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November 4, 2005

Professor
Department of Biology
Indiana University,
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Dear Prof. Brun:

I am writing to apply for a tenure-track position at the assistant professor level. The position was brought to my attention by an advertisement in *Nature Jobs.*

In my research, I focus on developing a mechanistic understanding of intertwined physical processes over a wide spectrum of length and time scales pertaining to complex biological systems. Multi scale theoretical approaches, rooted in statistical mechanics, combined with experimental observations in a synergistic manner are effective for gaining such insight. I was fortunate to work in that direction with Prof. Arup K. Chakraborty, where I studied signal transduction in T cells. My background makes me well suited to guide a research program that applies such theoretical and computational methods to solve fundamental problems in biology with genuine biomedical and technological importance.

I envisage my interests and background will allow me to fit well in the department of Biology and the Biocomplexity institute while at the same time the proposed research projects will complement the strengths of the present faculty members.

Enclosed please find my curriculum vitae, a list of references, a summary of my previous research, a brief research proposal and a statement of teaching interests. Please do not hesitate to contact me if you would like any further information. I look forward to hearing from you soon.

Sincerely yours,

Jayajit Das

Theoretical Modeling of Natural Killer Cell Immune Response

Jayajit Das

Immune responses in vertebrates are manifestations of intertwined processes spanning a wide range of length (from angstrom to micrometer) and time (nanoseconds to hours) scales. Advances in experimental techniques in last few years involving methodology to create transgenic animals and video microscopy imaging methods have enabled us to make quantitative measurements, and visualize the consequences of cooperative dynamics processes that occur on different scales. It is important to develop a mechanistic understanding that integrates different observations originating from the same underlying processes, and then use the gleaned physical insight to drive future experiments. My research will be focused on theoretical modeling, rooted in statistical mechanics, of such processes which can successfully describe cooperative processes in complex systems[1-4]. A key component of my research is to synergize the theoretical approaches with experimental observations. A hallmark of my research career has been close collaborations with leading experimentalists in the fields I have worked on and I will continue developing such collaborations in future. During my research career, I have specialized in applying principles from statistical mechanics to understand activation and signaling in T lymphocytes[5], in addition to studying single molecule and collective properties in polymer systems and magnets. In the projects described below, I will focus on understanding immune responses of NK cells, a special type of lymphocytes which are key players in the innate immune system in vertebrates. I will use multi-scale approaches combining complementary particle based methods[6,7] (Monte Carlo, Brownian and Molecular dynamics) and coarse grained field theoretic techniques[8,9] (Smoluchowski equation, Langevin dynamics) to investigate the activation and signaling of NK cells and their interactions with the members of adaptive immune system. The theoretical studies in conjunction with experiments (carried out by collaborators) will uncover fundamental mechanisms behind NK cell immune surveillance that may to help develop strategies for therapeutic interventions when things go awry.

Signal transduction in Natural Killer (NK) Cells

Natural Killer (NK) cells[10,11] provide the first line of defense against pathogens and tumor cells. The activation of NK cells depends on a subtle dynamic balance between the signals generated by its inhibitory and stimulatory receptors. However, mechanisms underlying NK cell recognition of potential target cells or tolerance to self tissues are not well understood[12-15]. The projects described below will focus on understanding the essential aspects of activation and signaling of NK cells based on computational modeling of the signal transduction pathways involving various receptor ligand binding interactions. Furthermore, this study will suggest approaches to control and engineer the immunological response of NK cells for therapeutic purpose[16-18]. The investigation will be synergistic with experimental work in other labs. In the following projects, *in silico* models involving the reaction pathways in the signaling network will be exploited in understanding the role played by different components (*e.g.,* receptor lignad binding, phosphoryation/dephosphoryation, feedback mechanisms) in the network. My

experience with modeling activation and signaling in T-cells using field theoretic[5] (Smoluchowski equation) particle based[19] (Monte Carlo) simulations will be useful in guiding the projects. My record of working synergistically with experimentalists also will be important for the future.

