

Since youth I have always desired to study science. As a knowledge seeker, reading books and journals have always been a major aspect of my existence and these qualities have consistently helped me during the development of my career. However, my learning is not confined to the seclusiveness of text, as the public seminar has become an outlet for my passions. Stony Brook University (SBU) and Brookhaven National Labs (BNL) both attract professional, accomplished experts whose work at the interface of medicine and engineering have enlightened a plethora of disease pathways resulting in novel treatments. Fortunately, many of these doctors, engineers, physicists and chemists enjoy presenting their work and I find myself consistently taking advantage of my ability to personally interact with them.

In 2004, I was fortunate enough to meet Lisa Miller, a beamline scientist at BNL. Following her seminar at SBU during which I asked many questions on the applications of synchrotron biosciences, I was invited to participate in a summer internship program. During a tour of the National Synchrotron Light Source (NSLS) I met several colleagues including my current PI, and quickly realized that their access to specialized equipment and outstanding projects would provide a phenomenal opportunity to conduct research.

My primary commitment to becoming a scientist is in hopes of contributing knowledge to the global scientific forum. I was attracted to the field of biomedical engineering due to the advanced quantitative analysis and multidisciplinary approach of uncovering novel medical treatments. The agglomeration of skills and knowledge from a diverse field set attracts experts from all scientific disciplines extensively impacting translational results. The proceeding application addresses the design of a high-throughput drug discovery tool to protein for small organic and peptide inhibitors. The proposed experiment can even be tested on proteins whose functions are unknown. Initial experiments have been performed on an erythroviral capsid protein, though any protein of interest can be tested.

Specifically, my experience is in the structural biology of viral capsid proteins. These geometrically distinctive assemblies fold into an icosahedral shell responsible for interacting with the host. This process involves attachment of the parvoviral capsid or envelope, in the case of retroviruses, to one or more cellular receptors. These mechanisms are constantly being examined by x-ray crystallography, cryo-electron microscopy, titration calorimetry and surface plasmon resonance to deduce protein-protein interactions. It has been demonstrated that multiple transmembrane glycoprotein and glycolipid receptors exist for many viral species. Important examples of receptors include CD4 glycoprotein for HIV and glycolipid globoside for B19 parvovirus. Upon binding, viral subunits experience a conformational change, revealing buried residues necessary for downstream coreceptor attachment or membrane fusion.

Advancements in x-ray protein crystallography have led to the rational design of many antiviral drugs approved by the FDA. While this method can disclose the detailed atomic configuration of a diseased protein, macromolecular crystallography is suboptimal for the colossal screening effort required to discover new pharmaceutical targets. Its strength resides in one's ability to discover covalent, hydrophobic, ionic, and sometimes hydrogen bonding interactions between protein-substrate complexes. Protein crystallography will reveal the unique ligand pose at the binding site illustrating coordinated interactions at atomic resolution. Many drugs can be designed with this information and co-crystallized with the protein under investigation. Unfortunately, the time for crystal growth often exceeds a year disallowing the method from becoming a high-throughput technique before finding crystallization conditions.

X-ray crystallography has the inherent ability of capturing the lowest energy state at which a biological macromolecule may slowly precipitate out of solution into crystals. However,

proteins and nucleic acids are dynamic structures which experience multiple transitions in response to thermodynamic, electrostatic and steric forces found in the environment. Other weaknesses lie in the difficulty of growing crystals resulting from protein insolubility, lipophilicity, post-translational modification or flexibility hindering ordered lattice formation. For example, the carbohydrate cloak surrounding HIV envelope glycoprotein gp120 prevents the native glycosylated state from being crystallized. Only the excisions of specific carbohydrate moieties make gp120 amenable to this technique. At the expense of viewing the original structure, a three-dimensional arrangement can still be found lacking these modified residues. This is significant because many epitopes on the surface of HIV are carbohydrates which initiate the humoral immune response.

As a student at Stony Brook University I was privileged to participate in a year long course entitled "HIV/AIDS Peer Education". This socially directed academic work in HIV public education demonstrated the necessity of new treatments and preventative measures. A major component of the class was open communication with students in the university context about HIV prevention, testing, and respect of peoples from all cultures and backgrounds. Throughout the year we focused on demographics most at risk and discussed the impact of public services on HIV transmission and risk education. As an HIV Peer Educator, our goal was to communicate with students about risk, determine approaches for reducing it, and motivate positive lifestyle changes. We provided ongoing support promoting behavior modification, for until a cure for HIV is found, education is the only means of prevention. Other aspects of the class included an analysis of white and male privilege and discussion of risk factors linked to socioeconomic conditions which encourages one to challenge assumptions made about those infected with HIV.

The proposed research will test new methods for discovering small molecules that alter the structure of pathological proteins. These assays will use high-energy synchrotron radiation to demonstrate conformational transitions, if evident, in a protein solution upon contact with a ligand. This is significant because deformations of a protein's folded structure are frequently indicative of a disappearance or alteration in function. Current methods for discovering drugs rely heavily on enzymatic assays which assess the cumulative generation of reaction products. The enzymatic assay is specific for the activity of a protein it was designed to quantify. This method allows pharmaceutical companies to compare binding affinities between ligands, though it comes at the expense of fabricating a new assay for each disease target. It also assumes that the protein's gene is well characterized, etiological role understood, and function known. However, the function is unknown for hundreds of thousands of proteins that play important physiological roles in the normal and disease states.

Funding of this project would allow graduate students to gain understanding of the synchrotron biosciences which have greatly contributed to the understanding of proteomic medicine. This innovative project seeks to harness synchrotron radiation circular dichroism and couple it to high-throughput screening (HTS) approaches. Additionally, the speed of small-angle x-ray scattering will be used to rapidly assess the conformation of proteins with and without ligands. These methods are at the forefront of biomedical engineering with potentiality for an immersive learning experience at the NSLS. Student education in these techniques will encourage future experimenters to consider tools not traditionally found in the laboratory environment, but are accessible at many synchrotrons worldwide. Specifically, an NSF grant would stimulate engineers to produce new systems invigorating a publicly available method of HTS. Current roadblocks exist in end station construction hindering HTS implementation, but could be solved by fabricating new designs.