Probing Cell Membrane And Biofilm Extracellular Matrix Interactions With Tumor Necrosis Factor-Alpaha and Interleukin-6 Through Molecular Dynamics Simulations Using A Detailed Chemical Description

Stephen DeSalvo
Probing Cell Membrane And Biofilm Extracellular Matrix Interactions With Tumor Necrosis Factor-Alpha and Interleukin-6 Through Molecular Dynamics Simulations Using A Detailed Chemical Description

A Capstone Project Submitted in Partial Fulfillment of the Requirements of the Renée Crown University Honors Program at Syracuse University

Stephen DeSalvo
Candidate for B.S. Chemical Engineering Degree and Renée Crown University Honors
May 2014

Honors Capstone Project in Chemical Engineering

Capstone Project Advisor: _______________________
Radhakrishna Sureshkumar, Dept. Chair & Professor

Capstone Project Reader: _______________________
Shikha Nangia, Assistant Professor

Honors Director: _______________________
Stephen Kuusisto, Director

Date: April 23, 2014
Abstract

Bacterial biofilms are a major cause of persistent infections and diseases with known antibiotic and host immune defense resistances. The interaction of signaling factors, namely small cytokines, with the biofilm and host cell is considered vital to the survival of bacterial biofilms. In this study, molecular dynamics (MD) simulations are performed using coarse-grained biomolecular systems to provide significant insight into medical therapeutic advancements in treating persistent and chronic infections. Specifically, MD simulations of the interaction between signaling factors tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) with a model biofilm matrix with detailed description were performed. Additional simulations were used to study TNF-α interaction with dextran polymer chains of varying monomer length. In each study, resultant potential of mean force curves were analyzed to quantify the energy associated with translocation of the signaling factors through the extracellular polymeric substances, as well as to highlight the effect of chain degradation on this translocation process. In some instances, polymer intramatrix interactions were studied to better understand the translocation energy findings. The translocation of TNF-α across a lipid bilayer was also performed using MD simulation techniques, and the resultant potential of mean force curve highlights the energy barrier associated with this process. Qualitative and quantitative assessment of membrane damage was also performed.
# Table of Contents

Abstract.........................................................................................................ii

1. Executive Summary .................................................................................. 1

2. Acknowledgements ................................................................................... 7

3. Advice to Future Honors Students .......................................................... 8

4. Introduction.................................................................................................. 9
   Bacterial Biofilms......................................................................................... 9
   Signaling Factors: Tumor Necrosis Factor-Alpha and Interleukin-6 13
   Molecular Dynamics Simulations................................................................. 14
   Research Goals and Objectives................................................................. 16

5. Computational Methodology....................................................................... 18
   Coarse Graining of Signaling Factor Cytokines and Matrix Proteins 18
   Lipid Bilayer Simulations........................................................................... 19
   Biofilm Matrix – TNF-α Simulations......................................................... 22
   Biofilm Matrix – IL-6 Simulations............................................................... 29

6. Results........................................................................................................... 31
   Lipid Bilayer Simulations........................................................................... 31
   Biofilm Matrix – TNF-α Simulations......................................................... 34
   Biofilm Matrix – IL-6 Simulations............................................................... 39

7. Discussion..................................................................................................... 42
   Lipid Bilayer Simulations........................................................................... 42
   Biofilm Matrix – TNF-α Simulations......................................................... 44
   Biofilm Matrix – IL-6 Simulations............................................................... 50

8. Conclusions................................................................................................. 52
   Simulation Summation............................................................................... 52
   Future Work............................................................................................... 54

9. References.................................................................................................... 55

10. Appendices.................................................................................................. 60
1. Executive Summary

Undoubtedly, the human body is one of the most complex scientific puzzles given the vast network of biological systems and component molecular structures. Many of the complexities that perplex so many are associated with interactions that happen in the body at a very small scale. This small scale is known as the “nano-scale.” Advances in medicine that target interactions on this level are grouped into a field of study appropriately termed “nanomedicine.”

One of the most important areas of study in nanomedicine is the development of therapeutic treatments for diseases that currently have no cure or treatment, or that are simply resistant to drug treatment. The efficacy of diseases has continually been a concern for medical research since the inception of chemical treatments to medical ailments. Diseases such as rheumatoid arthritis, a chronic inflammatory disorder most commonly characterized by severe joint pain and bodily tissue deterioration, and cystic fibrosis, a chronic disease of the lungs causing consistent coughing and irritation, are two such chronic diseases for which research in nanomedicine is ideally suited. Associated with many chronic diseases are bacterial biofilms, which are colonies of bacteria known to irreversibly adhere to nearly any surface including many in the body. These adherent bacteria secrete a thick matrix of polymer that effectively provides a protective barrier over the bacteria creating a “biofilm.” This complex matrix is known to make bacterial biofilms resistant to therapeutics. Therefore, it is clear
that biofilm matrices are of important interest to studies in the area of nanomedicine.

In order to solicit key nutrients and carry out key cellular functions, body cells excrete signaling factors to the surrounding environment. Many signaling factors (SF) are key to initiating an immune response to disease, including both tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). Tumor necrosis factor-alpha is a pro-inflammatory cytokine released by cells in response to some perceived attack on the cells. It is known to be associated with sepsis, cystic fibrosis, and rheumatoid arthritis. Similarly, interleukin-6 is both a pro-inflammatory and anti-inflammatory cytokine released by some cells, and it is known to be associated with such diseases and ailments as depression and rheumatoid arthritis. It is imperative for these signaling factors to make their way out of the host cell within the human body in order to solicit immune system support in response to a perceived infection. In the case where bacterial biofilms have formed on the surface of body cells, for instance along the lining of blood vessels or in the lungs, these signaling factors must travel through the cell membrane and then through the bacterial biofilm matrix before making their way to an immune system helper cell. Insight into this movement process, which is known as a translocation process, for TNF-α and IL-6 SFs may provide valuable information for the development of future therapeutics capable of treating chronic diseases and infections associated with bacterial biofilms. Given that some studies indicate as many as 90% of known infections involve the presence of bacterial biofilms to some degree, research in this area is of significant importance.
To study the translocation of these signaling factors through a basic host cell membrane and a bacterial biofilm matrix (EPS), computational models were developed that relied on the primary structural components of each. For the host cell membrane, the primary component is the lipid bilayer which forms as a result of surfactant molecules that have hydrophilic (water-loving) head groups and hydrophobic (water-resistant) tails. The lipid of choice for this model is known as a distearoylphosphatidylcholine (DSPC) lipid molecule. For the biofilm matrix, the main components include polysaccharide chains, which are also known as polymers, nucleic acids, such as DNA, and proteins, all in a water solution. These computational models can then be simulated interacting with the TNF-α and IL-6 SFs using molecular dynamics simulations.

Molecular dynamics simulations are computer-based simulations that solve Newton’s equation of motion numerically over time. By calculating the forces on each atom of known mass in a given system, the computer is able to derive the spatial coordinates, or position, of each atom over time. In essence, the computer can simulate the movements of atoms interacting with each other over a specified time scale. Using these molecular dynamics simulations, nanoscale dynamics can be studied and the interactions can be quantified using known equations underlying common transport phenomena. Of most interest in the molecular dynamics simulations studied here are the potential of mean force (PMF) curves for the translocation of SF molecules through the host cell membrane and through the biofilm matrix. The potential of mean force curve represents, essentially, the change in free energy over a reaction coordinate, or in
this case, over the pathway travelled by the SF molecule in moving from inside
the host cell to a location outside of the bacterial biofilm on the surface of the host
cell.

The free energy, known to chemists and chemical engineers as the Gibbs
free energy (ΔG), is representative of the spontaneity and favorability of a given
process. The free energy quantifies the difference in energy for a system at some
state A (the SF inside the host cell) and some state B (the SF outside the host
cell). Given that every system strives to be at the lowest possible energy, positive
values of ΔG indicate the requirement of energy input in order for a given process
to occur and negative values of ΔG indicate that the process will happen
spontaneously without energy input to the system. Therefore, the potential of
mean force curves extracted from molecular dynamics simulations provide a form
of energetic analysis quantifying the energy barrier (for positive values of ΔG) or
the stabilization energy (for negative values of ΔG) associated with the
translocation process. Furthermore, and more generally, the PMF curves provide
some insight into the favorability and likelihood of a given process occurring
naturally.

In experiments studied for this project, molecular dynamics simulations
were performed on four main types of systems in order to gain insight into the
energetics associated with TNF-α and IL-6 SF translocation processes. The first
system was TNF-α moving through a lipid bilayer that represents a cell
membrane. The second system was TNF-α moving through dextran polymer
chains (a basic polysaccharide), representing a generalized and basic biofilm
matrix. The third system was TNF-α moving through a more detailed biofilm matrix modeled using dextran polymer chains, amylase proteins, and DNA strands. The final system was a repeated study of the third system using the IL-6 SF rather than TNF-α. In each of these studies, PMF curves were extracted to determine the free energy along the reaction coordinates.