Understanding NK cell self tolerance: Recent experimental findings[14,15] have challenged the central dogma behind NK cell self tolerance which asserted that all NK cells possess at least one inhibitory receptor specific for self Major Histocompatibility Complex (MHC) in order to be tolerant to autologous cells. In one experimental study[15], NK cells from normal mice that lack all known inhibitory receptors specific for self MHC class I produced considerably less IFN-γ (can be used as a measure of NK cell activation) compared to the NK cells with inhibitory receptors when stimulated by cross-linking the NKR-P1C (an activating NK receptor) receptor with antibodies. However, explanation for such non-intuitive results will require understanding of the signaling pathways (Fig.1a) involving stimulatory (such as NKR-P1C)[12] and inhibitory (such as, KIR, NKG2A)[12] receptors in NK cells. The signal transduction pathways[20] in NK cells suggest a nonlinear relationship between the binding affinities of the peptide (self or non-self) molecules to NK cell receptors and NK cell effector functions like cytokine production. The non-linearities can arise from various co-operative mechanisms[21] associated with signaling, for example, proteins with multiple binding sites (*e.g.*, LAT), feedback loops or inhomogeneous spatial distribution of molecules on the cell membrane and in the cytosol favoring proximal signaling pathways. Co-operative mechanisms, as those described above give rise to multi-stable states in gene transcription pathways in *E. coli.*,[22,23] which may underlie the counter intuitive results for NK cell self tolerance. I will start the investigation by modeling the signaling pathways using kinetic Monte Carlo and coarse-grained field theoretic approaches.

Effect of stochastic nature of number and expression of inhibitory receptors on self non-self interactions: The NK receptors are acquired in a stochastic manner in the bone marrow[24]. Therefore, different NK cell will posses different types and number of stimulatory/inhibitory receptors. Since NK cells do not undergo clonal selection[10] like T-cells it is not clear how all the NK cells can be self-tolerant with a range of receptor expression. However, this may suggest a switch like response of the NK receptors. A systematic study of NK cell activation dependence on the stochastic nature of receptor expression will help develop an understanding the physical principles behind NK self-tolerance. I will use stochastic particle based simulations (kinetic Monte Carlo, Gillespie method[25]) for the study.

