

Non-ribosomal biosynthesis of peptides in planktonic cyanobacteria

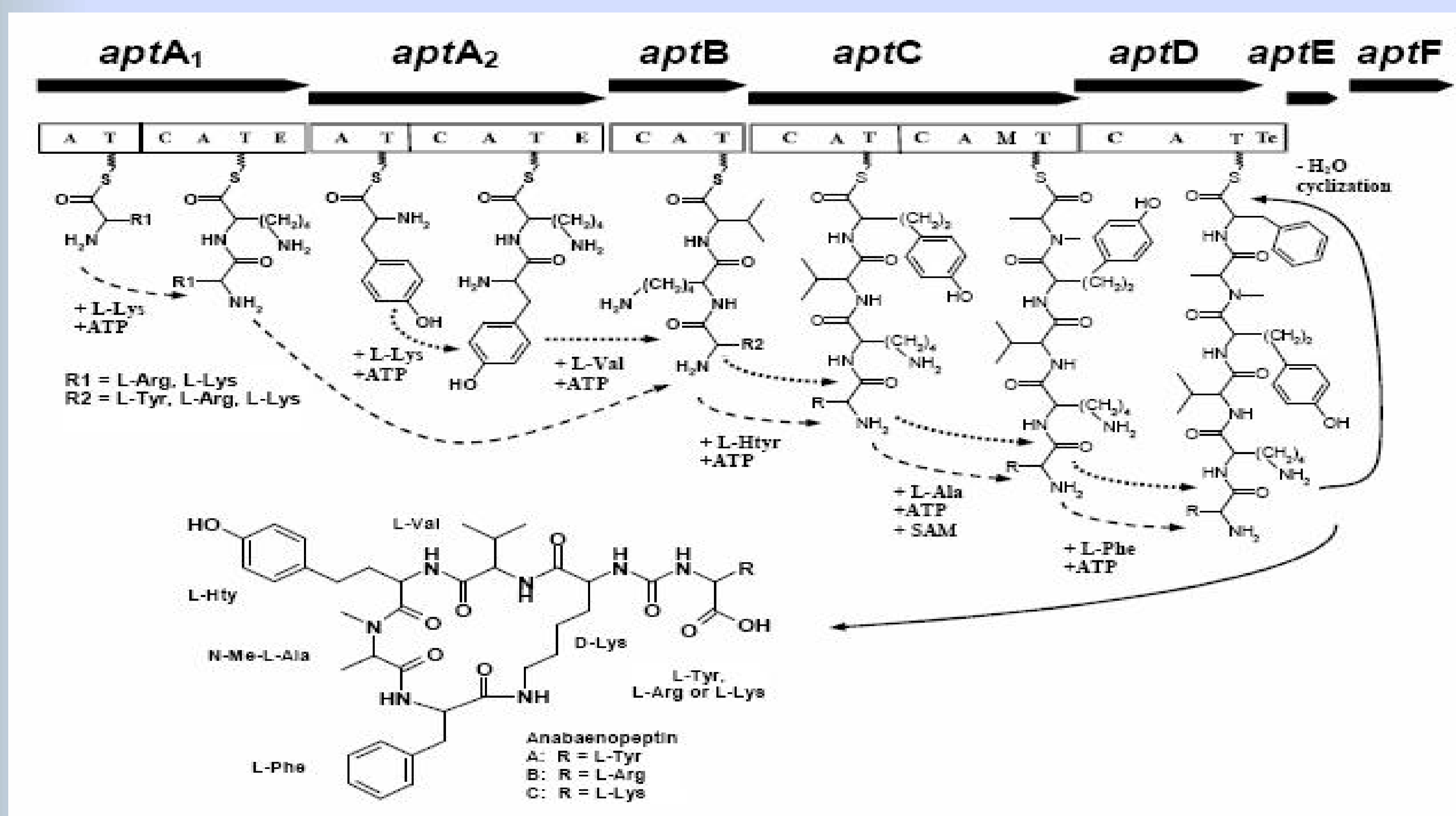
Leo Rouhiainen, David P. Fewer, Jouni Jokela and Kaarina Sivonen

Department of Applied Chemistry and Microbiology, P.O. Box 56, Viikki Biocenter, Viikinkaari 9, FIN-00014, University of Helsinki, Finland