To delve deeper into the analysis of the SF-EPS interactions, the effect of chain deterioration or “polymer chain chopping” was analyzed by simulating systems with different chain length polymers. Given that this chain degradation is an observed phenomena in bacterial biofilm matrices, it is of interest to study its effects on the translocation of SF molecules. Comparison of the PMF curves for the various systems of different polymer chain lengths allowed for analysis of the change in the ease of translocation over time as the polymer in the EPS breaks down. Furthermore, additional analyses were conducted on the simulated systems to highlight possible explanations for the observed PMF results. These additional analyses involved a quantitative and qualitative analysis of the polymer interaction with the SF molecules embedded within the matrix.

The results of the molecular dynamics simulations demonstrate, for the first time, quantifiable energies associated with the translocation of SFs through host cell membranes and model bacterial biofilm matrices. Furthermore, the studies demonstrate clear relationships between SF translocation process favorability and the polymer chain length of the bacterial biofilm matrix. Comparison of TNF-α and IL-6 simulations allow for conclusions to be drawn regarding the effect of SF diameter on the ease of translocation.
The computational studies conducted provide valuable insight into the mechanisms by which human cells secrete SFs to the body’s immune system. Although it is known that SF translocation through the cell membrane is an unfavorable process, which is verified by the simulations conducted in this study, and that adenosine triphosphate (ATP) energy molecules (or packets of energy supporting signal movement) must be used to assist in the translocation process, it was previously unknown as to how many ATP molecules were required. This study provides an answer. Furthermore, by understanding the energy landscape associated with translocation mechanisms while taking into account so many parameters in each of the four main studies, great advancements in therapeutics that can take advantage of the encouraging energy results may finally prove successful in ending chronic infections and overcoming the drug resistance of bacterial biofilms.
2. Acknowledgements

A special thank you is given to my advisor and mentor, Dr. Radhakrishna Sureshkumar. Through his guidance in chemical engineering and molecular dynamics simulation studies, I have grown immensely in ability and motivation. I also give a special thank you to Dr. Shikha Nangia for her continual support of my research on bacterial biofilms, for her assistance in troubleshooting simulation challenges, and for her innovative ideas that propelled my research towards results. I acknowledge graduate student Abhinanden Sambasivam for his guidance with code writing and complex simulation techniques.
3. Advice to Future Honors Students

As an engineering student, your Honors experience is sure to be unique in comparison to students from nearly all other backgrounds. The experience will be just as rewarding for you as it is for all other students, perhaps even more so, but the need to start early is pivotal. My primary piece of advice is to start freshman year in an engineering laboratory of interest to begin research and experimentation. After just a year, I assure you that numerous ideas will become apparent as to what you may want to study for your capstone project. From there, it is smooth sailing (relatively speaking, of course).

I found that many of my friends from other majors struggled most with finding a reasonable topic, as well as with “getting started”. As a student who started working in the area in which I knew I would end up conducting my research for my capstone project, this was not an issue. In fact, over the many years in which I worked in the lab, I found that the studies and research I conducted were useful in laying the foundation for my capstone work.

I strongly believe that you will get out of the Honors Program exactly what you put into it. I did not find that I had many more opportunities than other students outside of the Honors Program, generally, until I began to utilize the structure it sets up for academic success. To me, the Honors Program enables students to go into greater depth on topics of particular interest. Know why you are joining the program, and be prepared to motivate yourself to reach the objectives and goal you originally set when you became a member. The experience is certainly worthwhile if you are prepared for the exciting challenges it will present.
4. Introduction

Bacterial Biofilms

The human body is one of the most, if not the most, complex and intricate systems on Earth from numerous scientific standpoints. Of significant interest to many are the chemical structures that exist and chemical processes that occur within the human body, and most specifically, the micro- and nano-scale structures associated with cellular and atomic biochemical interactions. The study of such chemical structures and processes at the molecular level is essential to medical advancements associated with leading ailments, diseases, and infections. As a result, nanomedicine has grown significantly as its potential usefulness in mediating molecular interactions and aiding therapeutic agent transport through biological barriers in the human body has become apparent.\(^1\)\(^2\) The future of therapy development for human disease and infections lies in better understanding of human structures and cellular functions or interactions at the nano-scale level.

Nanomedicine has the potential to aid significant medical breakthroughs in those diseases and infections that are most troublesome, namely those that are persistent, chronic, or for which cures have not been established. Among the most involved components of persistent infections are bacteria. Planktonic bacteria aggregate on solid surfaces and become reversibly attached. Irreversible attachment begins when the bacterial community secretes a protective barrier of extracellular polymeric substances (EPS) that effectively encases the bacteria.\(^3\) This effectively establishes what is known as a bacterial biofilm.\(^4\)\(^5\) Not only is the development of bacterial biofilms in host tissue a major cause of infection at the
molecular level, but many biofilms have been associated with persistent and chronic infections. Biofilms are characterized as causing persistent inflammation and tissue damage. The nature of the biofilm life cycle, depicted in Figure 1 from a 2009 journal article by Richards and Melander, contributes in part to biofilm persistence. The ability of aggregates of bacteria to provide their own protective EPS matrix to afford growth and maturation ultimately leads to bacterial dispersion for colonization of nearby surfaces. At this time, the biofilm life cycle restarts again with aggregation and reversible attachment.

Bacterial biofilms have been known to establish themselves on nearly any natural surface including medical devices such as pacemakers, artificial heart valves, and catheters, on teeth in the form of dental plaque, and on human cardiac tissue and valves. In many realms, biofilm formation has been linked to opportunistic pathogens leading many to view biofilms as a virulence factor providing bacteria with a strategy for contributing to and sustaining infection.
Overall, the concern with bacterial biofilms rests in large part on their known resistance to antibiotics, some host immune defenses, and medical treatment.\textsuperscript{4,5,9} There are numerous theories characterizing the mechanism by which biofilms maintain such broad resistance, but the theory of persister cells appears to be among the most credible and logical. In a review of persister cells by Lewis, research is highlighted showing that antibiotics actually kill a majority of the cells living within the biofilm.\textsuperscript{10} However, as the antibiotic concentration diminishes over time, dormant persister cells become active to rebuild the bacterial colony and invoke infection relapses.\textsuperscript{10} Therefore, the biofilm community, most notably the EPS matrix providing a protective barrier to the underlying bacteria, is viewed generally as a safe haven for persister cells. Given this theory, it is reasonable to understand why many suggest that mechanisms focused on inducing biofilm detachment from natural surfaces may improve biofilm therapeutics and antibiotics.\textsuperscript{11-13}

An important consideration in studying biofilms is a detailed understanding of the components of the extracellular polymeric substance that effectively forms the biofilm matrix. The predominant component of the biofilm matrix, up to 97\%, is water.\textsuperscript{14} Studies have determined the main solid and semi-solid components to be polysaccharides (1-2\%), proteins (<2\%), and nucleic acids such as DNA and RNA (<2\%).\textsuperscript{14} Other trace components can be found in varying quantities between different biofilm strains in different environments. Polysaccharides are considered the most essential component of the biofilm, with proposed functions including promotion of cell adhesion to surfaces, protection of attached cells
within close proximity to one another, entrapment of toxic ions, storage of essential nutrients, and functional defenses against cells of the host immune system. Specific types of polymers, such as dextrans with 1,6-α-linkages, have been found in the typical biofilm EPS. Proteins in the biofilm matrix consist of both enzyme proteins and structural proteins. While structural proteins are involved with the formation and stabilization of the polysaccharide matrix, enzyme proteins such as polysaccharases have been identified as the source of polysaccharide chain degradation by systematically breaking polymer linkages. This enzyme action may severely threaten the biofilm integrity by attacking the ability of the EPS to sustain bacterial surface attachment. Although the source of extracellular DNA is debatable, the DNA components of the EPS have been cited for their role in assisting biofilm formation. Any study of the biofilm matrix should give particular attention to the polysaccharide, protein, and DNA components in aqueous solution.

In summation, bacterial biofilms have been described and identified as a culprit for persistent and chronic infections. The components of the protective matrix known as an EPS have been characterized by researchers highlighting opportunities for medical advancements in eliminating or proactively preventing the formation of biofilms. Clearly, there exist opportunities for further research into biofilm structures that focus on characterization of communication mechanisms between the host cell, the environment, and biofilms which effectively allow biofilms to sustain themselves.
**Signaling Factors: Tumor Necrosis Factor-Alpha and Interleukin-6**

Bacterial biofilm survival depends largely on its interaction with host cells through chemicals known as signaling factors. Cytokines, which are soluble proteins that provide cellular communication mechanisms providing a wide range of functions,\(^1\) are a dominant type of signaling factor believed to interact with biofilms and the host cells. Intercellular signaling molecules have profound effects on biofilm growth and studies have found them to facilitate differentiation of individual cells into complex multicellular structures.\(^2\) Two signaling factors of particular interest are the cytokines tumor necrosis factor-alpha (TNF-\(\alpha\)) and interleukin-6 (IL-6), each of which is considered proinflammatory in nature.

