I am delighted to find out about the open assistant professor, Biology position in Brooklyn College, CUNY and I am submitting my application for the position. I have over 14 years of experience in molecular biology, biochemistry and structural biology as well as over 6 years of experience in T cell immunology. The assistant professor role in Brooklyn College, CUNY will provide me the ideal platform to explore new immunotherapy opportunities as outlined below. In recent years, advances in immunotherapy has generated a significant amount of progress to the treatment of cancer. As described below, I expect that my laboratory will pursue biochemical and structural approaches to investigate avenues to modulate T cell signaling to target specific immunological conditions, particularly specific forms of cancer. In addition to my research goals, my extensive experience teaching laboratory courses at City College of New York, as well as in mentoring undergraduates and graduate students in their research work should provide an excellent foundation as for my educational and training goals in a multi-cultural environment.

Understanding basic biological processes has been my life’s quest since my high school days. I majored in biotechnology as an undergraduate in India where I was exposed to different areas in the life sciences as a research volunteer in Anna University’s SPIC bioprocess lab and at the Indian Institute of Science, the latter being a premier research institution. Following this experience, I chose to specialize in biophysics and structural biology during graduate studies. During my graduate research, I became deeply familiar with high-resolution NMR spectroscopy in addition to other biophysical approaches. Under the guidance of Dr. Ranajeet Ghose (graduate advisor, *see referee letter*), at CCNY, I worked on two different classes of proteins to understand their structure/function relationships. These include ASC2, that is involved in the activation of the caspase-1 pathway that is central to the inflammatory response. I also solved the structure of a novel phosphoesterase domain in bacterial DNA ligase, LigD, involved in non-homologous end-joining.

An interest stimulated in graduate school during my study of ASC2 and enhanced during the final years, was in immunology, in particular cancer immunology. I was ready to apply my expertise in protein structure and dynamics using NMR techniques into understanding immunological problems. I started working with Dr. Michelle Krogsgaard at New York University Medical Center who specializes in studying molecular and cellular events that contribute to T cell sensitivity to cancer antigens. In Dr. Krogsgaard’s laboratory in worked towards understanding molecular mechanisms involved in T-cell activation by applying a suite of biophysical techniques, which includes NMR, X-ray crystallography, 2D affinity, computation and functional studies. Based on these studies, I published a paper on the structural model of the T cell receptor (TCR)-CD3 complex in *Cells Reports*. The results from this work will form the basis of my research during the coming years. This work is unique and orthogonal to that which will be pursued by the Krogsgaard laboratory in the future (*see referee letter*).

Antigen recognition of peptide-major histocompatibility complexes (pMHCs) by T cells, a key step in initiating adaptive immune responses, is performed by the TCR bound to CD3 subunits (CD3γε, CD3δε and CD3ζζ). TCR recognition of cognate pMHC initiates a signaling pathway involving the CD3 subunits that leads to multiple outcomes including cytokine production, proliferation, thymic selection and differentiation to different T cell subtypes. Adoptive T cell therapy (ACT) using T cells transduced with engineered antigen-specific TCRs or chimeric antigen receptors (CARs) are promising strategies for improving anti-tumor responses and have also shown responses solid cancers. Although some success has resulted from targeting antigens that are upregulated or overexpressed (self-antigens), these antigens are not efficiently recognized by T cells because generally such specificities are negatively selected in the thymus. Affinity-enhanced TCRs for antigens expressed in tumors have resulted in cross-reactivity and severe toxicity. Therefore, new approaches that target different components of the TCR complex, including TCR-CD3 and intracellular CD3 signaling components, are required to enhance T cell responses towards tumor-associated antigens in order to improve upon existing therapies. A molecular level understanding of how TCR-pMHC interaction triggers intracellular signaling is still lacking. My proposed research will help identify how ligand binding to the TCR is transmitted to CD3 subunits and the intracellular machinery that is critical for governing T cell functionality, and will identify new molecular targets for improving therapeutic T cell responses in cancer and T-cell mediated autoimmunity.

