Molecular Dynamics Simulations of the NF-κB Inducing Kinase:

A Computational Study of the Effects of Mutations and Environmental Stress on Proteins and their Applications to Disease Prevention

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Section I.

I have wanted to help prevent diseases to improve people's lives ever since my uncle was diagnosed with cancer when I was ten. Seeing the debilitating effects that cancer has had on my uncle has pushed me to realize that, despite the advances in medicine today, there are still so many diseases we cannot eradicate. I found it frustrating that most cancers have no effective cures. The more I learned about cancer, the more I became fascinated by the physiological causes of cancer and how it develops in the body, and the amount of unanswered questions I had stimulated my curiosity. Why do people get cancer? How do cancerous cells develop from healthy cells? How can we prevent cancer? These were the scientific questions that I hoped to answer one day.

In my studies, I began to take my scientific education more seriously, and the subject of chemistry caught my eye. I realized that chemists play an important role in studying diseases and developing treatments and cures for them. My parents quickly noticed my passion for chemistry and disease prevention, and having backgrounds in chemistry and physics, they wholeheartedly supported my interests in science with scientific discussions at the dinner table and frequent trips to local science museums. With their encouragement, I spent my childhood reading subscriptions of *ChemMatters* from the American Chemistry Society and building science fair projects to present at school fairs. I loved watching YouTube tutorials to figure out how to use the Beer-Lambert law and Avogadro's number, and I was self-motivated to further expand my knowledge by reading research studies online. These papers inspired me to hope to one day contribute to the field of chemistry and medicine by conducting research in a laboratory.

That day arrived when I reached out to Dr. Ron Smaldone, a professor at the University of Texas at Dallas (UTD) who conducts experimental chemistry research. In his lab, I

experimentally created organic nano-porous gas-separation membranes to reduce pollution and prevent lung cancer. For the first time, I was able to apply my passion for science to solve real-word issues; through it all, my education became even more important to me. As my passion for science grew, I decided that I wanted an education emphasizing science and math, so I applied to the Texas Academy of Mathematics and Science, a college-level program for high school students at the University of North Texas (UNT). When I was accepted into the program, I decided that I wanted to broaden my experiences in scientific research so I began conducting research in another chemistry lab, this time dealing with computational molecular models under Dr. Angela Wilson.

At UNT, I began researching how mutations occur within cells. I focused on using computational chemistry techniques to advance the study of cancer prevention by analyzing protein kinases for therapeutics. I chose to study the NF-κB protein family because dysregulation of the NF-κB pathway has been linked to various cancers and autoimmune diseases. After reading literature about the protein family, I discovered that the NF-κB pathway is composed of two paths. I learned that, while the classical pathway is well-known, the alternative pathway is not. NIK, the NF-κB Inducing Kinase, plays a central role in this pathway, so I decided to employ molecular dynamics simulations to study NIK. I chose to use molecular dynamics simulations since they enhance the ability to analyze NIK at the molecular level, which offers important insight into its structure-behavior properties.

My role as a researcher in my computational chemistry research group has piqued my curiosity. It's beyond exciting to know that my studies could reveal links between protein structure and disease vital for disease prevention. Conducting research consumes not just my time but my mind as well; while others may spend their weekends at the movies, I am itching to

return to the lab and check on my NIK simulations to see if the structural fluctuations are large enough this time to be significant. The tantalizing thrill of possible discovery, and the knowledge that what happens here in a lab today could hold the key to someone's life tomorrow, is the foundation for my interest in science.

While conducting research requires an extremely large learning curve that can be challenging at times, it's also exciting for me to explore this new environment, grappling with and learning about the world of computational chemistry research. Learning how to navigate command line interface was like learning to take my first steps; learning how to use scripts to create commands and run simulations was like saying my first words. Today, I am able to employ molecular dynamics simulations to analyze protein on my own. While challenging, it's worth it because I know that my research advances therapeutic technologies that can potentially prevent diseases such as cancer. Also, I have gained real-world experience, providing a glimpse into the responsibilities of an actual job.

Conducting research has influenced my plan to become a professor of chemistry to focus on cancer prevention and research on the function of proteins kinases within phosphorylation cascades. I hope to explore the effect of these kinases on gene expression to further identify how cancer develops within healthy cells, advancing therapeutic methods that target oncogenic proteins. My experience in research has shown me how much there still is to discover and learn. I look forward to continuing my work in scientific research for cancer prevention and treatment.

Section II.

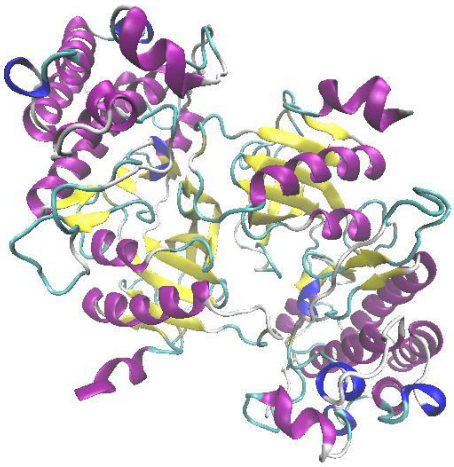
In the Computational Chemistry lab of Dr. Angela Wilson, I have contributed to understanding the signaling mechanisms associated with the onset and progression of

