16)
	(K)EFGAFTDSmPYSHFTQTVGHYELK(L)	95%	3.0369	0.4198	2	Oxidation (+16)
	(K)EFGAFTDSMPYSHFTQTVGHYELK(L)	95%	2.9496	0.4296	2	
	(K)EFGAFTDSMPYSHFTQTVGHYELK(L)	95%	3.3198	0.5568	2	
	(K)EFGAFTDSmPYSHFTQTVGHYELK(L)	95%	3.4939	0.6152	2	Oxidation (+16)
	(K)EFGAFTDSmPYSHFTQTVGHYELK(L)	95%	3.3684	0.6631	2	Oxidation (+16)
	(K)ELNEERDILNK(D)	95%	3.8304	0.2806	2	
	(R)FLSEEELPIFR(S)	95%	2.7851	0.378	2	
	(R)FLSEEELPIFR(S)	95%	2.4963	0.4163	2	
	(K)FTEDAEDAGVVK(F)	95%	1.9959	0.3554	2	
	(K)FTEDAEDAGVVK(F)	95%	3.5358	0.6532	2	
	(K)FTNLTAK(E)	95%	1.2302	0.2692	2	
	(K)FTNLTAK(E)	95%	1.5787	0.3538	2	
	(K)GFDPVLQIR(Y)	95%	2.4967	0.3163	2	
	(K)GFDPVLQIR(Y)	95%	2.2507	0.3845	2	
	(K)GFDPVLQIR(Y)	95%	2.1413	0.4036	2	
	(K)GFDPVLQIR(Y)	95%	2.2844	0.3904	2	
	(K)GFDPVLQIR(Y)	95%	2.9538	0.3921	2	

4	(K)GFDPVLQIR(Y)	95%	2.5997	0.4461	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	2.8412	0.2401	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	2.4667	0.3131	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.3666	0.3424	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	2.7831	0.4242	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.9852	0.4634	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.8669	0.4886	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.5689	0.4267	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.2907	0.4896	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.0903	0.574	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.9814	0.593	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.5542	0.5676	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.9852	0.5869	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.1655	0.6354	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.9709	0.7031	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.4741	0.6637	2	
	(K)GYNAEIIEYVGDFIKPINKPVYAAYTALK(K)	95%	2.7481	0.2927	2	
	(K)LAEGYETHLVGIK(N)	95%	1.9918	0.2948	2	
	(K)LAEGYETHLVGIK(N)	95%	1.9897	0.4241	2	
	(K)LAEGYETHLVGIK(N)	95%	3.696	0.5184	2	
	(K)LAEGYETHLVGIK(N)	95%	3.9148	0.5356	2	
	(K)NmDGLRKRNTKK(V)	95%	2.4187	0.2219	2	Oxidation (+16)
	(K)NNNNEVIAAcLLTAVPVMK(V)	95%	4.5889	0.4561	2	Carbamidomethyl (+57)

4	(K)NNNNEVIAAcLLTAVPVmK(V)	95%	4.4442	0.6011	2	Carbamidomethyl (+57), Oxidation (+16)
	(K)NNNNEVIAAcLLTAVPVMK(V)	95%	4.0564	0.6292	2	Carbamidomethyl (+57)
	(R)SFmEDTSESK(A)	95%	1.9839	0.3084	2	Oxidation (+16)
	(R)SFMEDTSESK(A)	95%	2.5511	0.4464	2	
	(R)SFMEDTSESKAFADRDDKFYYNRLK(Y)	95%	2.3005	0.2497	2	
	(K)TADDIIK(N)	95%	1.848	0.3038	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.8289	0.4453	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.3308	0.5202	2	
	(R)VLVPLAYINFDEYIK(E)	95%	4.0778	0.5866	2	
	(R)VLVPLAYINFDEYIK(E)	95%	4.1995	0.6168	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.8784	0.6191	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.8846	0.6358	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.8743	0.6169	2	
	(R)VLVPLAYINFDEYIK(E)	95%	4.0255	0.6248	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.8444	0.6269	2	
	(R)VLVPLAYINFDEYIK(E)	95%	4.645	0.6203	2	
	(R)YHSVLDLK(D)	95%	2.1603	0.4056	2	
	(R)YHSVLDLK(D)	95%	2.2131	0.4164	2	
	(R)YHSVLDLK(D)	95%	3.1015	0.3761	2	
	(R)YHSVLDLKDK(T)	95%	3.3904	0.4295	2	
5	(K)EFGAFTDSMPYSHFTQTVGHYELK(L)	95%	2.8076	0.3451	2	
	(K)EFGAFTDSmPYSHFTQTVGHYELK(L)	95%	3.8533	0.4398	2	Oxidation (+16)
	(K)EFGAFTDSMPYSHFTQTVGHYELK(L)	95%	3.8167	0.5562	2	

