

EXPRESSIONS OF INTEREST SOUGHT

Cyclotides - A Natural Plant Defence Mechanism

Summary of the Opportunity

Cyclotides are bioactive plant derived proteins with a novel structural framework known as the cyclic cystine knot (CCK) and which confers exceptional resistance to heat, chemical and enzymatic digestion. A postulated natural function of cyclotides' is in host-defence and a number of cyclotides have demonstrated insecticidal and antimicrobial activity.

UQ has proprietary technologies, IP rights and know-how relating to cyclotide genes, gene expression systems (constructs encoding cyclotides) and modified or grafted cyclotides (in a CCK framework) for use in agricultural plants.

We are seeking expression of interest from industry partners with an interest in working with leading UQ researchers in the field of cyclotides and peptide chemistry for development of transgenic crop plants, with cyclotide conferred resistance against agricultural pests.

Background to Cyclotides

Cyclotides are a topologically unique family of plant proteins ~28-37 amino acids that are exceptionally stable (survive boiling and human ingestion) and confer a broad range of biological activities, including uterotonic, antimicrobial, antiviral, anticancer and antidepressant activities. Traditionally cyclotides, in the form of plants, have been used as ethnobotanical agents (indigenous medicines). The natural function of cyclotides, however appears to be in plant defence, based on reported pesticidal activities, including insecticidal, nematocidal and molluscicidal activities. Individual plants typically contain dozens of cyclotides, expressed in several tissues (flower, leaf and stem) and in seeds, leading to their description as a natural combinatorial library. Plants presumably use this combinatorial library strategy to target multiple pests, or to minimize the possibility of individual pest species developing resistance to the protective cyclotide armoury.

More than 200 cyclotide sequences have been reported to date, although it is estimated that the family probably comprises around 50,000 members, making it a particularly large family of plant proteins. Until recently cyclotides had been found only in the Rubiaceae (coffee) and Violaceae (violet) plant families, apart from two atypical members in the Cucurbitaceae (squash) family (suggested cyclotide review article, Ireland DC *et al.*, J Nat Prod 2010).

The CCK framework

Cyclotides are arranged in a head-to-tail cyclised peptide backbone that is restrained by the cystine knot motif, which formed by six conserved cysteine residues. This cyclic cystine knot (CCK) framework is built from two disulfide bonds and their connecting backbone segments forming an internal ring in the structure that is threaded by the third disulfide bond to form an interlocking and cross braced structure (**Figure 1**). Superimposed on this cystine knot motif are a well-defined β -sheet and a series of turns displaying short surface-exposed loops.

The exceptional stability of cyclotides means that they have attracted attention as potential templates in peptide-based drug design applications. In particular, the grafting of additional bioactive peptide sequences into the cyclotide framework offers promise of a new approach to stabilise peptide-based therapeutics, thereby overcoming one of the major limitations of peptides, oral bioavailability. Grafting, or other cyclotide modifications, may also be used to enhance the natural function of cyclotides against their target.

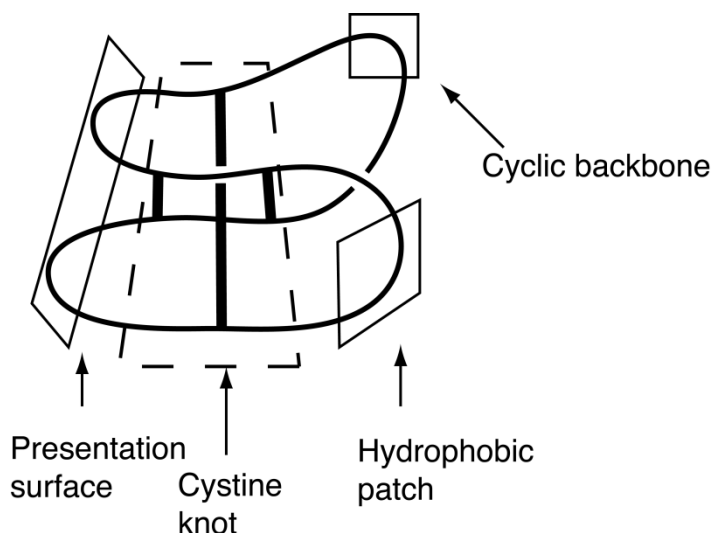


Figure 1. The CCK Framework, displaying the three disulfide bond cysteine knot structure, the hydrophobic patch and the surface, presentation area. The surface presentation area is ideal for grafting bioactive peptides to enhance the activity against suitable targets.