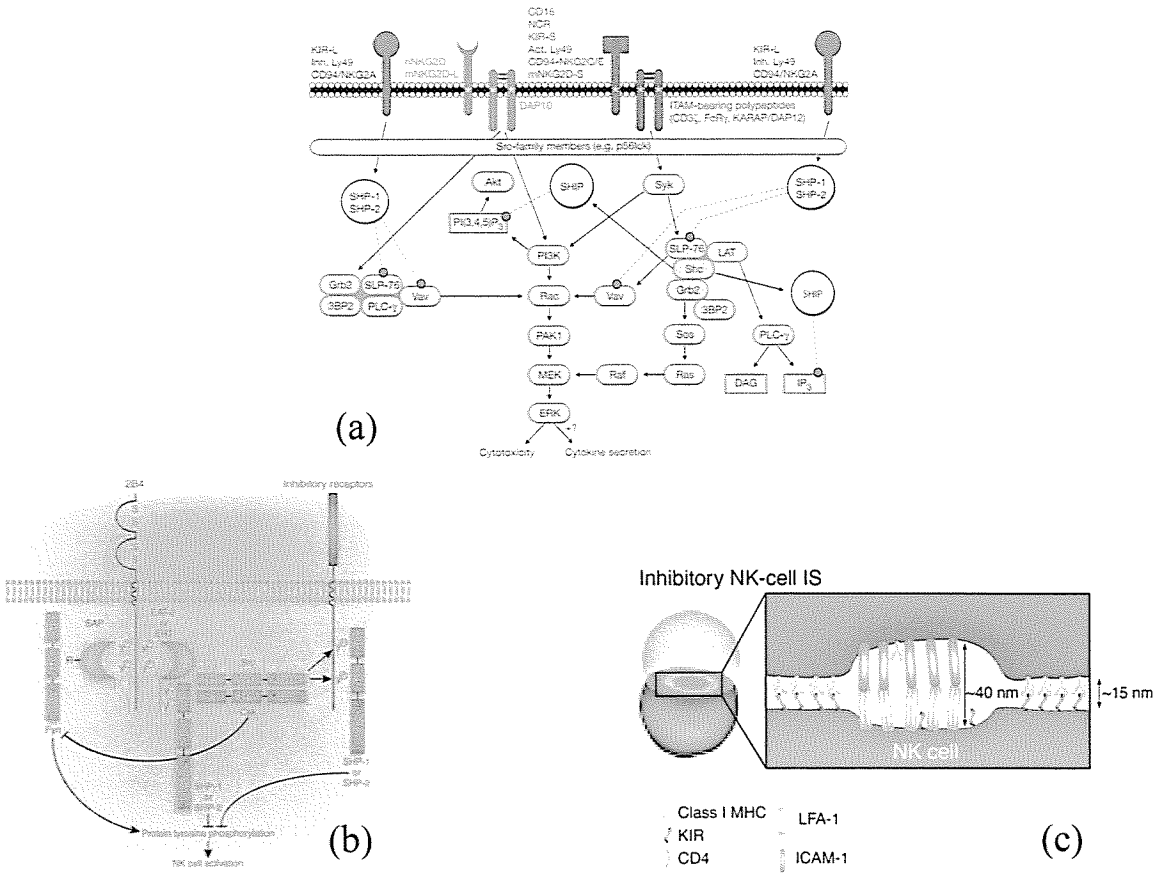

Figure 1 (a) NK cell signaling pathways [20]. m and h denote mouse and human respectively. * indicates charged amino acid. (b) Represent three different potential pathways for 2B4 signaling [26]. Adapter EAT-2 or ERT directly recruit phosphatases SHP1 or SHP2; EAT-2 or ERT activate Src kinase which phosphorylate ITIM leading to recruitment of SHP1/SHP-2; EAT-2 or ERT activate Csk which negatively regulates Src kinase Fyn. (c) Shows receptor lignad clustering. Activating receptors cluster in the cSMAC region.

Uncovering 2B4 receptor signaling pathways: 2B4 receptor[12], a member of a family of CD2 proteins, can mediate both inhibitory and stimulating responses[27,28] in NK cells, depending on the type of cytoplasamic adaptor molecules that are recruited after the receptor is phosphorylated. However, how different adaptors (e.g. SAP for activation or ERT for inhibition) generate such bipolar responses is not clear. Proposed 2B4 signaling pathways are complex and involve interaction with immunoreceptor tyrosine-based inhibition motifs (ITIMs) associated with other inhibitory receptors [26,27]. Modeling of 2B4 pathways will help test different hypothetical pathways (Fig. 1b) and help understand its role in self tolerance of NK cells, or NK cell mediated responses to viral infections that upregulate ligands (e.g. CD48) that activate 2B4. I will use coarse-grained field theoretic models to start the exploration of the signaling pathways.

Modulation of NK cell signaling by receptor clustering: Spatial migration of receptors, relevant to the lignads expressed on target cells, occurs in the inter-cellular junction as potential target cells are engaged by NK cells. The supra-molecular cluster of the migrated receptors, also known as immunological synapse[29] (Fig. 1c), modulates activation and signaling of NK cells[29,30]. Understanding the effects of this spatial organization in regulating NK cell activation and signaling will provide insights in analyzing results from experiments, at the same time will suggest ways to control NK immune response for therapeutic purpose. I will use coarse-grained field theoretic models to start the investigation.

Cross-talk between NK cells and dendritic cells (DC)

Dendritic cells present antigens to T-cells and B-cells and initiate adaptive immune responses[10]. Immature dendritic cells are resident in peripheral organs and at mucosal surfaces where they recognize foreign antigens by Toll like receptors (TLR)s, and upon maturation, they travel to the nearby lymphoid node and present the antigens to T-cells[10,11,18,31]. The interaction between immature DCs and NK cells can result in maturation or elimination of the dendritic cells[18]. Thus the crosstalk between NK cell and DCs regulates the ensuing adaptive immune response. The outcome of the interaction between DCs and NK cells depends on the delicate balance between the stimulatory and inhibitory responses generated during the interaction. However, the underlying mechanisms of the interaction are not known and will have implications in development of tumor vaccines or minimization of toxic side effects of immunotherapeutic drugs[17,18]. The projects described below are directed towards finding the essential processes that control the outcome of the interaction between the DCs and NK cells. The above projects will involve processes ranging from sub cellular pathways to dynamics of many cells. The results from the first set of projects will be used. Simulation techniques combining particle based methods (kinetic Monte Carlo, continuous time Monte Carlo) with coarse grained field based methods will be implemented for the study.