Background

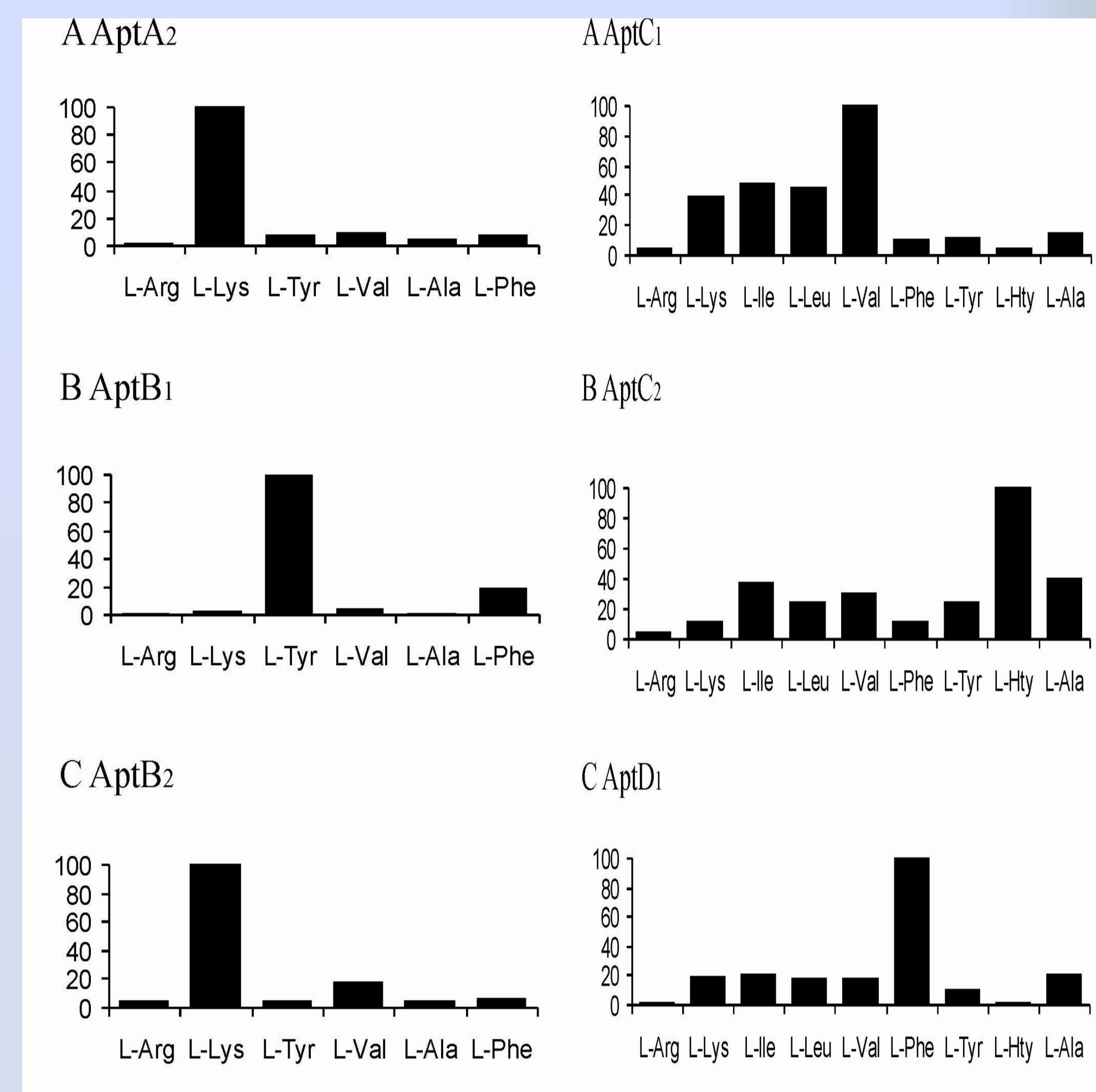
Cyanobacteria produce a large variety of peptides, many made by non-ribosomal peptide synthetases (NRPSs) and occasionally in combination with polyketide synthases (PKS). Non-ribosomal cyanobacterial peptides include protein phosphatase inhibitors, protease inhibitors and cytotoxic compounds. The NRPSs are large modular enzymes catalyzing the activation, modification and incorporation of amino acids or sometimes carboxylic acids into the peptide chain in a stepwise process. We have characterized the NRPSs of *Anabaena*, and *Nostoc* genetically and biochemically. The gene clusters coding for the biosyntheses of protease inhibitors anabaenopeptins from *Anabaena* strain 90, and mildly cytotoxic nostophycin from *Nostoc* strain 152 have been studied in the Finnish Program for The Centers of Excellence in Research started in 2008.

Alternative starter modules are employed by *Anabaena* to create structural variation of anabaenopeptins



Anabaenopeptin biosynthesis in *Anabaena* strain 90.

