

PhD Projects Available in B.A. Wallace Lab (Starting October 2006)

Structural Biology and Bioinformatics

Molecular and Structural Biology of Sodium Channels

Supervisor: Prof. B.A. Wallace, with Dr. N. Cronin

Sodium channels are physiologically-important membrane proteins, which are functionally related to a variety of disease states, including epilepsy and cardiovascular diseases. This project will entail the cloning and expression, isolation, purification and characterisation and crystallization of sodium channels from a range of prokaryotic and eukaryotic sources, and their heterologous expression in bacterial systems. In addition, molecular biology constructs of chimeras and isolated domains will be made in order to produce channels with altered functional characteristics. The structures of the channel proteins produced will be characterised by a number of techniques including circular dichroism spectroscopy, cryo-electron microscopy, X-ray crystallography and patch-clamp analyses. Additional goals will be to investigate the nature of their interactions with toxins and antiepileptic drugs with the ultimate goal of using rational drug design methodologies to develop new pharmaceutical agents against diseases. Thus this project will enable the student to gain experience in a large number of techniques, including molecular biology, structural biology, electrophysiology, bioinformatics and molecular modelling and graphics.

References

1. Cronin, N., O'Reilly, A., Duclouhier, H., and Wallace, B.A. (2003) Binding of the Anticonvulsant Drug Lamotrigine and the Neurotoxin Batrachotoxin to Voltage-gated Sodium Channels Induces Conformational Changes Associated with Block and Steady-State Activation. *J. Biol. Chem.* 278:10675-10682.
2. Cronin, N.B., O'Reilly, A., Duclouhier, H., and Wallace, B.A. (2005) Effects of Deglycosylation of Sodium Channels on their Structure and Function. *Biochemistry* 44:441-449.

Zinc Metalloproteases from *Mycobacterium leprae* and *Mycobacterium tuberculosis* as Targets for Rational Drug Design

Supervisors: Prof. B.A. Wallace and Dr. Nora Cronin

The zinc metalloprotease from *M. leprae* is a 667 amino acid protein belonging to the family of highly specific zinc proteases which include the endothelin converting enzymes (ECE) and NEP. These proteases are involved in the post-secretory processing and metabolism of vasoactive peptides such as endothelin. Endothelin was identified in 1988 as the most potent vasoconstrictor compound ever isolated and its crystal structure was solved in our lab in 1994. It has effects on both vascular and non-vascular smooth muscle. Its precursor peptide, "Big endothelin", however, has no vasoactivity, so inhibitors to the specific enzymes which cleave the precursor can effectively disable its vasoactive effects. *M. leprae* has 3 main targets – peripheral and neural tissues, small vessels (endothelial cells) and the monocyte-macrophage system. The bacilli survive and replicate within the Schwann cells and also within endothelial cells, from which they are released. The co-localisation of bacterial infection and proliferation with the expression of the mature peptide endothelin has led to the hypothesis of a role for the zinc metalloprotease in the pathogenesis of the disease. Hence knowledge of the 3-dimensional crystal structure of this enzyme (and in complex with inhibitors), which converts the inactive pro-peptide to the mature and active peptide, may provide a valuable basis for the design of new drugs. We have also cloned the related enzyme (80% homology) from *Mycobacterium tuberculosis*, another medically-important agent of infection.

Molecular and Structural Biology of Nicotinic Acetylcholine Receptors and their Interactions with α -Conotoxins

Supervisor: Prof. B.A. Wallace, with Dr. Robert W. Janes, Queen Mary College, University of London

Nicotinic acetylcholine receptors are physiologically-important membrane proteins, which are functionally related to a variety of disease states, including Alzheimer's and Parkinson's Disease, Autosomal Dominant Nocturnal Frontal Lobe Epilepsy, and Myasthenia gravis amongst others. This project will entail the, isolation, purification and characterisation of electropax acetylcholine receptors. The structures of the proteins produced will be characterised by a number of techniques including circular dichroism spectroscopy, patch-clamp analyses (the latter at the lab of our French collaborator, Dr. H. Duclohier), and crystallisation. Additional goals will be to investigate the nature of their interactions with the α -conotoxins isolated from sea snails with the ultimate goal of using rational drug design methodologies to develop new pharmaceutical agents against diseases. Thus this project will enable the student to gain experience in a large number of techniques, including biochemistry structural biology, electrophysiology, bioinformatics and molecular modelling and graphics.