Dependence of NK-DC interaction on the type of pathogens and NK/DC ratio: Host cells infected by pathogens express ligands that can be recognized by either DCs (through TLRs) or NK cells, or by both of them. Recognition of the ligands is followed by activation of the cells that engage the target cells. Activated DCs secrete cytokines[18,32] (IFN-α, IFN-β, IL-2, IL-12, IL-18) that can prime and proliferate NK cells, and cytokines of interferon type I (IFN-α, IFN-β) attack the infected target. Activated NK cells also secrete cytokines and cytolytes that can eliminate the infected targets. However, cytokines like, IFN-γ, and tumor necrosis factor (TNF) induced by NK cell activation help immature DCs to maturate[33]. The activated NK cells can lyse immature DCs expressing ligands for NK cell receptors NKp30 or NKG2A[18,34]. Tumor cells, on the other hand, can only activate NK cells, and upon activation NK cells can induce maturation in DCs[32]. NK cells or cytotoxic T-cells (CTLS), activated by mature dendritic cells eliminate the tumor targets. Thus the result of the interaction crucially

depends on the balance of expression of non-self and self-peptides, concentration of NK cells, immature and mature DCs, pathogens or tumor cells, and migration of NK cells, DCs and CTLs at the site of infection. Several studies found that the outcome of the NK-DC interaction depends on the ratio of NK cells to DCs: a low ratio favors DC survival and maturation and a high ratio eliminates immature DCs and inhibit DC maturation[18,34,35]. A computer modeling of NK-DC interaction will include results from the first set of projects to describe how a single NK cell interacts with another DC or an infected target, which will be then combined with more coarse grained models describing multi cellular cross talk among the players of innate (NK cell, DC) and adaptive immunity (CTL) and the target cells.

