Discovery of novel macro-cyclic peptide from *viola betonicifolia* (Violaceae)
Protein Discovery program

- **Wet Chemistry**
  - Standardization of in-house protein extraction protocols
  - Chromatographic techniques

- **Biochemical/biophysical characterizations**
  - Mass spectrometry, amino acid sequencing, NMR data

- **Biochemistry techniques**
  - Genomic DNA extraction, PCR amplification, TOPO cloning, colony screening, minipreps, and DNA sequencing

- **Bioinformatics**
  - Capacity to do;
  - Blast search,
  - Protein & DNA modeling, Manipulation & Visualization
  - 3-D model structure calculation

- **Collaborations**
  - Professor Daivd Craik’s Lab at the Institute of Molecular biosciences, University of Queensland, Australia
  - Professor Marilyn Anderson of the Biochemistry Department, La
Focus - discovery & characterization of defense-related proteins from plants

**Classes of Plant Defense Proteins**

- Plant defensins
- Knottins
  - ICKs
  - CCKs / cyclotides
Plant defensins

- 45-54 residues
- 4 disulfide bonds
- Prevalent in both monocot and dicot plants
- *In vitro* studies show host’s defence mechanism
  - Eg., Rs-AFP1 and Rs-AFP2 inhibit hyper-branching and growth of the hyphal tips of several pathogenic fungal strains
- Cysteine-stabilized α-helix β-sheet (CSαβ) motif
- Motif found in insect defensins and scorpion toxins

**3-D structure of plant defensins**
- Triple stranded anti-parallel β-sheet
- One α-helix
Knottins

- ICKs
  - discovered in insects, fungi, marine molluscs and plants
  - ~30 residues long
  - “cystine knot motif”
    - formed by three disulfide bonds; two of these bonds form a ring that is threaded by the third.
  - implicated in antagonistic behaviour against larger target proteins or receptors.

- CCKs/cyclotides
  - Has an added feature of a covalently cyclized backbone
  - Isolated from Rubiaceae, Violaceae and Cucurbitaceae plant families

Conotoxin MVIIA
Work on Plant defensin

- Screening for defensin in native PNG plants;
  - Curcurbitaceae
  - Tomatoe plants

- Cloning corresponding gene;
  - genomic DNA extraction
  - PCR amplification, TOPO cloning, cleaning, *E.coli* colony screen, minipreps, DNA sequencing

- Pichia expression

*Agarose gel electrophoresis profile of E.coli colony screen from TOPO cloning*

*SDS NuPAGE gel electrophoresis of the crude extracts of chickpea and faba*
DNA sequence

ER  Mature  Intron  Mature  Stop

KVCQRRSKTWSGPCINTGNCSRQCK
QQEDARFGACHRSGFGFACFCYFKC
Discovery of Novel cyclotide from *Viola betonicifolia novaguinensis* (Violaceae)

- Extraction
  - Solvent-solvent extraction and partition
- Chromatography
  - MALDI-TOF data
  - RP-HPLC (bulk up fractions)
- Amino acid sequence;
  - Back bone cyclization makes it impossible for direct sequencing
  - First reduction and alkylation with malemide
  - The reduced and alkylated species were confirmed by tandem mass spectrometry

RP-HPLC profiles of purified native Vb1 cyclotide (perforated line) and the reduced and alkylated [ra] species of Vb1 (bold line), which eluted almost 5 minutes earlier than the native peptide. (Inset): The mass profile of the reduced and alkylated species [ra].
Homology sequence alignment

- Sequence was 80% complete

- Full length sequence was achieved by rational analysis based on:
  - homologous sequence,
  - conserved residues in each intra-cysteine loops
  - and precise mass data

- Full deduced sequence;
  - SCVFIPCTVVSLLGCSCKVCYNGSPCAE
Cyclotides have diverse biological activities and to understand the basis for these activities three-dimensional structures have been investigated.