References

- Mielke, D.L. and Wallace, B.A. (1988) Secondary Structural Analyses of the Nicotinic Acetylcholine Receptor as a Test of Molecular Models. *J. Biol. Chem.*, 263, 8177-8182.
Janes, R.W. (2003) Nicotinic Acetylcholine Receptors: Alpha-Conotoxins as Templates for Rational Drug Design. *Biochem. Soc. Trans.* 31:633-635

Circular Dichroism and Synchrotron Radiation Circular Dichroism Spectroscopy of Sugars and Protein/Carbohydrate Interactions

Supervisor: Prof. B.A. Wallace

Glycosylation of proteins is important for their function, assembly and targeting. Proteins are often heterogeneously glycosylated, and have very different types of sugars and linkages at different sites. In addition, protein/carbohydrate interactions are important for signaling and inter-molecular interactions. There are many diseases that arise from incorrect glycosylation. This project will entail the use of circular dichroism spectroscopy to examine the structures of isolated sugars and glycoproteins and protein/carbohydrate complexes, to develop new methods for analyzing their structures.

It will have a significant Bioinformatics component, as well as experimental work, which will entail traveling to synchrotron sources (UK, USA, Denmark, China) for data collection.

References

- Wallace, B.A. (2000) Synchrotron Radiation Circular Dichroism Spectroscopy as a Tool for Investigating Protein Structures. *J. Synch. Rad.* 7: 289-295.
Cronin, N.B., O'Reilly, A., Duclohier, H., and Wallace, B.A. (2004) Effects of Deglycosylation of Sodium Channels on their Structure and Function. *Biochemistry*, 44:441-449.

Bioinformatics: spectroscopic data mining and analysis of native and mutant proteins

Supervisor: Professor B.A. Wallace

The proposed project will entail the development of new methods for data mining, analysis and archiving of circular dichroism and other spectroscopic data based on advanced applied mathematical and statistical tools. It will enable the comparison of properties of mutant and native proteins, produce a public data bank of these characteristics, and form the basis for identification of the structural basis of disease-related mutations. It will provide training for the student at the interface of the biomedical sciences and computing/IT.

References

- Lees, J.G., Smith, B.R., Wien, F., Miles, A.J., and Wallace, B.A.. (2004) *CDtool* – An Integrated Software Package for Circular Dichroism Spectroscopic Data Processing, Analysis and Archiving. *Analytical Biochemistry* 332:285-289.
- Whitmore, L. and Wallace, B.A. (2004) DICHROWEB, An Online Server For Protein Secondary Structure Analyses from Circular Dichroism Spectroscopic Data. *Nucleic Acids Research* 32:W668-673.

Molecular modeling of pyrethroid binding to insect and mammalian sodium channels

Supervisor: Prof. B.A. Wallace, with Dr. Martin Williamson, Rothamsted Research

This project focuses on computational modelling of the interaction of insecticide pyrethroid ligands with insect and mammalian sodium channels, with *in-silico* models generated taking into account known sodium channel mutations, which have previously been shown experimentally to affect the efficacy of the pyrethroid molecules.

It may also include experimental work to examine the binding of insecticides to sodium channels, and will entail biochemical purification of membrane proteins and may also involve cloning and expression of insect ion channels in bacterial systems.

Reference

- Cronin, N., O'Reilly, A., Duclohier, H., and Wallace, B.A. (2003) Binding of the anticonvulsant drug lamotrigine and the neurotoxin batrachotoxin to voltage-gated sodium channels induces conformational changes associated with block and steady-state activation. *J. Biol. Chem.* 278:10675-10682.