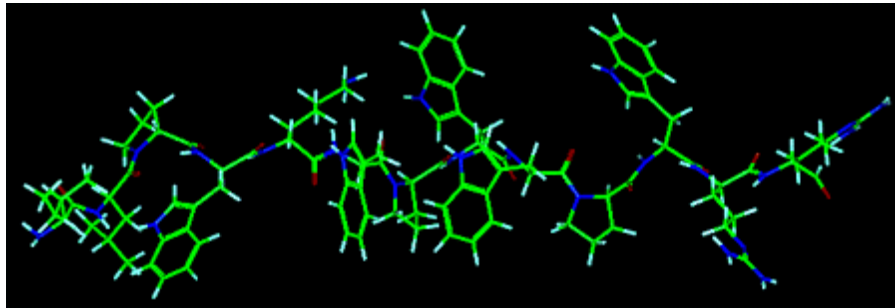


Cationic Antimicrobial Peptides

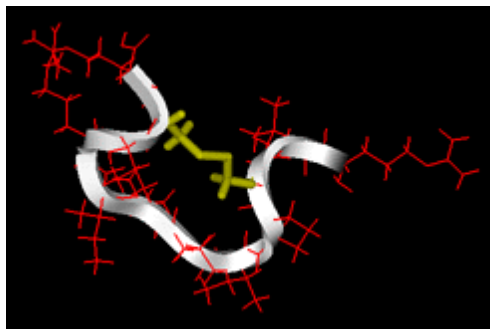
Our work on cationic antimicrobial peptides arose from the convergence of two lines of research. The first was our work on self promoted uptake for which we had demonstrated that polycationic antimicrobials such as polymyxin B and aminoglycosides are taken up across the outer membrane by a novel pathway that involves binding of these polycations to the divalent cation



binding sites on LPS, and disruption of these sites, leading to an increased permeability of the outer membrane to probe molecules, but more significantly to the polycation itself. It occurred to us as early as 1984 that cationic

antimicrobial peptides such as insect cecropins and mammalian neutrophil defensins might also access this pathway. The second line of research involved a series of studies on the mechanism of killing of *Pseudomonas aeruginosa* by phagocytic cells, and our resultant investigations on the mechanism of action of rabbit defensins from macrophages and neutrophils which we showed in 1986 were able to access the self promoted uptake system. The difficulties that we experienced in isolating substantive quantities of defensins led us to attempt to devise a recombinant DNA method for manufacturing such antimicrobial cationic peptides. After about 4 years we managed to devise a procedure for general manufacture of recombinant cationic peptides in sensitive bacteria. This technology, and several classes of peptides, were spun out into [Micrologix Biotech Inc](#) (now renamed Migenix), a Toronto Stock Exchange listed company (trading symbol: MGI) established in Vancouver around this technology. Micrologix has now taken peptides into clinical trials and one of these has just started a phase IIIb clinical trial to confirm efficacy in preventing colonization and tunnel infections associated with the implantation of central venous catheters. Another company that has licensed in a large amount of lab technology in the antimicrobial peptide arena is [Helix Biomedix](#).

The lab has been studying four structural classes of cationic peptides: (1) amphipathic alpha-



helices based on a fusion of silk moth cecropin and bee melittin, (2) extended peptides based loosely on the structure of the cattle neutrophil peptide indolicidin, (3) beta-sheet peptides including beta-hairpin peptides based on the horseshoe crab peptide polyphemusin, and cyclic beta-sheet peptides synthesized by our collaborator, Dr. Bob Hodges from the University of Alberta in Edmonton, based on the general structure of bacterial gramicidin S, and (4) loop peptides cyclized with a single cysteine disulphide based on cattle

neutrophil bactenecin. We have studied the mechanism of action of representatives of most of these classes. Basically these peptides interact with the surface of Gram negative bacteria and are taken up by self-promoted uptake. They then insert into the cytoplasmic membrane under the

influence of the transmembrane electrical potential gradient (which in bacteria is about -150 mV oriented internal negative so as to electrophorese the cationic peptides towards the membrane). They assemble in the membrane into multi-state channels which we have described via the “aggregate model”, and in many cases cross the cytoplasmic membrane to access cytoplasmic targets, or in some cases permeabilize the cytoplasmic membrane barrier. It should be stated that in the past many people in the antimicrobial peptide field favoured the latter mechanism for most peptides; however, our own published evidence appears to be more consistent with cytoplasmic targets for many peptides. The better cationic peptides act very rapidly (within minutes) to kill cells and have very broad ability to kill microbes including the most important Gram negative and Gram positive pathogenic bacteria as well as fungi like *Candida albicans*. They are by and large unaffected by the most common clinical mechanisms of antibiotic resistance, and in our hands do not easily select resistant mutants even after multiple passages on sub-MIC doses of cationic peptides. We have been able to demonstrate that certain α -helical peptides are effective against systemic infections of mice by *P. aeruginosa*, and are also effective against chronic rat infections when delivered by aerosol.

The ability of these peptides to access the self promoted uptake system and permeabilize the outer membrane explains in part one of their more useful properties. Since the outer membrane is normally a semi permeable barrier to conventional antibiotics, we would predict that cationic peptides, in overcoming this barrier, would promote the activity of such conventional antibiotics. We have demonstrated this *in vitro* for selected cationic peptides, and further demonstrated that one peptide, CP-26, can reverse all of the major clinically important mechanisms of antibiotic resistance in *P. aeruginosa* including de-repressed beta-lactamase, DNA gyrase mutations and efflux-mediated multiple antibiotic resistance. The peptides also demonstrate synergy with the host defence molecule lysozyme and different peptides demonstrate synergy with each other.

Our current studies are concentrating on solving the three dimensional structures of selected peptides, rational computer-assisted design of new peptide variants for structure activity relationship studies and random design of variant peptides by peptide array procedures. We recently solved the structure of several peptides by NMR. We are also interested in the ability of peptides to interact with mammalian cells as described in more detail under [innate immunity studies](#), and in particular are interested in the ability of peptides to cross membranes, an activity that in mammalian has been ascribed to a group of peptides termed cell penetrating peptides. Studies on the mechanisms of action of the peptides against bacteria and viruses and on mechanisms of resistance continue in the lab.

We have evaluated different methods for MIC determination for cationic peptides and have proposed a standard method as described in our [Methods](#) section.