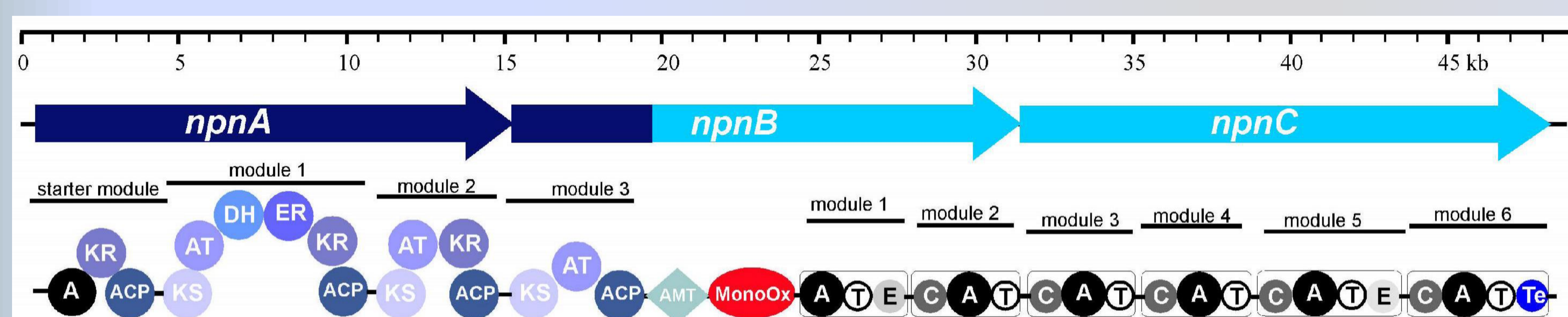
The genes *aptA1- aptD* code for NRPSs, *aptE* putatively the change of peptide bond to ureido bond and *aptF* ABC transporter. Anabaenopeptin synthetase of *Anabaena* spp. makes use of alternative starter modules with high substrate specificity to assemble a group of peptides with limited structural variance. Anabaenopeptin synthesis constitutes a novel exception to the colinearity rule of non-ribosomal peptide production. Abbreviations of the domains: A, adenylation; PCP, peptidyl carrier; C, condensation; E, epimerase domain; M, N-methyl transferase; Te, thioesterase.



Biochemical characterization of anabaenopeptin synthetase by ATP/PPi exchange assay.

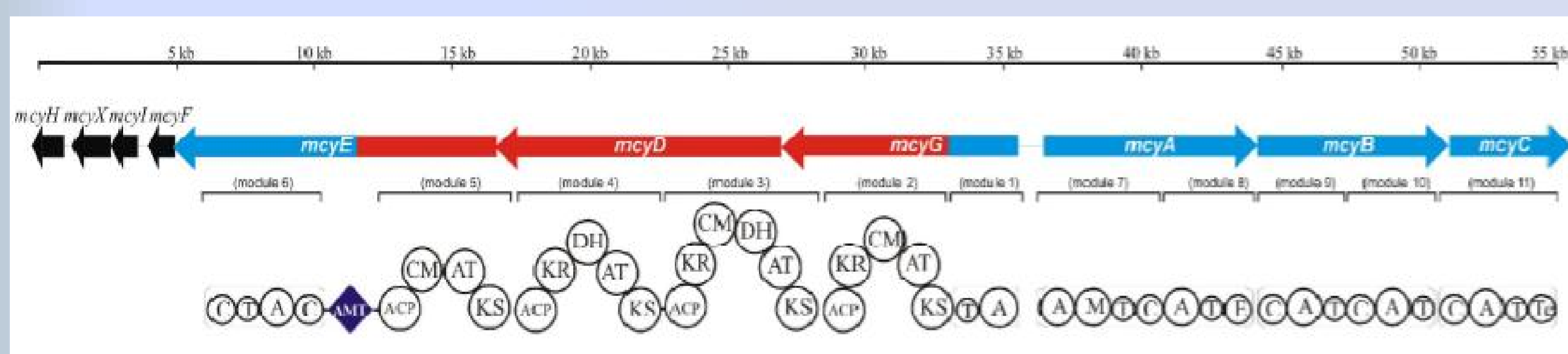
Anabaena sp. strain 90 is recalcitrant to genetic manipulation so the substrate specificities of expressed adenylation domains were studied. Six of the eight adenylation domains were overexpressed in *E. coli* BL21 Star (DE3) and their substrate specificities determined in an ATP-PPi exchange assay.

NRPSs and NRPS/PKSs characterized from *Nostoc* 152



Structure and genetic organization of the mixed PKS-NRPS, nostophycin synthetase, from *Nostoc* strain 152.

The genes *npnA* and *npnB* encode mixed NRPS/PKS and *npnC* NRPS. Abbreviations of the domains: A, adenylation; KR, ketoreductase; ACP, acyl carrier; KS, ketosynthase; AT, acyltransferase; DH, dehydratase; ER, enoylreductase; AMT, aminotransferase; MonoOx, mono-oxidase; T, peptidyl carrier; E, epimerase; C, condensation; Te, thioesterase.



Structure and genetic organization of the mixed PKS-NRPS, microcystin synthetase, from *Nostoc* strain 152.

