Project Proposal for Join A*STAR Graduate Scholarship (Overseas) with the University of Cambridge: **"A Computational & Experimental Platform for Modulation of Innate Immunity"**

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Background and Current Situation

The innate immune system represents the initial gateway to almost all mammalian inflammatory responses to invading microbes. The main "sensors" in this system are the Toll-like receptor (TLR) family of proteins, which are specialized for binding diverse ligands ranging from cell wall components to nucleic acids. TLR4 is a family member of particular interest, since it is specialized for recognizing bacterial surface lipopolysaccharide (LPS) [1], which serves as an early sign of infection that prepares the immune system to counteract further illness. TLR4 is associated with a range of infectious, allergic, inflammatory, and malignant diseases, whilst an uncontrolled TLR4 response can cause septic shock, a major cause of deaths in intensive care units [1, 2]. *It would therefore be useful to safely and effectively manipulate TLR4 with novel drugs, both to develop adjuvants in vaccine formulation, and to counteract inflammatory diseases.* Unfortunately there is great variation in the structure of LPS, and subtle alterations can profoundly and unpredictably affect the TLR4 response [1].

Objective and Approach

Traditional structure-based drug design approaches are hampered by the large and complex nature of molecules like LPS. Recently we have begun to tackle this problem by applying computational methods to rationalize experimental structure-activity relationships for naturally occurring LPS molecules [3]. In particular, a molecular simulation approach enables us to probe the structure and dynamics of biological macromolecules such as TLR4 in atomic detail [4], providing insights into the mechanisms of receptor signalling [3]. In this project we will extend our knowledge of the key determinants of TLR4 selectivity and signalling, towards the development of novel therapeutic treatments, by combining bioinformatics in the group of Dr Peter Bond with molecular biochemistry and immunopharmacology in the group of Prof Clare Bryant. We will use computational approaches to model the effects of a ligand library on receptor stability, to understand the energetic determinants of recognition, and to predict the higher order structure of ligands which is likely to affect bioavailability within the body. Our data will be used to iteratively guide live cell screening and spectroscopic experiments. We will focus on a library of synthetic amino-alkyl glucosaminide phosphate (AGP) molecules which been shown to mimic the immunomodulatory activity of LPS analogues. They have the advantage that their chemical moieties can be easily modified, enabling systematic testing of structural components on receptor regulation. We will develop models and parameters for the AGP analogues, and use modelling and simulation to study their recognition by TLR4; systematic variation of their chemical constituents will be carried out to test the effects on stability and dynamics of the receptor complex. The AGP compounds will be experimentally screened for activity using a well-established cell line expressing the TLR4 system and a transcriptional reporter assay. We will also develop protocols to establish the thermodynamics of AGP ligand binding and recognition, enabling us to directly compare experimental and theoretical measurements. This will greatly improve our prospects of rationally designing novel immunomodulatory compounds. In addition, we have developed a series of cross-species TLR4 chimeras, providing a unique opportunity to identify parts of the protein complex exclusively involved in ligand recognition and signal transduction. AGP "screening" will be performed experimentally and in silico across modelled human, mouse, and equine receptor variants. Similar approaches will be used to analyse several naturally occurring mutations in TLR4 associated with disease states will, helping us to understand the molecular basis for hyporesponsiveness and hence disease. Collectively, we will obtain a deep understanding of the fundamental molecular mechanisms of recognition and signalling by TLR4 which will allow rational compound design for the development of novel immunomodulators.

References

1. Bryant et al, **2010**. *Nat. Rev. Microbiol.* 8:8-. The molecular basis of the host response to lipopolysaccharide.
2. O'Neill et al, **2009**. *Pharmacol. Rev.* 61:177-. Therapeutic targeting of Toll-like receptors for infectious and inflammatory diseases and cancer.
3. Paramo et al. **2013**. *J. Biol. Chem,* 288:36215-. The Structural Basis for Endotoxin-Induced Allosteric Regulation of the TLR4 Innate Immune Receptor.
4. Paramo et al. **2014**. *J. Chem. Theory Comput.* 10:2151-. Efficient characterization of protein cavities within molecular simulation trajectories.