*Nature of the TCR-CD3 Extracellular Interface:* The first part of my research concerns solving the structure of extracellular TCR-CD3 complex. Earlier, I had identified parts of the TCR constant domain that interacts with CD3 subunits using chemical shift perturbation analysis and modeled the TCR-CD3 structure. However, this model does not include interface epitopes from the CD3 side. I would use solution NMR to assign individual CD3γε and CD3δε extracellular subunits and perform chemical shift perturbation analysis with unlabeled TCR to identify TCR interaction residues in CD3s. This will enable me to create a more complete model of TCR-CD3 complex computationally. Further, I will also use paramagnetic relaxation enhancement (PRE) to calculate the structure of the TCR-CD3 complex. Incorporating single paramagnetic tags onto specific TCR regions and titration with labeled CD3 subunits would accomplish this by generating distance restraints that can be used for structure computation. The results from this research help understand mechanistic basis of early stages of T cell activation and identify the critical interaction residues on CD3 and TCR obtained from PRE based structure. I will then identify critical mutations in the TCR-CD3 interface that enhance TCR-CD3 interactions using computational approaches. These new TCR clones against various different antigens will be expressed in T cell lines and their CD3 affinities will be tested by tetramer and functional assays. The functional clones will be transfected into TCR retrogenic mice and their ability to survive thymic selections and functionality will be analyzed. I expect that this integrated biochemical/biophysical, cellular and *in vivo* approach to be the hallmark of my research operation.

*Nature of Intracellular Conformational Changes Induced on CD3 by TCR Binding:* Another major research area my laboratory is expected to pursue concerns the intracellular components of TCR-CD3 signaling complex. Antigen binding to TCR leads to conformational changes resulting in CD3 subunit reorientation, which in turn likely regulates the binding of downstream signaling proteins to CD3 cytosolic regions. The TCR lacks intracellular signaling domains but instead interacts with CD3 subunits, each possessing intracellular (IC) intracellular tyrosine based activation motifs (ITAMs) for phosphorylation. The structural features of the CD3 IC regions are largely unknown but some are known to interact with the plasma membrane. Recently, cholesterol has been shown to play an active role in TCR regulation between active and inactive conformations. My research will address this difference in conformation states of the intracellular CD3 subunits induced by cholesterol, primarily by NMR spectroscopy. Upon antigen ligation, the IC CD3 ITAMs become amenable to Lck phosphorylation and subsequent ZAP-70 interaction. However there are other important regulatory/adaptor proteins such as Nck, Esp8, Numb and WASP that are required for immunological synapse formation and actin reorganization. I will also study the critical structural features in the IC CD3 responsible for these interactions through NMR and mutational analyses. Together, this information will provide insights on the molecular basis of proximal signaling involving CD3 cytosolic regions, which can be pharmacologically targeted to create better T cells with enhanced signaling.

I expect that I will further expand my research goals to include animal tumor models. Given the strong fundamental bent and the ultimate translational importance of my research, I expect to be able to apply for federal grants using the results that can be obtained rapidly following once my laboratory has been set up. I expect that I will use the R01 mechanism for funding through the NIAID (or the NIGMS) or the early career MIRA mechanism through the NIGMS. At Brooklyn College, I expect to also have access to the SCORE mechanism. Given that my proposed research has tremendous potential, as we would be able to modulate TCR signaling pharmacologically using antibodies, modified T cells, chimeric antigen receptors and small molecules to target specific immunological conditions and particularly cancer, partnerships with the pharmaceutical industry are also possible. I have experience in this sort of partnership through mycurrent

Given the highly multi-disciplinary nature of my postdoctoral research, I have had significant experience in establishing strong collaborations with scientists with complimentary skills e.g. Dr. Cheng Zhu (2D binding kinetics) and Dr. Timothy Cardozo (computation biology, *see referee letter*). I expect that my experience of working in a collaborative environment will enable me, in my independent career, to work with researchers who have complimentary skills. This will allow me to expand on the complexity of problems that can be tackled in my laboratory.

As mentioned above, I have significant teaching experience in CCNY, which has a multiethnic and multicultural student population similar to Brooklyn College. I was actively involved in teaching and mentoring undergraduate students at the CCNY where I taught general chemistry and physical chemistry laboratory courses for 6 years. I have supervised undergraduate students and rotation students in their research work (at CCNY and NYU) and those were the times when I realized my commitment to the next generation of STEM trainees that I decided to pursue a career in academia.

The facilities at Brooklyn College would provide me the ideal platform to perform my research, including access to NMR facilities at New York Structural Biology Center (NYSBC) and the newly established NMR, mass-spectrometry and crystal screening facilities at the ASRC. My research will be interdisciplinary and compliments into the widespread portfolios of research undertaken in the Chemistry department at Brooklyn College. With my expertise in biophysics I expect to establish collaborations with several faculty with complimentary skills. Dr. Shaneen Singh (bioinformatics and computational biology), Dr. Barbara Studamire (retroviral-host protein interactions), Dr. Peter Lipke (cell adhesion) and Dr. Anjana Saxena (cell signaling) come to immediately mind. Close interactions with other faculty will certainly increase the potential for other collaborations.

I would like to thank Brooklyn College for this opportunity to submit my application for this position and I hope that the search committee will give an opportunity to showcase my potential.