Title: Development of Coarse-Grained Models for Protein Aggregation for Understanding Mechanisms Associated with Immune Responses.

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Project Description:

Protein aggregation – the incorrect folding and subsequent self-assembly of proteins into fibrillar structures – is a serious problem in both biomedical and biotechnological fields[1]. A number of diseases, known as amyloid diseases (these include Alzheimer's disease and type II diabetes), are associated with this process. The efficacy of protein therapeutics is often hampered by aggregation occurring during bioprocessing, storage and delivery. In addition, these aggregates are known to enhance immune responses to the monomeric form of the protein, leading to adverse antibody mediated events in treatment with therapeutic proteins products. Developing a strategy to control aggregation requires an understanding of the thermodynamic and kinetics of this polymerization process.

Dr. Shea (UCSB) has considerable expertise in simulations of proteins. Dr. Gnanakaran has experience extending molecular simulation methods to address immunological problems. We propose to combine our expertise to develop simplified models for protein aggregation with the goal of providing a structure-based mechanistic understanding of the first steps involved in allergic responses. While the computational study of the entire aggregation process using fully atomic models is simply prohibitive, coarse-grained models offer the possibility of extracting the underlying principles governing the thermodynamics and kinetics of aggregation. Our specific aims include:

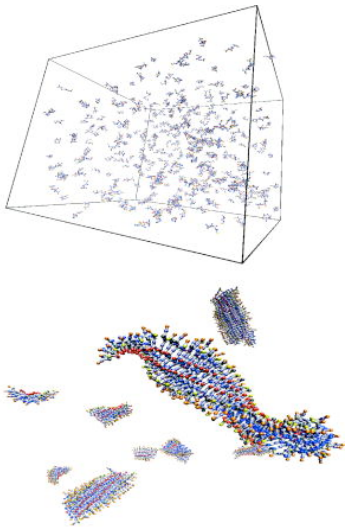
1. The development of novel coarse-grained protein models for the study of aggregation
2. The study of antigen induced receptor aggregation on mast cell membrane surfaces

We anticipate that interactions with the LANL research team will offer a unique R&D and educational experience for the student in the areas of mathematical modeling, computational biology and high performance computing.

Research Aim 1: Development of Coarse-grained models for protein aggregation

Much of the work in the field of coarse-grained protein models has focused on off-lattice models in which only the backbone is represented. While these models have been extremely successful for the study of protein folding, they lack certain key elements for fibrillization. Specifically, they lack side chains (responsible for hydrophobic interactions, electrostatic interactions and packing interactions), backbone hydrogen bonding (a major structural component for the formation of β-sheets, and the assembly and stabilization of the fibril) and finally chirality (a key feature to generate the overall twist of the fibril). A solution is to implement a model that incorporates both backbone and sidechain descriptions. We propose to develop such a mid-resolution model that possesses enough structural complexity to assemble into realistic fibrillar structures, while still remaining simple enough to allow the study the aggregation of several hundred peptides over time scales exceeding μs using Langevin Dynamics. The polypeptide in our model will retain most of the backbone degrees of freedom and one interaction center representing the side group. We will consider four different types of side chains: hydrophobic (H), polar uncharged (P), positively charged (C) and negatively charged (A).

Thus far, we have successfully designed a model for a short 7-residue peptide (a protein fragment) that can assemble into fibrils (see figure below that depicts the initial and final snapshots of a simulation of the self-assembly of 400 peptides from monomers into fibrils)[2-3]. Our force field includes bonded (bond



stretch, bond angle and dihedral) and non-bonded (Lennard-Jones and electrostatic) interactions. The next critical step (and the focus of Aim 1) is to develop a model for a protein. This is a significantly more complex undertaking for the following reason. The short peptides considered thus far do not misfold: rather, they assemble from extended β-strand conformations. Proteins, on the other hand, first misfold to a compact, non-native structure prior to aggregation. The study and design of these systems will be particularly interesting as it will allow us to investigate the competition between folding and aggregation. We will investigate various factors that affect the thermodynamics and kinetics of aggregation, including sequence and β-sheet propensity. We will investigate how these parameters affect the structure of protein oligomers and fibrils and generate the corresponding phase diagram.