Both the TNF-\(\alpha\) and IL-6 cytokines have been noted for their importance in initiating primary aspects of the host defense response to infection.\(^3\) Gogos et al. confirmed the involvement of TNF-\(\alpha\) and IL-6 in immune responses to sepsis and septic shock. High levels of TNF-\(\alpha\) were found in patients with severe sepsis, especially non-survivors, and it is also predicted that early prediction of septic shock can be identified by conjointly high levels of TNF-\(\alpha\) and IL-6.\(^4\) In studying rheumatoid arthritis, it has been determined that TNF-\(\alpha\) and its associated pro-inflammatory response plays an important role in the pathogenesis of the disease.\(^5\) IL-6 has also been implicated for prolonging inflammatory responses in rheumatoid arthritis patients, causing chronic inflammation.\(^6\) High levels of TNF-\(\alpha\) and IL-6 proinflammatory cytokines have been found in cystic fibrosis patients, a debilitating and chronic lung disease.\(^7\) Involvement of these signaling factors in many other diseases and infections have been noted.\(^8-10\)
It has been determined that cell-to-cell signal inhibition could aid in biofilm treatment,\(^{20}\) which makes the study of signaling factor interaction with biofilm and host cell structures of particular interest to the medical field. Other hypotheses suggesting the ability of the biofilm matrix to serve as a sink sequestering signaling molecules also makes studying the interaction between signaling molecules and EPS important.\(^{29}\) Prior research is conclusive in its suggestion that the study of TNF-\(\alpha\) and IL-6 signaling factor interaction with the host cell and bacterial biofilm could provide significant insight into broad medical treatments.

**Molecular Dynamics Simulations**

Molecular dynamics (MD) are computer simulations that numerically solve Newton’s laws of motion to allow analysis of complex phenomena on nanosized length scales over picosecond to microsecond timescales. MD simulations provide a unique tool for verification of laboratory experiments and extrapolation from laboratory results. Using this *in silico* technique, researchers are able to study interactions and analyze findings that are not accessible in the lab setting. This is particularly important in nanotechnology where the study of small-scale interactions that are difficult to conduct in a typical laboratory setting is easily completed using simulation techniques.

Lemkul and Bevan have demonstrated an ability to assess the stability of amyloid \(\beta\)-peptide (A\(\beta\)) involved with Alzheimer’s disease using molecular dynamics simulations by determining for the first time the binding free energies
associated with the Aβ protofibril. In another study, Lemkul and Bevan showed how molecular dynamics could be utilized to determine the free energy associated with protein extraction from a palmitoyloleoylphosphatidylethanolamine (POPE) lipid membrane. A plethora of simulations have been conducted involving nanoparticle translocation across lipid bilayer membranes of various types, including dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DMPC), dimyristoylphosphatidylethanolamine (DMPE), and dioleoylphosphatidylcholine (DOPC) lipids. In each instance, the authors relate their results to important medical advancements, namely insight into drug delivery enhancement. The wealth of simulation history and capabilities highlights the importance of using molecular dynamics to advance the field of nanomedicine. As many have already noted, research into drug delivery hinges on developing nanoparticulate systems capable of preventing, controlling, and treating diseases.

One major limitation in molecular dynamics is computational power. With increasing system size, the required computation time increases exponentially as the number of interactions that must be calculated increases. This limitation is particularly evident when conducting atomistic simulations of biomolecular systems, which when solvated frequently exceed 150,000 atoms. Such systems, with their innate complexity, encounter a prohibitive bottleneck when simulated under the all-atom description. As a result, many have begun to utilize a technique known as coarse graining to alleviate these computational issues. Using the MARTINI framework, four-heavy (non-hydrogen) atoms are mapped into a single
coarse-grained (CG) bead. Several advantages arise from using this computational methodology including reduction of the number of degrees of freedom in the system and an ability to simulate using larger timesteps. The ultimate result is decreased computational cost and faster simulations. As shown in the recent work of Sangwai and Sureshkumar on shape and phase transitions in surfactant micelles, and by Nangia and Sureshkumar on the effect of charge and shape anisotropy on the translocation of nanoparticles through cell membranes, large systems can be simulated using MD under the MARTINI framework without loss of essential chemical description.

There is currently no research involving molecular dynamics simulations of bacterial biofilms or their associated interactions with signaling factors. Given the power of MD simulations as an experimental tool for probing translocation processes, a detailed study of host cell, bacterial biofilm, and signaling factor interactions may provide results with broad implications on drug therapies, specifically drug delivery.

**Research Goals and Objectives**

The primary goal of this study is to provide significant insight for the first time into the interaction between signaling factors, bacterial biofilms, and the host cell in order to direct future drug delivery and infection therapeutic solutions. A multitude of objectives are presented throughout this study. One such objective is to assemble useful model biofilm matrices based on prior research of predominant EPS components. Second, atomistic signaling factors are coarse-grained to allow
the simulation of large biomolecular systems on effective timescales. Third, *in silico* experimentation is utilized to quantify the energy associated with the interaction of TNF-α with a distearoylphosphatidylcholine (DSPC) lipid bilayer, a generalized biofilm matrix, and a detailed biofilm matrix. Additional studies are conducted to assess the interaction of IL-6 with a detailed biofilm matrix. Potential of mean force calculations are used to quantitatively describe, for the first time, the free energy profile associated with signaling factor interaction with the host cell and biofilm matrix. Furthermore, qualitative and quantitative assessment of membrane deformation is provided for all simulations involving the lipid bilayer.

Studies indicate that polysaccharide chain degradation through the action of polysaccharase may have altering effects on biofilm integrity and signaling factor mobility.\textsuperscript{7,11,12,17,42} Therefore, within the MD simulations of both the generalized and detailed biofilm matrices, specific attention is given to the effect of polymer chain length on the translocation process in order to simulate the degradation effect caused by extracellular proteins in the biofilm EPS. Novel methods are developed to allow the results from these MD simulations to effectively test the hypotheses only “proposed” thus far by previous researchers.
5. Computational Methodology

All molecular dynamics simulations were performed using the GROMACS package, versions 4.0.3 and 4.5.4. All simulations were performed using the MARTINI force field for coarse-grained systems. Periodic boundary conditions were defined in the x-, y-, and z-dimensions for each simulated system. The neighbor list was updated every 1 step, and generally a timestep of 0.02 ps was used for all MD simulations. Short-range van der Waals and electrostatic interactions were calculated using shifted potentials with a cutoff of 1.2 nm. Pressure coupling was applied isotropically using the Parrinello Rahman barostat with a reference pressure of 1.0 bar, a compressibility of $10^{-5}$ bar$^{-1}$, and a coupling time constant of 1.0 ps. Temperature coupling was applied using the Nosé-Hoover thermostat with a coupling time constant of 0.5 ps. These settings apply to all MD simulations conducted in this study unless stated otherwise.

Coarse Graining of Signaling Factor Cytokines and Matrix Proteins

The initial protein structures for TNF-$\alpha$ and IL-6 were obtained from PDB entries 1TNF and 1IL6, respectively, provided by the RCSB Protein Data Bank. The MARTINI coarse graining tool for protein structures, known as Martinize, was utilized to coarse grain the atomistic TNF-$\alpha$ into a 984 bead structure and atomistic IL-6 into a 363 bead structure. Given the nature of the three component polypeptide chains comprising TNF-$\alpha$ to separate, an elastic network was placed over the protein using a spring force constant of 500 kJ mol$^{-1}$.
Comparison between the atomistic and coarse-grained structures using the Visual Molecular Dynamics (VMD) simulation tool is provided for each signaling factor in Appendix I.

To simulate the effect of polysaccharide chain degradation in the biofilm EPS, as well as to add chemical description to the biofilm matrix using extracellular proteins, the polysaccharase glucoamylase was retrieved from PDB entry 1KUM provided by the RCSB Protein Data Bank. Martinize was used to coarse grain atomistic glucoamylase into a 234 bead structure. VMD visualizations of the atomistic and coarse-grained enzyme structures are provided in Appendix I.

DNA, another major component of the biofilm matrix, was downloaded from the MARTINI web site. The double stranded, course-grained DNA structure with 12 base pairs was provided to MARTINI users by Syma Khalid. A VMD representation of the coarse-grained DNA structure is shown in Appendix I.