SwissProt and NCBI blast search showed 83% identity to vhr1.

- Lowest energy structure of vhr1 was chosen to construct a 3-dimensional structure for vb1.
- The model Vb1 structure was generated using Modeller within the Homology module of InsightII (Accelrys).
- The best structure was subjected to further energy minimization using the Optimize suite within the Builder module of InsightII.

Structure of Vb1 (red) superimposed over the backbone of vhr1 (blue). (B). The ribbon representation of the backbone chain of Vb1 showing the secondary structure elements.
The side chain orientation of Lys 23 in loop 5 protrudes into the solvent front in vhr1 but in Vb1 it is attracted towards the Asp adjacent to it via electrostatic interactions (upper panel).

Lys23 and Asp22 are actually involved in an H-bond (previous slid)

If the Lys residue plays any significant biological roles, such as receptor interactions, then its role in Vb1 will be reduced since the electrostatic interactions between Asp22 and Lys23 will effectively render the charges neutral (lower panel)

Structures of Vb1 (red) and vhr1 (blue) superimposed over their backbone chain. The side-chain orientations of Lys23 and Asp22 are labeled.

Molecular surface plots of Vb1 colored by polarity using different color codes. The one to the right represent a 180 degrees rotation around the vertical axis.
Antimicrobial test

- The peptide was tested for its antibacterial properties against the following pathogenic strains:
  - *Bacillus subtilis*,
  - *Staphylococcus aureus* and
  - *Eschericia coli*

- No inhibition observed at concentrations tested

- The peptide is perhaps not an anti-bacterial agent, otherwise the electrostatic interaction between Lys23 and Asn22 could undermine its potency
Further work - Agrochemicals

- PNG cash crops constantly under attack by pest and diseases
  - Cocoa pod borer (*Conopomorpha cramerella* Snellen)
    - PNG Cocoa industry is at the brink of collapsing
  - Coffee
    - *Oribius* Weevil attack on coffee cherries in Simbu
  - Oil Palm (*Elais guineensis*)
    - Nematodes, weevils, beetles, planthoppers,
    - Most recently report of deadly ants invading Bougainville island

- Threatens PNG economy;
  - Improved pest management technique required
  - Collaborative efforts between different fields of science is essential

- What we would like to do;
  - Test discoveries on different agricultural targets
  - Identify leads and reconfirm activity
  - Solve primary structure (amino acid sequence)
  - Extract corresponding genes
  - Express on stable transgenic plants to assess their activity
Pest attacks coffee

K50 million Simbu industry under threat

COFFEE in Simbu province is at risk of the Oribius weevil, an insect extended into a type of snout. Hosts plants of Oribius include ajibika, capsicum, avocado, ginger, and bibitiga — common coffee crops.
Pharmaceutical applications

- ICKs display wider spectrum of bioactivity and include uterotonic, hemolytic, anti-cancer, insecticidal, antimicrobial and anti-HIV

- Demonstrate exceptional stability due to cystine knot motif
  - For instance kalata B1 survives boiling temperature and the acidic environment of the gastro intestinal tract

- Knottins (ICKs and CCKs) are attractive alternative for combinatorial drug design and also themselves as drug candidate

- It is hoped that novel members with unique bioactivity will be discovered if this work continues with adequate financial support
Conclusion

- PNG biodiversity vs. economic values
  - PNG and its pacific neighbors houses some of the most unique and diverse flora and fauna of the world. The potential to harness the biological (molecular and genetic) resources and translate them into economic values is immense and is beckoning at our door steps.

- Need to “Build Research Culture”
  - Collaboration
    - Have standing collaboration with the Institute of Molecular Biosciences, UQ and Biochemistry department, La Trobe University.
    - We also would like to be involved with researchers from other institutions within the pacific and other French colonies to develop our discoveries
  - Adequate Funding
    - We have already attracted some funds for this research from the OHE through STI and hope to attract more funds from other sources to support the research.
EM Tasol

Questions Please