Research Aim 2: Study of receptor aggregation on mast cell surfaces

Protein aggregation in vivo often occurs primarily on cell surfaces rather than in the bulk. An important instance of aggregation on surfaces occurs in the early event of the immunological response in allergic reaction[4]. As in many cell signaling events, an allergic reaction is initiated after a given ligand binds to a cell surface receptor and information is transmitted across the cell membrane. Signaling is initiated when the ligands cause the receptors to aggregate among themselves or with other membrane bound proteins. We propose to extend the coarse-grained models developed in Aim 1 to address the following fundamental processes associated with allergic reactions: receptor oligomerization, ligand-receptor binding and ligand-induced receptor aggregation. Initial studies will focus on aggregation on surfaces with varying degrees of hydrophobicity, hydrophilicity and charge, and hence different levels of interactions between the protein and the surface. Further studies will involve the use of more realistic coarse-grained membrane models in which the lipids are explicitly represented.

We will focus primarily on the high-affinity Fc receptor, FcεRI. When an antigen cross-links IgE antibodies bound to this receptor, an allergic reaction is initiated. The FcεRI receptor is expressed as an $\alpha\beta\gamma_2$ tetramer in mast cells and basophils[5]. Gaining a molecular understanding of the aggregation of these proteins on cell surfaces is highly relevant to understanding the innate immune response mechanism since both IgE antibodies and mast cells are concentrated on mucosal tissue.

We expect this initiative to fill the existing gap in LANL in the area of coarse-grained spatial models of protein-protein and protein-membrane interactions. Though several other efforts have been ongoing at LANL on lipid membranes, our focus is on developing a coarse-graining approach that can capture protein-protein interactions in the context of a membrane. Establishing such a capability is extremely valuable to LANL's ongoing and future efforts on biosecurity. LANL has well established experimental programs in the areas of bio-detection and antibacterial therapies. However, a strong counterpart in the areas of molecular simulations and structural modeling is severely lacking. Many molecular recognition and signal transduction processes in host-pathogen interactions involve time and length scales that are beyond atomistic level description. This initial collaboration with UCSB will serve as a seed to establish capabilities in coarse-grained modeling of protein-membrane systems with emphasis on host-pathogen interactions and immune response. In addition to NIH, we intend to target funding agencies such as DTRA, DARPA, DHS for studying various mechanisms associated with the innate immune response which constitutes the body's first line of defense against invading pathogens.

References

1. F. Chiti and C. M. Dobson, Protein Misfolding, Functional Amyloid and Humam Disease. Annu. Rev. Biochem. (2006) 75: 333-66.

2. G. Bellesia and J.-E. Shea, Self-assembly of beta-sheet forming peptides into chiral fibrillar aggregates. J. Chem. Phys. (2007) 126: 245104.

3. G. Bellesia and J.-E Shea, Effect of beta-sheet propensity on peptide aggregation. J. Chem. Phys. (2009) 130: 145103.

4. H. J. Gould and B. J. Sutton. IgE in allergy and asthma today. Nature reviews Immunology. (2008) 8: 205-217.

5. R. M. Lynch, T. Shen, G. Gnanakaran and C. A. Derdeyn. Appreciating HIV-1 Diversity: Subtypic Differences in Env. AIDS Res. And Hum. Retroviruses (2009) 25: 237-248.

Budget Justification

We are requesting funds for a graduate student for a period of two years. We expect to garner sufficient preliminary results during this time period to successfully apply for an NIH grant to continue this collaborative project on a long term basis.

We are requesting $5000.00 in the first year to purchase 3 nodes to add on to an existing cluster at UCSB. These nodes will be used exclusively by the student working on this project. Larger scale simulations will be run at the LANL facilities.

Listed below is our budget for the UCSB side (Shea):

Year 1:

Student travel to LANL: $1000.00
Computer: $5000.00
Payrollfor 6 months (2 quarters): $13249.56
Tuition/Fees for 6 months (2 quarters): $7638
Total: $26887.56

Year 2:

Student travel to LANL: $1000
Computer (over 5K): $0
Payroll for 9 months (3 quarters): $19874.34
Fees/Tuition for 6 months (2 quarters): $7638
Total: $28512.34

Listed below is our budget for the LANL side (Gnanakaran):

Year 1 (no tax added):

Student Salary for 3 months: $21612.44
Computer Equipments: $0
Time and Effort for LANL personnel: $0
Travel to UCSB by LANL personnel: $1500.00
Total: $23112.44

Year 2 (no tax added):

Student Salary for 3 months: $20487.66
Computer Equipments: $0
Time and Effort for LANL personnel: $0
Travel to UCSB by LANL personnel: $1000.00
Total: $21487.66

Total for UCSB and LANL:

Year 1: $50000.00
Year 2: $50000.00