**Lipid Bilayer Simulations**

MD simulations were utilized to self-assemble a DSPC lipid bilayer with 493 lipids in a box with dimensions 15×15×6 nm$^3$ in the presence of MARTINI CG water. Energy minimization was performed using the steepest descent algorithm to ensure minimal forces on each atom and optimized molecular geometries. Following minimization, selected atoms of each lipid in the bilayer were restrained for simulation purposes using 1000 kJ mol$^{-1}$ nm$^{-2}$ forces defined only in the z-dimension. The isothermal-isobaric ensemble (NPT) was employed
to relax the system using the velocity rescale thermostat coupling water and DSPC at 300 K for 40 ns of simulation time. Using techniques similar to prior lipid bilayer simulations, semi-isotropic pressure coupling was used to allow box size fluctuations in the z-dimension only. All position restraints were then removed, and the water molecules were deleted from the system.

The coarse-grained TNF-α molecule was centered in a box of dimensions 11×16.2×25 nm³ at the coordinates (5.5, 8.1, 20.5) along with the self-assembled DSPC lipid bilayer centered at (5.5, 8.1, 10). This afforded approximately 5 nm of space between the bottom of the signaling factor and the top plane of the lipid bilayer. The box was solvated with 38,634 coarse-grained water molecules. The system was then energy minimized using a steepest decent algorithm to reduce the forces on each particular atom to a minimum, as well as to optimize the geometry of all molecules in the system. An NPT equilibration was performed for 40 ns using velocity rescale temperature coupling of the DSPC lipids and TNF-α signaling factor with water at 300 K. The equilibration was carried out using semi-isotropic pressure coupling which allowed fluctuations in box volume in the z-dimension only. The canonical (NVT) ensemble was utilized as an additional system equilibration and relaxation step with velocity rescale temperature coupling of TNF-α and the DSPC lipids with water at 300 K. In order to introduce initial interactions between all atoms of the system, a short 20 ns MD simulation was performed using the Berendsen barostat and temperature coupling at 300 K. This step also allowed for additional system equilibration.
In order to effectively sample the translocation process in a reasonable amount of time, an umbrella sampling technique was employed where a biasing potential forced a translocation to occur at a faster-than-normal rate. Analysis of the results removed this biasing force in order to maintain simulation accuracy. A center-of-mass (COM) pull simulation was performed over a distance of 25 nm for a total simulation time of 2.7 ns. This was achieved by applying a constant force of 1000 kJ mol$^{-1}$ nm$^{-2}$ to the COM of the TNF-$\alpha$ protein. The trajectory of the protein over a 16 nm distance was cut into 121 umbrella sampling windows. Measured with respect to the protein COM, the spacing between each window was approximately 0.2 nm from a distance 8 nm to 4.2 nm above the lipid bilayer COM, and from a distance 4 nm to 8 nm below the lipid bilayer COM. However, the window spacing was roughly 0.1 nm from 4.2 nm above the bilayer COM to 4 nm below the bilayer COM when measuring with respect to the protein COM.

Within each umbrella sampling window, 1 ns of NPT equilibration was performed to relax the lipid bilayer and TNF-$\alpha$ structures in their unique configuration. An extensive, 100 ns molecular dynamics simulation was performed in each window to simulate the interactions at that location along the translocation trajectory. Using GROMACS version 4.5.4, the built-in g_wham utility was employed to remove the biasing potential from the umbrella sampling technique using the weighted histogram analysis method.$^{50}$ The utility extracted a potential of mean force curve from the translocation process and provided a sampling histogram to showcase the quality of the simulated results.
Using the free GridMAT-MD software developed by Allen, Lemkul, and Bevan, deformation of lipid bilayer membranes is analyzed.\textsuperscript{51} The x- and y-vectors of the box were scaled to fit on a grid of 30 points by 30 points. This means that, in order to analyze the membrane effectively using the GridMAT-MD program, the actual vectors along the x- and y-dimensions were sliced into 30 sections. The program analyzed the distance from each PO4 and NC3 atom of each DSPC lipid in the top leaflet of the lipid bilayer to its nearest neighbor in the bottom leaflet of the bilayer. The analysis process was repeated, but this time measuring from the bottom leaflet upward to the nearest neighbor in the top leaflet of the bilayer. The average of these two calculations was provided in the program’s final output.

\textit{Biofilm Matrix – TNF-\textalpha{} Simulations}

A system was manufactured of fourteen coarse-grained dextran chains with 100 monomer \(\alpha\)-D-glucopyronosyl residues with (1,6) linkages in a box of dimensions 15\(\times\)15\(\times\)10 nm\(^3\). The dextran system was solvated with CG water using the GROMACS genbox utility. Steepest decent energy minimization was performed to ensure a minimal force no greater than 50 kJ mol\(^{-1}\) nm\(^{-2}\) was exerted on any given atom, as well as to optimize all molecular geometries. The NVT ensemble was used for 5 ns of simulation time to relax the system with velocity rescale temperature coupling of water and the polysaccharide matrix at 300 K. This final system structure is termed the generalized biofilm matrix because it consists solely of standard dextran polysaccharide chains in water, which is a
primary component of biofilm EPS securing biofilm surface attachment and serving as an essential protective barrier for bacterial cells.

The water was removed from the generalized matrix system, and the box was expanded to dimensions $15 \times 15 \times 25 \text{ nm}^3$. The coarse-grained TNF-α molecule was centered in a box at the coordinates $(7.5, 7.5, 20)$ along with the generalized matrix of dextran chains centered at $(7.5, 7.5, 10)$. This afforded approximately 1.5 nm of space between the bottom of the signaling factor and the top plane of the generalized model EPS matrix. The box was solvated with 32,752 coarse-grained water molecules, 12% of which were replaced with CG antifreeze water. This was utilized to prevent crystallization of the water during the simulation. The methodologies employed for system energy minimization and equilibration were identical to those described earlier for the system of TNF-α with the DSPC lipid bilayer.

The umbrella sampling technique was applied again to effectively sample the translocation of TNF-α through the generalized EPS matrix. A COM pull simulation was performed over a distance of 16 nm for a total simulation time of 2.66 ns by applying a constant force of 1000 kJ mol$^{-1}$ nm$^{-2}$ to the COM of the TNF-α protein. The $z$-coordinate was defined as the reaction coordinate ($\xi$). The trajectory of the protein over the 16 nm distance was cut into 81 umbrella sampling windows with the spacing between each window being approximately 0.2 nm. The windows were taken from a distance 8 nm above the matrix COM to a distance 8 nm below the generalized matrix COM.
To analyze the effect of polymer chain length on the translocation process of the signaling factor through the model biofilm matrix, it is important that all system attributes be held constant except for dextran monomer length. This means that the pull path must be held constant in all experiments. Performing umbrella sampling MD simulations for systems of various chain lengths by the traditional method will require unique pull simulations which inherently introduce variability in the pull path. Therefore, after having created 81 windows along the trajectory of TNF-α through the generalized EPS matrix of 100-mer length dextran chains, three new systems were manufactured by effectively chopping the 100-mer chains into smaller units. Each of the 81 windows was reproduced into unique systems of 50-mer, 20-mer, and 10-mer chains. In this case, since the number of atoms of dextran are not changing, but the chain length is getting smaller, all variables are effectively held constant except for polysaccharide chain length. A simple C++ code was written to systematically renumber the dextran chains for each of the 81 structure files for the 100-mer system to 50-mer chains, then to 20-mer chains, and so on. In this manner, all four systems sampled (for each of the four chain lengths) have originated from a single pull simulation containing the same number of dextran and water atoms. This innovative methodology is new to molecular dynamics studies, and will be considered very useful for many others in their future computational experiments. The full C++ code can be found in Appendix III.

Equilibration for 1 ns using the NPT ensemble was performed for the 100-mer dextran chain system. For the 50-mer, 20-mer, and 10-mer systems, 5 ns
simulations using the NPT ensemble were performed in order to relax the newly
constructed configurations created by chopping larger dextran chains. The
methodologies employed for energy minimization, equilibration, and umbrella
sampling MD simulations in each window for each of the four systems were
identical to those described earlier for the system of TNF-α with the DSPC lipid
bilayer.

In most cases, simulations are performed multiple times to confirm a
result, and the average calculated result is reported. Given the large computational
power necessary to perform a single umbrella sampling simulation, let alone for
four unique systems, it is computationally prohibitive to repeat the sampling
process numerous times. However, in order to improve statistical convergence
and set error limits on the simulation results, the GROMACS g_wham utility was
invoked four times on each system over varying, overlapping 10 ns time spans.
Figure 2 depicts this simulation technique. From this bootstrap analysis, it was
feasible to maximize the data extraction from the single simulation completed for
each of the four systems. Potential of mean force curves were extracted from each
system using this methodology.