oncogenesis. Specifically, my work has focused on the dysregulations within the nuclear factor (NF)-κB signaling pathway, as aberrations within this pathway are associated with various immune responses. Although there are many ways in which NF-κB activation can be triggered, previous studies have found that the release of the NF-κB dimers are regulated by two main pathways. Of these two pathways involved in NF-κB activation, the more well-known is the classical (also called the canonical) pathway, which relies on the activation of the catalytic sub-units of the IκB kinase (IKK) complex to target NF-κB1. In contrast, the lesser-known alternative (or non-canonical) pathway is dependent on the activation of the NF-κB Inducing Kinase (NIK), which activates NF-κB2. Thus, I chose to study the NIK protein kinase because its importance as a central component in the alternative pathway makes it a prime target for disease-prevention mechanisms. I aim to demonstrate that shedding light on how NIK maintains its structural integrity for its functions will reveal potential ways of manipulating the activity of NIK, thereby improving the understanding of disease prevention via therapeutics.

While previous research on NIK focuses, in the main, on crystallizing a static structure, these crystallographic studies are unable to demonstrate how the protein actually behaves within a living cell. To achieve a better understanding of NIK functionality, I employed computer simulation and modeling techniques to offer stronger insight into why structure-function properties of NIK are dependent on their intracellular microenvironments. I decided to investigate the properties of NIK via computer simulations rather than use traditional experimental techniques. I did so because computational studies reduce human error while enhancing the ability to observe the NIK at the atomic level and in slower time frames. I was mindful, moreover, that molecular-dynamics simulations illustrate how a dynamic protein interacts with its environment, thus simulating the protein's behavior. By investigating both the

inactive and active states of NIK and how it responds to gradient intracellular ionic strengths, I contributed further insight into the effects of altered microenvironments and phosphomimetic mutations on NIK. Knowing how the structure-behavior properties of NIK change will result in the development of therapeutics that stabilize NIK and subsequently prevent dysregulation of the NF-κB pathway. That, in turn, stands to lead to disease prevention.

To obtain an accurate model of the NIK structure on which to conduct molecular dynamic simulations, I used the initial coordinates of the tertiary structures of NIK from the Protein Data Bank (PDB ID: 4DN5), a database of biological molecules resolved via x-ray crystallography. I then added solvent to the NIK protein structure via LEaP, a program that built the models for atomistic simulations, to properly simulate an intracellular environment. In order to compare the active and inactive states of NIK, I introduced the S549D phosphomimetic mutation in the dimer, since previous research has mentioned that the mutation may result in an active state of NIK. I hoped that investigating the inactive and activated states of NIK would show how dimerization changes upon activation. Once I fully prepared the systems, I employed the molecular dynamics simulation software from AMBER to propagate the positions and velocities of the systems and run calculations. I displayed the finished simulations via VMD and Chimera to help visualize and analyze NIK's structure and behavior. My use of computational techniques to calculate the time-dependent behavior of NIK provides detailed information on the fluctuations and conformational changes of the protein that cannot be found experimentally.



Molecular Dynamic Simulation of the NF-κB Inducing Kinase (NIK) Structure

I discovered that the S549D mutation induces conformational changes that make the NIK dimer more exposed to solvent. Additionally, I found that the presence an ion gradient within the solvent led to greater flexibility in NIK's structure, indicating that NIK is sensitive towards environmental changes. The enhanced exposure induced by the mutation, combined with NIK's sensitivity towards ions, allowed me to identify local conformational changes in the C-terminal and N-terminal regions. These domain fluctuations result in changes in NIK's catalytic behavior, since they have been previously acknowledged as sites where NIK can bind or signal to molecules such as TRAF3. My findings are supported by my discovery that the presence of the ionic buffer alters hydrogen bonding networks and results in changes in the bonding patterns near Thr559, the site of phosphorylation. I concluded that the changes in intracellular environment, along with the S549D mutation, result in changes in NIK's phosphorylative behavior, subsequently causing the protein to deviate from its normal kinase functions within the NF-κB alternative pathway. My research provides insight that scientists can use to design therapeutics that specifically target these problematic domains in order to prevent the onset of diseases and cancers. Keeping in mind that changes in intracellular ion concentrations perturb the structural features and dynamics of NIK, my results allow for adjustments in therapeutic delivery methods to more strongly control the regulation of inflammatory pathways. I was able to reveal further insights into the NIK structure and behavior by pinpointing potential target areas for therapeutic design, allowing for the development of effective therapeutics and drug-delivery to combat the dysregulation of NF-κB pathways to prevent diseases.

My research identified important domains on NIK's structure that are susceptible to changes that can cause mutations. Because a protein's structure dictates its function, understanding correlations between changes in NIK's structure leading to NF-κB pathway

dysregulation and subsequent activation of incorrect genes can provide crucial stepping stones to both healing and preventing NIK-pathway related diseases. The insight that my research provides into NIK will directly affect therapeutic and drug-delivery mechanisms as well as techniques for effective treatment. Scientists interested in studying NF-κB pathways and pharmaceutical companies developing NIK aberrant functionality prevention mechanisms alike will be able to make advancements based on the data that this research provides. Using my research data, these groups will be able to develop and test therapeutics to stabilize NIK's structurer and prevent dysregulation of the NK-κB pathway, which will benefit the entire population.