# Structural and Mechanistic Studies of Bioactive Peptides

A thesis submitted for the Degree of Doctor of Philosophy

by

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# ~ STATEMENT OF ORIGINALITY ~

This thesis contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent for a copy of this thesis, when deposited in the University Library, to be available for loan and photocopying.

Tara Louise Pukala
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# ~ ABSTRACT ~

Venoms, toxins and host-defence systems constitute rich sources of biologically active molecules, many of which have enormous therapeutic and biotechnological potential. In particular, peptides are often a significant component of these chemical arsenals, and are fundamentally important as biological effector molecules. The research presented in this thesis is centred on the isolation and investigation of peptides from both frogs and spiders, and endeavours to probe the important structural and mechanistic features of these bioactive compounds.

The skin peptide profiles of interspecific hybrids between the green tree frog *Litoria caerulea* and the magnificent tree frog *Litoria splendida* have been investigated in a nine-month survey. Fourteen peptides were characterised primarily using mass spectrometry, of which three had not been identified previously in the skin secretions of either parent. A number of these peptides are antibacterial agents, while others effectively inhibit the formation of nitric oxide by neuronal nitric oxide synthase. Implications for the genetics and expression of amphibian dermal peptides are also discussed.

The majority of frogs of the genus *Litoria* contain at least one peptide in their glandular secretion capable of inhibiting the formation of nitric oxide by the enzyme neuronal nitric oxide synthase. This was proposed to occur by preventing the association of the regulatory cofactor, $Ca^{2+}$-calmodulin, with its binding site on the enzyme. Non-covalent binding of the amphibian peptides to calmodulin in the presence of $Ca^{2+}$ has been confirmed using electrospray ionisation mass spectrometry, by the observation of complexes in the gas phase with a 1:1:4 calmodulin/peptide/$Ca^{2+}$ stoichiometry. In addition, the structure and binding interactions of caerin 1.8, a potent nitric oxide synthase inhibitor, have been further probed using mass spectrometry and nuclear magnetic resonance spectroscopy techniques.

Recently a number of small, disulfide-containing neuropeptides of the signiferin and riparin families have been characterised from the skin secretion of frogs of the *Crinia* genus. Of these, signiferin 1 and riparin 1.1 are both ten residue peptides with similar primary sequences, however appear to have a significantly different spectrum of bioactivity. Although both act at cholecystokinin-2 receptors, signiferin 1 is smooth muscle active while riparin 1.1 is not, and instead causes proliferation of lymphocytes. The three-dimensional structures of these peptides were determined using nuclear magnetic resonance spectroscopy and restrained molecular dynamics calculations. Both signiferin 1 and riparin 1.1 adopt β-turn type conformations, however differences in these structures may be responsible for the variation in biological activity noted for these peptides.

The dermal secretions of most Australian frogs contain at least one broad-spectrum peptide antibiotic, and often a series of peptides with differing activity to afford greater protection against microbial pathogens. Solid state nuclear magnetic resonance spectroscopy studies were carried out to investigate the interaction of a number of these antibacterial peptides with anionic model membranes, and the results are compared with work previously reported using neutral lipids. It appears the peptides may have a different mode of interaction with the membranes depending upon the charge of the lipid head group.

The cupiennin 1 peptides have been identified in the venom of the neotropical wandering spider, *Cupiennius salei*, and demonstrate potent wide-spectrum antibacterial activity. Primary sequence analysis of these peptides suggests a unique amphipathic structure distinctly different from that of other potentially helical cationic antimicrobial peptides isolated thus far. Using nuclear magnetic resonance spectroscopy and restrained molecular dynamics calculations, cupiennin 1a was found to adopt an α-helical structure with a flexible central hinge region in membrane mimicking solvents. Following this, nuclear magnetic resonance spectroscopy methods were used to further probe the antibacterial and the newly identified neuronal nitric oxide synthase inhibitory activity of this peptide.