In addition to the simulations performed using the generalized matrix, it
was of significant interest to compare results from umbrella sampling simulations
with a more detailed biofilm matrix. In order to build the matrix, three DNA
strands were put in a box of dimensions 15×15×10 nm³ with even spacing
between each strand along the x-coordinate. Similar to the previous simulations,
fourteen coarse-grained dextran chains with 100 monomer α-D-glucopyronosyl
residues with (1,6) linkages were fit into the box around the DNA. Two CG glucoamylase structures were added to the new system with geometric centers at the coordinates (3.5, 9.7, 7.6) and (9.9, 4.5, 2.0) within the $15 \times 15 \times 10$ nm$^3$ box. The box was solvated with a total of 20,780 CG water molecules, of which 10% were converted to antifreeze water to prevent solvent crystallization. To neutralize the charge associated with the extracellular components (polysaccharide chains, proteins, and nucleic acids), 92 sodium ions were added to the system. The resulting system is referred to as the detailed biofilm matrix given its extensive chemical description involving all of the most important EPS components identified in prior research. Figure 3 depicts a top view and side view of the detailed biofilm matrix using VMD software.

The detailed matrix system was energy minimized using the steepest descent algorithm to ensure forces no larger than 100 kJ mol$^{-1}$ nm$^{-2}$ on any given
bead. An NPT equilibration was then conducted for 20 ns with temperature and pressure coupling of the water and the matrix components. Temperature was held constant using velocity rescaling. An MD production run was performed on the equilibrated structure for 20 ns under the NVT ensemble. Subsequently, a short NPT run using Berendsen pressure coupling for 2 ns of simulation time was performed for additional matrix equilibration. Following the final NPT, the box dimensions grew slightly to 16.75×16.75×11.2 nm³.

**Figure 3.** Visualization of the detailed biofilm matrix consisting of three DNA strands (red, blue, green, and yellow), two glucoamylase proteins (purple), and 100-mer dextran polymer chains (orange). Both top and side views of the EPS matrix model are provided.

The solvent water beads and ions were removed from the system, and the box dimensions were expanded to 16.75×16.75×26 nm³. The signaling factor TNF-α was positioned above the matrix along the z-coordinate. The system was solvated with 58,253 CG water beads and 6,482 antifreeze water molecules to prevent solvent crystallization. 92 sodium ions were placed back into each system to restore charge neutrality. Energy minimization was performed on the entire system using the steepest descents algorithm. Position restraints were then defined for the signaling factor, DNA, and extracellular proteins using a force constant of
1000 kJ mol\(^{-1}\) nm\(^{-2}\) in order to prevent significant movement of these system molecules during processing. The system was simulated for 200 ps under the NPT ensemble using velocity rescaling for temperature control to introduce initial interactions, and this was followed up by an additional NPT equilibration for 20 ns. The system was simulated for 20 ns in an MD production run with the position restraints removed from all system particles.

The umbrella sampling technique was applied to effectively sample the translocation of TNF-\(\alpha\) through the detailed EPS matrix. A COM pull simulation was performed for a total simulation time of 2.5 ns by applying a constant force of 1000 kJ mol\(^{-1}\) nm\(^{-2}\) to the COM of the TNF-\(\alpha\) protein. The \(z\)-coordinate was defined as the reaction coordinate \((\xi)\). The trajectory of the protein over the 16 nm distance was cut into 82 umbrella sampling windows with the spacing between each window being approximately 0.2 nm. The windows were taken from a distance 8 nm above the matrix COM to a distance 8 nm below the detailed matrix COM.

Using the same technique described earlier, the 82 windows produced from the single pull simulation were used in conjunction with a C++ code to create three other unique systems with dextran polymer chains of 50mer, 20mer, and 10mer lengths. The methodologies employed for energy minimization, equilibration, and umbrella sampling MD simulations in each window for each of the four systems were identical to those described earlier for the system of TNF-\(\alpha\) with the generalized EPS matrix. The bootstrapping method described in Figure 2
was implemented using the GROMACS g_wham utility to extract potential of mean force curves from each system.

**Biofilm Matrix – IL-6 Simulations**

From the established and equilibrated detailed EPS matrix, the solvent water beads and ions were removed from the system, and the box dimensions were expanded to 16.75×16.75×26 nm\(^3\). The signaling factor IL-6 was positioned above the matrix along the z-coordinate. The system was solvated with 58,492 CG water beads and 6,509 antifreeze water molecules to prevent solvent crystallization. 92 sodium ions were placed back into each system. Energy minimization and NPT equilibration processes were carried out in the same fashion as that described for the TNF-\(\alpha\) and detailed biofilm matrix. The system was simulated for 20 ns in an MD production run with the position restraints removed from all system particles except for the IL-6 signaling factor, which remained restrained. Following this simulation, the IL-6 position restraints were removed.

The umbrella sampling technique was applied to effectively sample the translocation of IL-6 through the detailed EPS matrix. A COM pull simulation was performed for a total simulation time of 3.0 ns by applying a constant force of 1000 kJ mol\(^{-1}\) nm\(^{-2}\) to the COM of the IL-6 protein. The z-coordinate was defined as the reaction coordinate (\(\xi\)). The trajectory of the protein over the 16 nm distance was cut into 82 umbrella sampling windows with the spacing between each window being approximately 0.2 nm. The windows were taken from a
distance 8 nm above the matrix COM to a distance 8 nm below the detailed matrix COM.

Using the same technique described earlier, the 82 windows produced from the single pull simulation were used in conjunction with a C++ code to create three other unique systems with dextran polymer chains of 50mer, 20mer, and 10mer lengths. The methodologies employed for energy minimization, equilibration, and umbrella sampling MD simulations in each window for each of the four systems were identical to those described earlier for the system of TNF-α with the generalized EPS matrix. Instead of using the bootstrapping method described in Figure 2, the built-in bootstrapping method of the GROMACS g_wham utility, which produced four unique profiles, was used to extract an average potential of mean force curve from each system. The analysis was conducted over 18 ns by disregarding the first 2 ns of simulation in each window.
6. Results

**Lipid Bilayer Simulations**

Visual representations of the molecular dynamics simulations for TNF-α translocation across a DSPC lipid bilayer in aqueous solution are shown in Figure 1. In the figure, the signaling factor is represented in yellow while the lipid bilayer is represented with green, blue, and red hydrophilic head groups and blue hydrophobic carbon chains. The trajectory of the signaling factor and deformation of the bilayer are depicted throughout translocation process by proceeding from (a) to (b) to (c). It is important to note that this process is unlikely to occur spontaneously, and these simulation results are based on a biased potential applied to the TNF-α cytokine to allow experimental analysis on a reasonable timescale.

**Figure 1.** Visualization of umbrella sampling utilizing molecular dynamics simulations where TNF-α, yellow, is pulled through the DSPC lipid bilayer.
The results from umbrella sampling of the system along the trajectory depicted in Figure 1 are summarized in the potential of mean force plot in Figure 2. The plot showcases the energy barrier associated with TNF-α translocation across the bilayer as the cytokine moves from state (a) to state (b) to state (c). The energy barrier is quantified as 1,341 kJ mol\(^{-1}\).

![Potential of mean force curve for the translocation of TNF-α across a DSPC lipid bilayer.](image)

The lipid bilayer deformation is quantitatively described by surface and contour plots provided in Figure 3. The plots in Figure 3 depict bilayer thickness with respect to the xy-plane where increased color variation indicated heightened variability in the lipid bilayer thickness. Visualization of the system configuration accompanies the plots for reference purposes. Additional calculations to quantify the bilayer deformation were performed, such as analysis of the area per lipid head group, and these results are summarized in Appendix II.
Figure 3. Qualitative and quantitative description of the lipid bilayer deformation associated with translocation of TNF-α, yellow, through a DSPC lipid bilayer. Surface plots show the change in lipid bilayer thickness as the simulation proceeds.
**Biofilm Matrix – TNF-α Simulations**

Visual representations of the simulated systems for TNF-α interaction with a generalized biofilm matrix of only dextran polysaccharide chains in aqueous solution are shown in Figure 4. In the figure, the signaling factor is represented in yellow while the each dextran chain is represented using dynamic bonds of various colors to allow for chain differentiation. Dextran chain lengths are (a) 10 monomers, (b) 20 monomers, (c) 50 monomers, and (d) 100 monomers.

![Figure 4. Visualization of the simulated system of TNF-α, yellow, interaction with dextran polysaccharide chains of (a) 10mer, (b) 20mer, (c) 50mer, and (d) 100mer lengths.](image)

The results from molecular dynamics simulations and umbrella sampling of each of the four systems depicted in Figure 4 are summarized in the potential of mean force plot in Figure 5. The plot allows for convenient comparison between the systems and showcases the energy differences associated with TNF-α translocation through the matrix of dextran chains. It is important to note that the plot contains polynomial fits of the true data to exclude noise from the overall results. On average, the energy is quantified as 71.3 kJ mol\(^{-1}\) for 10mer chains, 60.2 kJ mol\(^{-1}\) for 20mer chains, 49.3 kJ mol\(^{-1}\) for 50mer chains, and 20.2 kJ mol\(^{-1}\) for 100mer chains.
Figure 5. Potential of mean force curves highlight the average stabilization energies associated with TNF-α translocation through a matrix of dextran polysaccharide chains of 10mer (purple), 20mer (red), 50mer (green), and 100mer (blue) lengths.