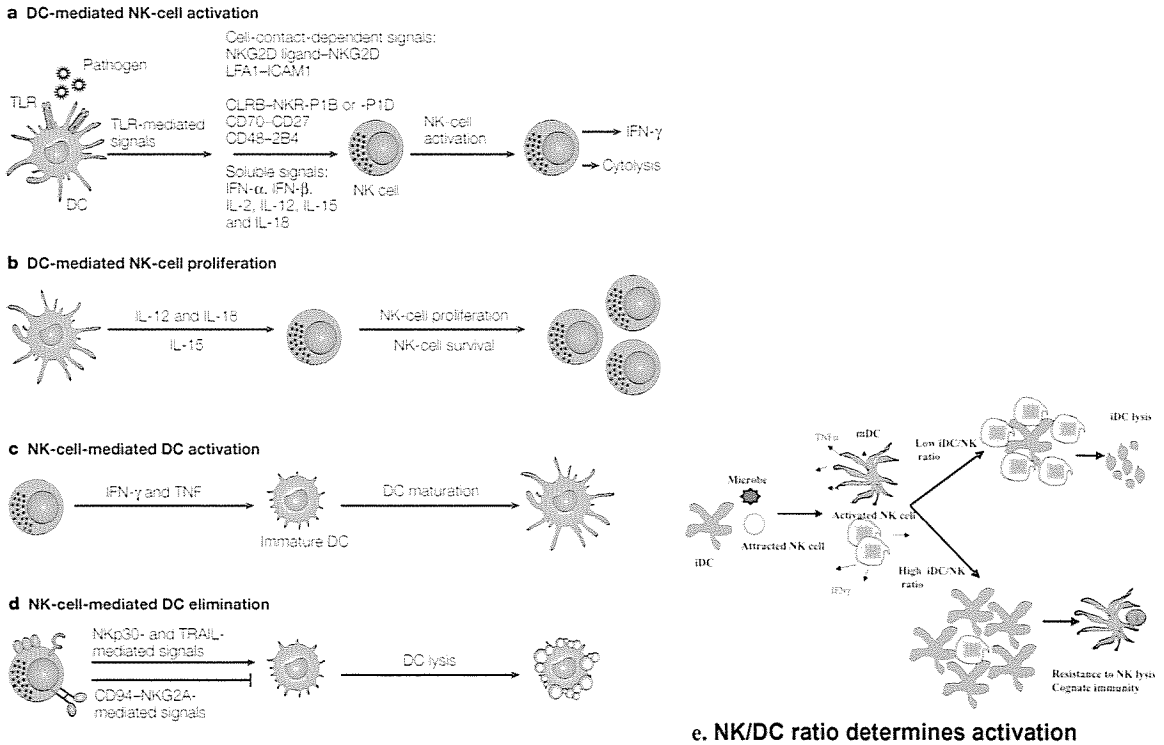

Figure 2 (a,b,c,d) Results of NK/DC interactions. (e) DC maturation of elimination depends on NK/immature DC ratio.

Effect of receptor clustering on NK-DC interactions: NK-DC contact interactions are important in their mutual activation or elimination of immature DCs[18]. Receptor clustering at the contact region of NK cell and DC can modulate the ensuing signaling[36]. As in the case of T-cells where the signaling depends on receptor clustering and receptor-ligand binding rates, NK-DC interactions may also be controlled by analogous

factors. The project will quantify the modulation of NK/DC signaling due to receptor ligand clustering. Coarse grained field theoretic or kinetic Monte Carlo methods, similar to that of used to study T-cell signaling can be employed for that purpose. The results will be combined with the entire signaling network of NK-DC interactions described in the previous project.

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Summary of Previous Research

Jayajit Das

My research has been focused on understanding cooperativity between different physical processes over a wide spectrum of length (10Å-100 mm) and time (nanoseconds to minutes) scales. A variety of computational techniques and analytical tools acquired during my research career enabled me to elucidate mechanisms that give rise to complex spatio-temporal mesoscale characteristics. A hallmark of my research has been collaborations with leading experimental groups in the fields I have worked on. I strive to integrate theoretical insights with experiments with a view towards impacting mechanistic understanding of problems that are of genuine bio-medical and technological interest.

With Prof. Arup K. Chakraborty, Massachusetts Institute of Technology(MIT), I studied activation and signaling of T lymphocytes. In particular, we investigated how activation and signaling of T cell receptors(TCRs) depend upon clustering of the receptors (immunological synapse formation) and the quality of antigen using *in silico* models. We show, using our computer simulations and synergistic experimental studies in Prof. Andrey Shaw's group(Washington Univ., St. Louis), that TCR signaling in the immunological synapse depends non-linearly on antigen quality. For example, a weaker agonist stimulates more signaling in absence of TCR clustering than the wild type agonist which induces synapse formation. Furthermore, our study demonstrates how the nonlinear relationships between synapse formation, antigen quality and TCR signaling and degradation can give rise to apparently conflicting experimental results. We are currently studying the effect of self peptides and antagonists on TCR signaling. These studies may unravel fundamental mechanisms underlying T cell activation that may also be useful for understanding the origins of certain autoimmune disorders.

I developed a coarse grained model for dendronized polymer chains which have the potential to be building blocks for important applications in nanotechnology and biomimetics. This work was done in collaboration with Prof. Arup K. Chakraborty, at the University of California, Berkeley (UCB) when he was there and Prof. Jean M. J. Fréchet (UCB). The rigidification of the backbone chain upon changing the density and branching ratio of the dendron units attached to it is reflected in our Monte Carlo simulations which are in excellent agreement with the experiments done by Prof. J. M. J. Fréchet's group. The dynamics of the single chain reveals that the backbone chain carrying large generation dendron units is a *single molecule glass,* a first example of such a novel macromolecule.We also investigated the self-assembly of dendronized chains in solution using a field theoretic computer simulation method which is more efficient than particle based simulations (Monte Carlo or Molecular Dynamics). We find different phases from lamellar to gyroid phases and nematic phases in the system. The phases we uncover may be useful for making bio-mimetic ion channels.