UQ Technologies

We recently reported the discovery of cyclotides in plant tissue from the Fabaceae (legume) plant family, the third largest family of flowering plants on earth comprising 18,000 species, many of which are important in human nutrition e.g. peas and beans (Poth *et al*). These findings were further corroborated in an independent study by Nguyen *et al*. The discovery of cyclotides in Fabaceae and the associated gene structure highlight the potential of developing commercial transgenic, cyclotide expressing, crop plants. This is because Fabaceae plants (i) are more amenable to transfection than members of the Rubiaceae or Violaceae plant families and (ii) include commercially important crop species, for example soybean. A range of commercially beneficial traits could further be incorporated in engineered cyclotides, including superior insect protection.

Cyclotides from the Rubiaceae and Violaceae are biosynthesised via processing from dedicated precursor proteins encoded by multi-domain genes which contain one, two or three cyclotide domains. The genetic origin of the cyclotides from Fabaceae was expected to be similar, however, we have reported an unexpected biosynthetic mechanism in which cyclotide domains are encoded within an albumin precursor. Interestingly, we have also recently reported a similar albumin precursor origin of the cyclic peptide Sunflower Trypsin Inhibitor 1 (SFTI-1) (Mylne *et al*). These findings suggest that cyclic peptides might be more common than has previously been realised and probably have evolved multiple alternative mechanisms for their production.

Applications

There is an opportunity to commercially exploit UQ's technologies, know-how and IP rights in relation to cyclotides and the CCK framework for a number of applications:

- generating cyclotide encoding gene cassettes;
- expressing one or more cyclotides in plants and;
- expressing grafted or modified cyclotides in plants.

POC work

UQ have generated transgenic *Arabidopsis thaliana* plants, using UQ's proprietary cyclic peptide expression systems, for the simultaneous expression of multiple cyclic peptides, or cyclotides in seeds. MALDI MS was further used to corroborate the presence of correctly folded, cyclic peptides of the predicted masses.

Research Leaders

Professor Craik leads a group of ~30 researchers who are focused on the use of peptide chemistry and NMR spectroscopy to determine the structures of proteins that are important in drug-design programs and in agriculture. They have a particular interest in the discovery and structural characterisation of novel protein topologies. They aim to determine the mechanisms of biosynthesis and evolutionary origin of circular proteins and to apply protein-engineering principles to explore applications of circular proteins in drug design and agriculture. Circular proteins are particularly stable and thus have advantages over conventional proteins.



They undertake protein-engineering studies in which we modify protein frameworks either by "grafting" new biologically active epitopes onto them, or by stabilising them by cyclisation. They currently have molecules under development for the treatment of multiple sclerosis, cardiovascular disease, cancer and chronic pain. They also study the structures of a range of toxins from cone snails, spiders and snakes and use this information to understand their mode of action against ion channels and other receptors.

IMBcom

IMBcom is The University of Queensland's commercialisation company for the Institute for Molecular Bioscience (IMB). IMBcom have extensive experience in identifying and protecting intellectual property, devising development and business strategies, raising capital, licensing and founding start-up companies. Several of IMBcom technologies have progressed to Phase II.

UQ's Intellectual Property Portfolio

A provisional patent application was filed in April 2011 to protect the gene encoding Fabaceae cyclotides. UQ also owns a family of patents in regard to a generic cyclotide gene and to a platform technology in regard to grafting and modifying cyclotides in the CCK framework.

IMBcom Contact Details

Dr Amanda Smith

a.smith@imbcom.com.au

+61 (7) 3346 2188

Key Publications

1. Isolation, sequencing, and structure-activity relationships of cyclotides. Ireland DC, Clark RJ, Daly NL, Craik DJ., J Nat Prod 2010 73:1610-1622.
2. Discovery of an unusual biosynthetic origin for circular proteins in legumes. Poth AG, Colgrave ML, Lyons RE, Daly NL, Craik DJ., PNAS 2011 Jun 21;108(25):10127-32
3. Discovery and Characterization of Novel Cyclotides Originated from Chimeric Precursors Consisting of Albumin-1 Chain a and Cyclotide Domains in the Fabaceae Family. Nguyen GK, Zhang S, Nguyen NT, Nguyen PQ, Chiu MS, Hardjojo A, Tam JP., JBC 2011 Jul 8;286(27):24275-87.
4. Albumins and their processing machinery are hijacked for cyclic peptides in sunflower. Mylne JS, Colgrave ML, Daly NL, Chanson AH, Elliott AG, McCallum EJ, Jones A, Craik DJ., NCB 2011 May;7(5):257-9.