The relationship between polymer chain length and the average energy difference associated with the translocation process is summarized in Figure 6. The linear fit has a coefficient of determination ($R^2$) of 0.9858 and is given by Equation 1:

$$\Delta E_{av} = -0.539(N) + 76.539$$  \hspace{1cm} (1)

where $\Delta E_{av}$ is the average energy difference for the translocation process in kJ mol$^{-1}$ and $N$ is the dextran polymer chain length. Error bars are provided in Figure 6 based on the standard deviations of the average energy differences.

The radial distribution function, $g(r)$, defined as the average from the TNF-α COM to each individual dextran chain in the matrix, is depicted for each generalized matrix system of varying dextran chain lengths in Figure 7. The dextran chain radius of gyration is plotted as a function of chain length for the
10mer, 20mer, and 50mer systems in Figure 8. The relationship plotted in Figure 8 can be described by Equation 2:

$$\Delta E_{av} = -0.539(N) + 76.539$$

(2)

where $r_g$ is the radius of gyration of the dextran chains in nm and $N$ is the dextran chain length. A similar plot is provided in Figure 9 for the ratio of the dextran chain radius of gyration to that of the TNF-α signaling factor. The relationship plotted in Figure 9 can be described by Equation 3:

$$\frac{r_{g, \text{dextran}}}{r_{g, \text{protein}}} = 0.11N^{0.51}$$

(3)

where $r_g$ is the radius of gyration of the TNF-α signaling factor, which has been determined to be $2.13 \text{ nm} \pm 0.002 \text{ nm}$. Both Figures 8 and 9 contain error bars that describe the standard deviation in the experimental data points.

**Figure 6.** Relationship between dextran chain length, N, and the average energy difference for the translocation of TNF-α through a generalized biofilm matrix. Error bars demonstrate the standard deviations of the average energy differences.
Figure 7. Plot of the radial distribution function, $g(r)$, between the protein center of mass and the dextran polysaccharide chains of 10mer (purple), 20mer (red), and 50mer (green) lengths.

Figure 8. Plot of the radius of gyration of dextran chains in the generalized matrix as a function of the dextran chain length, $N$. 
Figure 9. Plot of the ratio of the radius of gyration of dextran chains in the generalized matrix to the radius of gyration of the TNF-α signaling factor as a function of the dextran chain length, N.

The results from molecular dynamics simulations and umbrella sampling of each of the four systems involving the detailed biofilm matrix and TNF-α are summarized in the potential of mean force plot in Figure 10. The plot allows for convenient comparison between the systems and showcases the energy differences associated with TNF-α translocation through the matrix of dextran chains, DNA, and glucoamylase molecules. On average, the energy is quantified as 13.3 kJ mol$^{-1}$ for 10mer chains, 22.6 kJ mol$^{-1}$ for 20mer chains, 12.3 kJ mol$^{-1}$ for 50mer chains, and 12.4 kJ mol$^{-1}$ for 100mer chains. The experimental data in Figure 10 is provided without fitting in order to provide an accurate representation of the data.
Figure 10. Potential of mean force curves highlight the energy barriers associated with TNF-α translocation through a detailed biofilm matrix of DNA, protein, and dextran polysaccharide chains of 10mer (purple), 20mer (red), 50mer (green), and 100mer (blue) lengths.

**Biofilm Matrix – IL-6 Simulations**

The results from molecular dynamics simulations and umbrella sampling of each of the four systems involving the detailed biofilm matrix and IL-6 are summarized in the potential of mean force plot in Figure 11. The plot allows for convenient comparison between the systems and showcases the energy differences associated with IL-6 translocation through the matrix of dextran chains, DNA, and glucoamylase molecules. On average, the energy is quantified as 20.4 kJ mol⁻¹ for 10mer chains, 17.9 kJ mol⁻¹ for 20mer chains, 22.4 kJ mol⁻¹ for 50mer chains, and 30.7 kJ mol⁻¹ for 100mer chains.
Figure 11. Potential of mean force curves highlight the energy barriers associated with IL-6 translocation through a detailed biofilm matrix of DNA, protein, and dextran polysaccharide chains of 10mer (purple), 20mer (red), 50mer (green), and 100mer (blue) lengths. The relationship between polymer chain length and the average energy difference associated with the translocation process is summarized in Figure 12. The linear fit has a coefficient of determination ($R^2$) of 0.9025 and is given by Equation 4:

$$\Delta E_{av} = 0.130(N) + 16.982$$

where $\Delta E_{av}$ is the average energy difference for the translocation process in kJ mol$^{-1}$ and N is the dextran polymer chain length. Error bars are provided in Figure 12 based on the standard deviations of the average energy differences.
Figure 12. Relationship between dextran chain length, $N$, and the average energy difference for the translocation of IL-6 through a detailed biofilm matrix. Error bars demonstrate the standard deviations of the average energy differences.
7. Discussion

**Lipid Bilayer Simulations**

From many of the systems studied experimentally, the primary result is the extraction of a potential of mean force curve. The potential of mean force curve represents an average over all of the configurations of the system along a given reaction coordinate, and this can be used to evaluate the free energy difference ($\Delta G$) between two states of the system. The Gibbs free energy is an important property in thermodynamics and chemical processes because of its insight into the spontaneity and favorability of such processes occurring. The profile observed for the translocation of tumor necrosis factor alpha through a DSPC lipid bilayer, as shown in Figure 2, demonstrates a large energy barrier. It has been proposed that adenosine triphosphate (ATP) molecules work in the presence of a protein to overcome the energy barrier associated with translocation of a molecule across a membrane.\(^{31}\) The energy barrier of 1,341 kJ mol\(^{-1}\) is equivalent to a need for 38 ATP molecules, which is a significant amount of energy.

It should be noted that the potential of mean force curve presented in Figure 2, which does not represent fitted data, shows an exceptionally smooth transition from one state to another along the reaction coordinate. This may be attributed to the large simulation time of 100 ns permitted within each of the 121 windows used to produce the PMF curve. Although this simulation timescale may be unnecessarily large, it is important to note that the results are clear.

The qualitative and quantitative description of lipid bilayer deformation provided in Figure 3 provides a more detailed assessment of the translocation
process energetically quantified in Figure 2. Visualization of the lipid membrane as the simulation progresses shows that as the signaling factor approaches the bilayer, bending at the contact point occurs. Bending of the lipid bilayer continues until the stress becomes so great that pore formation results, allowing the signaling factor to pass through the membrane. As the translocation continues, partial rupture of the lipid bilayer is observed around the formed pore. However, with further simulation time, it is likely that the lipid bilayer will reform in order to minimize hydrophobic lipid tail contact with the solvent water molecules.

The contour plots provided in Figure 3 provide further insight by showing that the bulk of the lipid bilayer that does not encounter the TNF-α molecule remains intact throughout the translocation process. These contour plots, which depict bilayer thickness for various points in time during the translocation process, highlight a major decrease in bilayer thickness around the area of the TNF-α contact as the translocation process advances. Although bilayer thickness does increase in non-contact areas in response to physical stresses, the thickness is consistent in these areas allowing the bilayer to remain essentially intact.

Area per lipid headgroup and bilayer thickness calculations were performed as a function of translocation time. These calculations allow additional quantitative analysis of the clear membrane deformation. Data from the analysis are presented in Appendix II, and the results are summarized in Table 1 below. Table 1 showcases the change in the area per lipid headgroup in both the top and bottom leaflets of the DSPC lipid bilayer for various windows. Higher number
windows correspond to snapshots of the system further into the translocation process, mimicking the change in the system demonstrated in Figure 3.

Table 1. Area per lipid headgroup data summary for both the top and bottom leaflets of the DSPC lipid bilayer.

<table>
<thead>
<tr>
<th>Window</th>
<th>Area Per Lipid Headgroup (sq. Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Top Leaflet</td>
</tr>
<tr>
<td>1</td>
<td>66.02</td>
</tr>
<tr>
<td>2</td>
<td>71.50</td>
</tr>
<tr>
<td>3</td>
<td>68.81</td>
</tr>
<tr>
<td>4</td>
<td>34.24</td>
</tr>
</tbody>
</table>

Table 1 demonstrates that the area per lipid headgroup is consistent for both the top and bottom leaflet prior to TNF-α interaction (window 1). As the simulation progresses, the area per lipid headgroup in the top leaflet increases to accommodate the bending of the bilayer upon TNF-α contact, while the bottom leaflet experiences decreased area per lipid headgroup from the increased stresses caused by shifted lipids in the top leaflet. Ultimately, after the TNF-α signaling factor has passed through the lipid bilayer, there is clear deformation of the top and bottom leaflets with significantly deviating area per lipid headgroups. In fact, the bottom leaflet area suggests the lipids are no longer in an ordered arrangement. Visualization also shows that disorder is occurring around the formed pore to assist in the translocation of the signaling factor.