5	(K)EFGAFTDSmPYSHFTQTVGHYELK(L)	95%	3.7671	0.4996	2	Oxidation (+16)
	(K)EFGAFTDSmPYSHFTQTVGHYELK(L)	95%	3.0922	0.6498	2	Oxidation (+16)
	(K)EFGAFTDSMPYSHFTQTVGHYELK(L)	95%	3.6848	0.594	2	
	(K)EFGAFTDSmPYSHFTQTVGHYELK(L)	95%	3.5795	0.6809	2	Oxidation (+16)
	(R)FLSEEELPIFR(S)	95%	2.0485	0.3769	2	
	(R)FLSEEELPIFR(S)	95%	2.7497	0.4121	2	
	(K)FTEDAEDAGVVK(F)	95%	1.9638	0.4533	2	
	(K)GFDPVLQIR(Y)	95%	2.1422	0.3535	2	
	(K)GFDPVLQIR(Y)	95%	2.1592	0.4089	2	
	(K)GFDPVLQIR(Y)	95%	2.6116	0.4633	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	2.6922	0.1972	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	1.7411	0.2801	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.1907	0.2819	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	2.5757	0.4736	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.0204	0.4715	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.9776	0.5301	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.9382	0.5711	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.4929	0.4983	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.5438	0.5409	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.3158	0.6045	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.942	0.5477	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.3527	0.5304	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.4723	0.5824	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.8811	0.5919	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.3781	0.6364	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.6041	0.6651	2	

5	(K)LAEGYETHLVGIK(N)	95%	3.9174	0.577	2	
	(K)LAEGYETHLVGIK(N)	95%	3.8285	0.568	2	
	(K)LAEGYETHLVGIK(N)	95%	2.6841	0.5416	2	
	(K)LAEGYETHLVGIK(N)	95%	2.9621	0.5854	2	
	(R)LQEEHGNELPISAGFFFINPFEVVYYAGGTSNAFR(H)	95%	3.7704	0.2389	2	
	(K)NNNNEVIAAcLLTAVPVmK(V)	95%	2.0647	0.304	2	Carbamidomethyl (+57), Oxidation (+16)
	(K)NNNNEVIAAcLLTAVPVMK(V)	95%	4.9015	0.5321	2	Carbamidomethyl (+57)
	(K)NNNNEVIAAcLLTAVPVMK(V)	95%	5.5906	0.6454	2	Carbamidomethyl (+57)
	(K)NNNNEVIAAcLLTAVPVmK(V)	95%	5.4649	0.6472	2	Carbamidomethyl (+57), Oxidation (+16)
	(K)PVYAAYTALK(K)	95%	2.3179	0.5104	1	
	(K)RPENKKAHNKR(D)	95%	2.3335	0.3519	2	
	(R)SFMEDTSESK(A)	95%	2.1611	0.4715	2	
	(R)SFMEDTSESK(A)	95%	3.2271	0.4827	2	
	(R)SFMEDTSESKAFADRDDKFYYNRLK(Y)	95%	2.4895	0.2766	2	
	(R)VLVPLAYINFDEYIK(E)	95%	2.1824	0.4472	2	
	(R)VLVPLAYINFDEYIK(E)	95%	2.9151	0.5825	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.8551	0.6046	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.9115	0.6095	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.841	0.6573	2	