We also studied the ordering transition in crosslinked diblock copolymers using coarse-grained field theoretic methods (Random Phase Approximation, Replica theory). These materials have applications as, actuators or artificial muscles. The experiments performed

by Prof. Nitash Balsara's group, UCB, are synergistic with our theoretical work. Our calculations also helped to gain insight into the novel ordered solid phase of this system.

Prior to joining Prof. Arup Chakraborty's group, I collaborated with Prof. Uwe Täuber (Virginia Tech.) as a postdoctoral fellow on investigating the dynamics of flux lines in high temperature superconductors using Monte Carlo simulations. High temperature superconductors have important applications in sensors (SQUID), high field permanent magnets and superconductive electronics. The major result of our study was the identification of the type (point or extended columnar) and the spatial organization (random or ordered) of defects or pins, introduced in the material to reduce energy dissipation, from voltage noise and voltage-current characteristics data measured in experiments.

As a graduate student with Prof. Madan Rao, Raman Research Institute, Bangalore, India, I studied the cooperativity between dissipative and inertial dynamics in addition to an external drive in a model of a isotropic magnet using Langevin simulations and dynamic renormalization group calculations. The motivation was to study the generic effects of these mechanisms in a simple model system. An important result of our study was: external drives give rise to a *spatio-temporal chaotic behavior* in the system that can be *controlled* to replace the chaotic phase by spatially periodic helical steady states.

I feel my experience developing and applying coarse grained models in conjunction with a variety of simulation techniques (particle-based and field theoretic) and analytical methods to complex problems in biology and materials engineering makes me well qualified to pursue the research program outlined in the accompanying proposal.

Statement of Teaching

Jayajit Das

I believe teaching is an integral part of an academic career. Not only is it a rewarding experience to see students develop the expertise in the subject and the interest to seek further knowledge, but teaching provides me with expanded perspectives on subjects not directly related to my research; this understanding of a different subject, in turn, can sometimes impact one's own research. As a graduate student and postdoctoral scholar, I had a number of teaching and advising opportunities that ranged from supervision of individual research projects to giving lectures in introductory courses. I feel my training makes me well qualified to teach core undergraduate engineering courses like thermodynamics, fluid mechanics and mathematical methods. I would also welcome the opportunity to develop and teach interdisciplinary graduate courses like Systems Biology of Cell Signaling.

My teaching objectives differ depending on the type of the course, but they are bound by the same underlying motive -- to impart knowledge with clarity which can be applied creatively. For undergraduate courses, it is important to relate the course material to the latest technological and industrial applications in addition to creating an interest in the subject. In graduate courses, I feel it is essential to invoke creativity, encourage questioning in every student, and make them aware of the interdisciplinary aspects of a subject.

My teaching strategies are structured by my own classroom experiences and the discussions I have had with my fellow students and colleagues. I believe fostering mutual respect, trust and a good relationship between the teacher and students should be at the heart of the strategy. Here, I outline some of my specific ideas for undergraduate and graduate courses.

Undergraduate Courses: A carefully planned syllabus that reaches out to students from different backgrounds, levels of maturity and interests, is essential. Assignments aimed at learning the core concepts of the subject should be supplemented with challenging, but not open-ended, problems for the advanced students. Formation of small study groups and assigning each of them multifaceted short projects will encourage discussions, and collaboration as well as enhance leadership qualities. For instance, in a course on thermodynamics, the pros and cons of using different equations for describing various real gases can be debated in-group discussions. I would encourage exercises involving in-class presentations which will train the students to share and present their results and thoughts.

Graduate Courses: The courses will be designed to invoke creativity and expose students to various computational and analytical techniques in order to enhance their problem solving abilities. I will assign problems that will involve topics from latest research. Group discussions, in-class presentations of such problems, and even writing up the work in the form of a research paper in the case of new results will be strongly encouraged. An essential part of the whole strategy is to give the student the freedom to think creatively and to ask questions.