**Biofilm Matrix – TNF-α Simulations**

In modelling the bacterial biofilm extracellular polymeric substance, the main constituents of water and polysaccharides were used to conduct a simple analysis. In the model bacterial biofilm matrix simulations with TNF-α, the first
goal was to determine the effect of polysaccharide chain length on the translocation process. It is known that polysaccharase action causes chain degradation, and it may be hypothesized that this action has considerable effects on the interaction of the biofilm with its host environment. Glucoamylase, which is a known polysaccharide exo-hydrolase present in many microorganisms, carries the responsibility of cleaving glucose units by hydrolyzing $\alpha$-1,4 and $\alpha$-1,6 linkages. This makes glucoamylase ideal for breaking down dextran polysaccharide chains with $\alpha$-D-glucopyranosyl residues consisting of (1,6) linkages. The effect of this action on the chain length was simulated using molecular dynamics simulations of various systems where every parameter was controlled while only dextran monomer length varied between 10 and 100.

The simulated systems depicted in Figure 4 produced the potential of mean force curves shown in Figure 5. From this figure, it is clear that there is a relationship between dextran chain length and the translocation process favorability. Given that all of the energy differences are negative from the initial state the state where the signaling factor is embedded within the EPS matrix, it is clear that this translocation is favorable and is likely to occur spontaneously. This energy difference can be thought of as a signaling factor stabilization energy where the TNF-$\alpha$ signaling factor finds more favorable interactions while embedded within the matrix as opposed to resting outside of the matrix. For the 10mer chains, Figure 5 shows the greatest stabilization. Additionally, the 100mer chains appear to provide the least amount of stabilization. In fact, Figure 5 suggests that the degree of stabilization decreases as the dextran chain length
increases when considering all simulated systems. When the average stabilization energy over 4 analyses for each system is plotted as a function of dextran chain length, $N$, as shown in Figure 6, it is clear that a linear relationship exists that fits perfectly within the uncertainties of the simulations. Therefore, it has been determined that a linear relationship exists between the stabilization energy experienced by the signaling factor and the polymer chain length, and this relationship is given by Equation 1.

The results of these simulations with a generalized biofilm matrix have broad implications. Research theories that perhaps small molecules may become trapped, or sequestered, within the EPS matrix due to favorable interactions have been confirmed with the molecular dynamic simulations studied here\textsuperscript{29}. The minimum energy attained within the matrix provides a comfortable home for the TNF-$\alpha$ signaling factor. Additionally, the simulations allowed for some analysis of the polymer matrix and its effect on protein stabilization. The theory of polymer brushes suggests that polymer chains attach one polymer end to a surface or interface while the other end is forced to stretch away from the surface\textsuperscript{55}. This phenomenon is known to occur for several types of interfaces, including curved surfaces\textsuperscript{56}. Furthermore, it is proposed that stabilization of interfaces occurs as a result of the polymer attachments\textsuperscript{55-57}, which may provide an explanation for the observed energy stabilizations observed for TNF-$\alpha$ translocation through a polymer matrix.

Figure 7, which demonstrates the radial distribution functions for dextran polymer configuration with respect to the TNF-$\alpha$ COM for various dextran chain
lengths, shows that the arrangement of polymers is dependent on the chain length. Dextran chains that are 10mer in length show a greater density and more well-defined peak around 4.5 nm from the TNF-α center of mass, which suggests a systematic distribution and regular arrangement. Contrarily, the 50mer chains have a less well defined peak, suggesting a more irregular arrangement. This suggests that polymer brush formation in the 10mer chain length case may be providing additional stabilization to the TNF-α signaling factor, thus making the translocation process more favorable.

So why is polymer brush formation more difficult for the longer 50mer dextran chains? The answer to this question can be ascertained by comparing visualizations of the SF-EPS interactions for the 10mer and 50mer systems, which are shown in Figure 13. The figure clearly depicts contrasting interactions for the 10mer (top) and 50mer (bottom) systems. In the case of the 50mer polymer chains, there is a greater tendency for the chains to interact at more than just the end points to take up larger surface areas, which effectively screens interactions between the SF and other 50mer chains. In the 10mer system, where the dextran polymer chains are shorter, there is less chance of screening which increases the ability for polymer brush formation and, thus, greater SF stabilization.

Figure 8, which demonstrates the change in the dextran radius of gyration as a function of $N$, depicts an increase in $r_{g,dextran}$ following a power law relationship with an exponent of 0.51. Figure 9 shows a similar relationship in depicting the ratio of the dextran radius of gyration to that of the TNF-α protein as
Figure 13. Visualization of the TNF-α interactions with the dextran polymers for the 10mer [(a) and (b)] and 50mer [(c) and (d)] systems from both side and top views.

a function of $N$. These results suggest that the polysaccharides in the generalized biofilm matrix experience θ-like solvent in line with the Rouse model.

For the first time, molecular dynamics simulations have been used to detail the energetics surrounding translocation processes of communicator proteins through cell membranes and bacterial biofilm matrices. The details of the results from these simulations may be easily related to drug delivery and therapeutic development research. However, the model utilized for the EPS matrix of the biofilm is generalized and very basic. As noted in the introduction to biofilms presented here, there are numerous other EPS constituents other than water and polysaccharides. This led to the second goal of this study, which was to quantify the energetics surrounding the translocation process through a detailed
biofilm matrix with comparison of the results to the previous simulations. In order to add additional detail to the biofilm, proteins and DNA were added to the polysaccharide and water matrix. Specifically, two alginate lyase proteins were used to simulate the effect of polysaccharide protein action and three DNA strands provided nucleic acid content. The simulated matrix is depicted in Figure 3.

Potential of mean force curves, shown in Figure 10, provide the raw, unfitted data from the simulations using the detailed biofilm matrix. From this figure, unlike in the case of the generalized biofilm matrix, there does not appear to be any clear relationship between the average stabilization energy and the polymer chain length. For nearly all four cases, the results suggest that there is little to no energy change associated with the translocation of the signaling factor into the detailed biofilm matrix. In fact, the process initially appears to be unfavorable with a small energy barrier for each of the translocation processes. This trend is vastly different from the previous simulation results even though the only difference is the presence of proteins and DNA strands in the biofilm matrix. These results suggest that the interaction between the TNF-α SF and the non-polysaccharide matrix components is significant. The presence of the protein and DNA extracellular components, likely due to their rigidity when compared to the loose polymer chains, increase the free energy of the system when compared to the case involving their absence.
**Biofilm Matrix – IL-6 Simulations**

The third goal of this study was to analyze the effect of signaling factor size on the translocation process. In order to meet this goal, a smaller interleukin-6 signaling factor replaced the larger tumor necrosis factor-alpha signaling factor in the detailed biofilm simulations. The resulting potential of mean force curves shown in Figure 11 from the translocation processes suggest that the process is energetically favorable. In fact, in comparing the average stabilization energy associated with translocation through a biofilm matrix of various chain lengths, the opposite trend to that realized with TNF-α through the generalized matrix is observed. As the polymer chain length increases, the average stabilization energy increases as well, suggesting a direct relationship rather than an inverse relationship. The relationship between $\Delta E_{av}$ and $N$ is depicted in Figure 12 and quantified by Equation 2. It should be noted that the slope of the trendline in this case is positive, whereas the slope was negative in the case of TNF-α translocation through the generalized matrix.

These results are interesting because it suggests, first and foremost, that the signaling factor size strongly affects the translocation process. This result is reached when comparing the PMF curves for the unfavorable TNF-α translocation through a detailed biofilm matrix with the PMF curves given in Figure 11 suggesting a favorable process for translocation of IL-6 through the same matrix. Although only two signaling factors are provided for comparison, the only difference between the two simulations is the size of the SF. The results presented in this study suggest that the favorability of SF translocation through the biofilm
matrix increases as the size of the SF decreases. This result is expected given that smaller SF molecules undoubtedly find easier diffusion pathways through a given polymer matrix than larger molecules. Intuitively, smaller molecules can move faster and fit into more pores than their larger counterparts.