5	(R)VLVPLAYINFDEYIK(E)	95%	4.7115	0.4921	2	
	(R)VLVPLAYINFDEYIK(E)	95%	5.3317	0.4697	2	
	(R)YHSVLDLK(D)	95%	2.9153	0.3376	2	
	(R)YHSVLDLK(D)	95%	2.5794	0.2597	2	
	(R)YHSVLDLK(D)	95%	2.0959	0.3878	2	
	(R)YHSVLDLK(D)	95%	2.3946	0.3359	2	
	(R)YHSVLDLK(D)	95%	2.3117	0.4447	2	
	(R)YHSVLDLK(D)	95%	2.6753	0.4349	2	
	(R)YNFYGVSGK(F)	95%	2.1073	0.3088	2	
	(R)YNFYGVSGK(F)	95%	2.2127	0.4321	2	
6	(K)AFADRDDKFYYNR(L)	95%	2.8946	0.3241	2	
	(K)DIEKRPENKKAHNKRDNLQQQLDANEQK(I)	95%	2.5067	0.2803	2	
	(K)EFGAFTDSMPYSHFTQTVGHYELK(L)	95%	2.7334	0.3898	2	
	(K)EFGAFTDSMPYSHFTQTVGHYELK(L)	95%	2.9545	0.418	2	
	(K)EFGAFTDSmPYSHFTQTVGHYELK(L)	95%	3.7106	0.4329	2	Oxidation (+16)
	(K)EFGAFTDSMPYSHFTQTVGHYELK(L)	95%	3.8545	0.5762	2	
	(R)FLSEEELPIFR(S)	95%	3.4808	0.3861	2	
	(K)FTEDAEDAGVVK(F)	95%	2.2151	0.5087	2	
	(K)FTEDAEDAGVVK(F)	95%	3.9413	0.5577	2	
	(K)FTNLTAK(E)	95%	1.6276	0.3672	2	
	(K)GFDPVLQIR(Y)	95%	2.4032	0.3978	2	
	(K)GFDPVLQIR(Y)	95%	2.9894	0.4362	2	
	(K)GFDPVLQIR(Y)	95%	2.5624	0.4576	2	
	(K)GFDPVLQIR(Y)	95%	2.7795	0.4635	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	2.182	0.2914	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	2.7318	0.3854	2	

6	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	2.9076	0.402	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	2.8712	0.5428	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.7394	0.5278	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.2636	0.4923	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.4504	0.4695	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.6661	0.5785	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	3.9738	0.5813	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.2002	0.5993	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.4892	0.6225	2	
	(R)GPVIDYENQELVHFFFNELSK(Y)	95%	4.4412	0.6604	2	
	(K)LAEGYETHLVGIK(N)	95%	1.8676	0.5717	2	
	(K)LAEGYETHLVGIK(N)	95%	3.1284	0.5389	2	
	(K)LAEGYETHLVGIK(N)	95%	2.6081	0.5021	2	
	(K)LAEGYETHLVGIK(N)	95%	4.0605	0.5483	2	
	(K)LAEGYETHLVGIK(N)	95%	3.5461	0.5956	2	
	(K)LAEGYETHLVGIK(N)	95%	2.3948	0.6071	2	
	(K)NNNNEVIAAcLLTAVPVMK(V)	95%	4.3057	0.6365	2	Carbamidomethyl (+57)
	(K)NNNNEVIAAcLLTAVPVMK(V)	95%	5.2522	0.6737	2	Carbamidomethyl (+57)
	(R)SFMEDTSESK(A)	95%	2.7233	0.5283	2	
	(K)TADDIIK(N)	95%	1.5545	0.1994	2	
	(R)VLVPLAYINFDEYIK(E)	95%	2.3536	0.3564	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.7383	0.5229	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.5922	0.5891	2	
	(R)VLVPLAYINFDEYIK(E)	95%	3.75	0.6443	2	

6	(R)VLVPLAYINFDEYIK(E)	95%	4.2015	0.6522	2	
	(R)VLVPLAYINFDEYIK(E)	95%	5.1525	0.4842	2	
	(R)YHSVLDLK(D)	95%	2.7918	0.4518	2	
	(R)YHSVLDLK(D)	95%	2.6475	0.3004	2	
	(R)YHSVLDLK(D)	95%	2.5334	0.4405	2	
	(R)YHSVLDLK(D)	95%	2.8394	0.3679	2	
	(R)YHSVLDLKDK(T)	95%	3.3734	0.2244	2	
	(R)YNFYGVSGK(F)	95%	1.7362	0.5057	2	

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