However, an explanation is also in order for the direct relationship between IL-6 stabilization through a detailed biofilm matrix and the dextran polymer chain length versus the inverse relationship for TNF-α stabilization through a generalized biofilm matrix and the dextran polymer chain length. Although IL-6 is smaller than TNF-α, lending to increased favorability amongst the chains, the detailed biofilm matrix appears to cause decreased translocation favorability for the SF. Therefore, without further studies, it is nearly impossible to say with any certainty why the IL-6 SF encounters greater stabilization with increased dextran chain lengths as opposed to smaller chain lengths. One may theorize that the polymer brush theory breaks down when other elements are present in the matrix. For example, it may be the case that intramatrix interactions between the polymer, lyase protein, and DNA components adversely affects the IL-6 SF translocation. In the case of the smaller dextran chain lengths forming more lyase protein stabilizing interactions than the larger dextran chain lengths, it may be that the IL-6 SF has a more difficult time overcoming the intramatrix interactions to afford its own stabilization interactions with the matrix polymers. In any case, further studies of IL-6 in a generalized biofilm matrix are necessary in order to gain insight into the direct relationship between SF stabilization energy and the dextran polymer chain length.
8. Conclusions

*Simulation Summation*

For the first time, molecular dynamics simulations have been used to show the energy barrier associated with TNF-α signaling factor translocation through a DSPC lipid bilayer modeling a mammalian cell membrane. The energy associated with this unfavorable translocation process is 1,341 kJ mol$^{-1}$, and the unfavorable interaction results meet expectations. Quantitative and qualitative analysis of the lipid bilayer deformation was performed, and clear pore formation is present upon translocation of the TNF-α SF through the lipid bilayer.

A generalized biofilm matrix model was created using coarse-grained dextran chains in solution. Matrices of various dextran chain length were synthesized in order to simulate the effect of polysaccharide action and chain degradation on the translocation of SFs through the EPS. TNF-α translocation through this generalized matrix was found to be favorable, suggesting the process will occur spontaneously. Furthermore, the average stabilization energy was found to increase linearly with decreasing chain length, $N$, demonstrating a clear and quantifiable inverse relationship. The radial distribution functions measured from the TNF-α COM to the dextran chains of the matrix for chains of various length suggest increased uniformity in the configuration of the chains around the SF with decreasing chain length. Furthermore, the ratio of the radius of gyration of the dextran chains to that of the TNF-α SF is shown to increase with increasing $N$. These results demonstrate that polymer brush formation around the TNF-α SF may be more likely for shorter dextran chains than longer dextran chains, which
would account for the more uniform configuration of dextran chains found in the systems with smaller dextran chain lengths. The larger chains are theorized to effectively screen increased polymer end attachments to the SF, which decreases the amount of SF energy stabilization observed during the translocation process.

Simulations were also conducted using a detailed biofilm matrix which incorporated more EPS components. The matrix was manufactured to include lyase proteins and DNA in addition to dextran polysaccharides, all in solution. Translocation of the TNF-α SF through this detailed biofilm matrix produced PMF curves that showed unfavorable translocation interactions. The results suggest that the interactions between the SF and the non-polysaccharide components are significant.

Additional simulations were performed using a detailed biofilm matrix and a smaller signaling factor, interleukin-6, in order to determine the effect of SF size on the translocation process. Unlike with the TNF-α SF, the resultant PMF curves demonstrated a favorable translocation process for IL-6 movement through biofilm matrices of various dextran chain length. Therefore, it was determined that smaller molecules interact more favorably with the biofilm matrix than larger molecules, as expected. However, the average energy stabilization associated with IL-6 translocation was found to increase with increasing chain length, demonstrating a direct relationship. This contrasts greatly with the earlier results for TNF-α translocation through a generalized biofilm matrix of dextran in solution. Further simulations are necessary to determine the exact cause of this result. It is hypothesized that this contrasting result is a consequence of increased
intramatrix interactions that cause complications for SF stabilization upon translocation through the detailed biofilm EPS.

The results and conclusions reached as a result of these molecular dynamic simulations are useful in many applications, most especially in future biofilm therapeutics aimed at overcoming the drug resistant properties of bacterial biofilms. Insight into the energetic properties of molecule translocation through the host cell and biofilm EPS, as provided in this study, brings the research community one step closer to finding a chemical solution to effective biofilm treatment.

**Future Work**

It is recommended that future studies simulate the effect of chain length on the translocation of IL-6 through a generalized matrix of dextran polymers. The potential of mean force curves, in addition to matrix analysis using radial distributions functions and radius of gyration calculations, may provide useful insight into the direct relationship observed for IL-6 SF stabilization and polymer chain length. It is also recommended that a more detailed cell membrane be modeled using lipopolysaccharides and transmembrane proteins in order to better model the interactions between SFs and the host cell. The effects of ionic solutions and pH on the translocation process may also be of interest, given their better approximation of realistic biofilm environments than charge-neutral and neutral pH models.
9. References


27. Champsi, J.; Young, L. S.; Bermudez, L. E., Production of TNF-α, IL-6, and TGF-β, and expression of receptors for TNF-α and IL-6, during murine *Mycobacterium avium* infection. *Immunology* 1995, 84, 549-554.


10. Appendices

Appendix I – Atomistic and Coarse Grained TNF, IL-6, Glucoamylase, and DNA

Figure 14. Visualization of TNF-α based on its (a) atomistic description and (b) coarse-grained description.

Figure 15. Visualization of IL-6 based on its (a) atomistic description and (b) coarse-grained description.
Figure 16. Visualization of glucoamylase based on its (a) atomistic description and (b) coarse-grained description.

Figure 17. Visualization of a DNA strand in its coarse-grained description provided by MARTINI.
Appendix II – Area Per Lipid Headgroup Analysis

Window 1

**TOP:** 65.99 sq. Å

**BOTTOM:** 67.34 sq Å

![Area per lipid head group tessellations for the top and bottom leaflets of the DSPC lipid bilayer for the case where the TNF-α SF has not yet begun its approach to the surface. The area per lipid head group is demonstrably generally homogeneous in both leaflets.](image)

**Figure 18.** Area per lipid head group tessellations for the top and bottom leaflets of the DSPC lipid bilayer for the case where the TNF-α SF has not yet begun its approach to the surface. The area per lipid head group is demonstrably generally homogeneous in both leaflets.
**Figure 19.** Area per lipid head group tessellations for the top and bottom leaflets of the DSPC lipid bilayer for the case where the TNF-α SF just begins to contact the top leaflet of the bilayer. The area per lipid head group is seen to account for the bending stress associated with TNF-α contact. The first glimpse of pore formation is evident.
Figure 20. Area per lipid head group tessellations for the top and bottom leaflets of the DSPC lipid bilayer for the case where the TNF-α SF has made its way into the lipid bilayer. The area per lipid head group in both leaflets show the formation of a clear pore allowing for the translocation. Uncontacted areas remain relatively homogeneous.
Window 4

**TOP:** 34.24 sq. Å  
**BOTTOM:** 1264.25 sq Å

**Figure 21.** Area per lipid head group tessellations for the top and bottom leaflets of the DSPC lipid bilayer for the case where the TNF-α SF has passed through the lipid bilayer. The area per lipid head group in the top leaflet is highly variable, and it is clear that the bottom leaflet has become destroyed to the point of being ill-defined.
Appendix III – C++ Code for Cutting Polymer

Chain Lengths

```cpp
#include <iostream>
#include <string>
#include <sstream>
#include <fstream>
using namespace std;

int i = 0, m = 1, n = 457;
string str1, str2, str3, str4, str5;
string Integer2String, StringReplace;
stringstream convert;
stringstream convert2;
ofstream outputFile;

int numfiles;

int main()
{
    cout << "Please enter the number of files to be analyzed: " << endl;
    cin >> numfiles;
    ++numfiles;

    string begin;
    cout << "Please enter the beginning of the input file name: " << endl;
    cin >> begin;

    string exten;
    cout << "Please enter the input file extension: " << endl;
    cin >> exten;

    string edit;
    cout << "Please specify the additional text to append to output file name: " << endl;
    cin >> edit;

    int j;
    cout << "Enter the number of lines to skip: " << endl;
    cin >> j;

    cout << "Thank you. The program is now running." << endl;

    while(i<numfiles) {
        str1 = begin;
        convert << i;
        Integer2String = convert.str();
        str2 = Integer2String;
        str3 = exten;
```
str4 = str1 + str2 + str3;
Integer2String.clear();
convert.str("");
std::ifstream file(str4.c_str());
std::string line;
unsigned int line_number(1);
str5 = str1 + edit + str2 + str3;
outputFile.open(str5.c_str());
while (std::getline(file, line))
{
    if (line_number > j)
    {
        if (n < 527)
        {
            if (m == 61)
            {
                m = 1;
                n++;
                if (n == 527)
                {
                    goto perm;
                }
            }
        }
    }
    convert2 << n;
    StringReplace = convert2.str();
    line.replace(2,3,StringReplace);
    outputFile << line << "\n";
    line_number++;
    m++;
    goto stop2;
}
perm:
outputFile << line << "\n";
line_number++;
stop2:
StringReplace.clear();
convert2.str("");
}
outputFile.close();
str5.clear();
double complete;
complete = (((double)i/(double)numfiles) * 100); 
cout << "Status: " << complete << "%" << endl;
++i;
n = 457;
}
outputFile.close();
cout << "Done!\n";
cout << "Done!\n";
return 0;