The genes *mcyA-mcyC* encode NRPSs, *mcyG* and *mcyE* mixed NRPS/PKS, *mcyD* PKS, *mcyF* epimerase, *mcyI* dehydrogenase, *mcyX* unknown function and *mcyH* ABC transporter. Abbreviations of the domains: A, adenylation; T, peptidyl carrier; KS, ketosynthase; AT, acyltransferase; CM, C-methyltransferase; KR, ketoreductase; ACP, acyl carrier; DH, dehydratase; ER, enoylreductase; AMT, aminotransferase; M, N-methyltransferase; E, epimerase; C, condensation; Te, thioesterase.

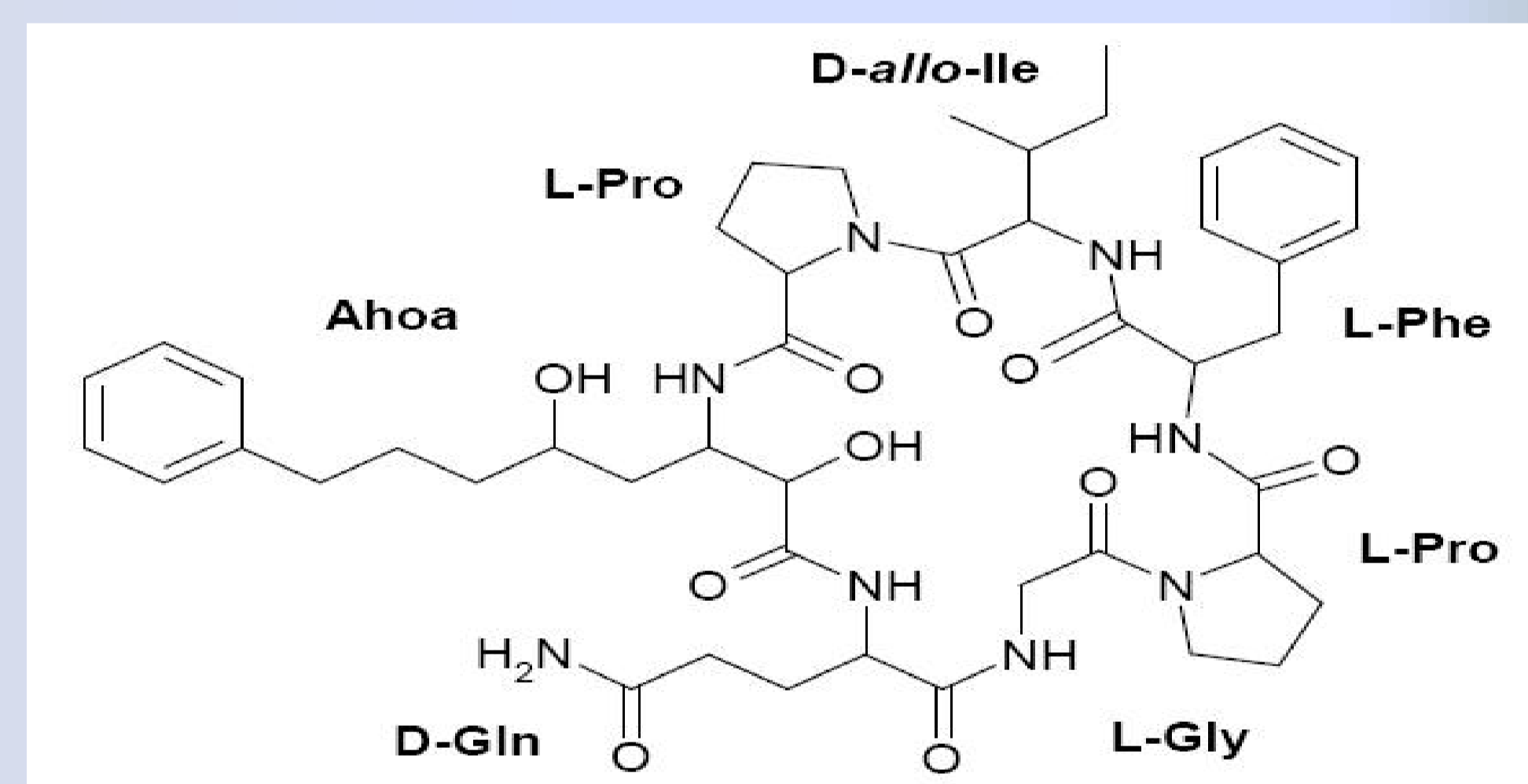
Future work

We need a method to manipulate genetically *Anabaena* 90 and anatoxin producing *Anabaena* 37, and we are working to develop conjugation system. The electroporation method used has shown to be ineffective. The biochemical characterization of the peptide synthetases will be continued.

Publications in 2008 - 2010

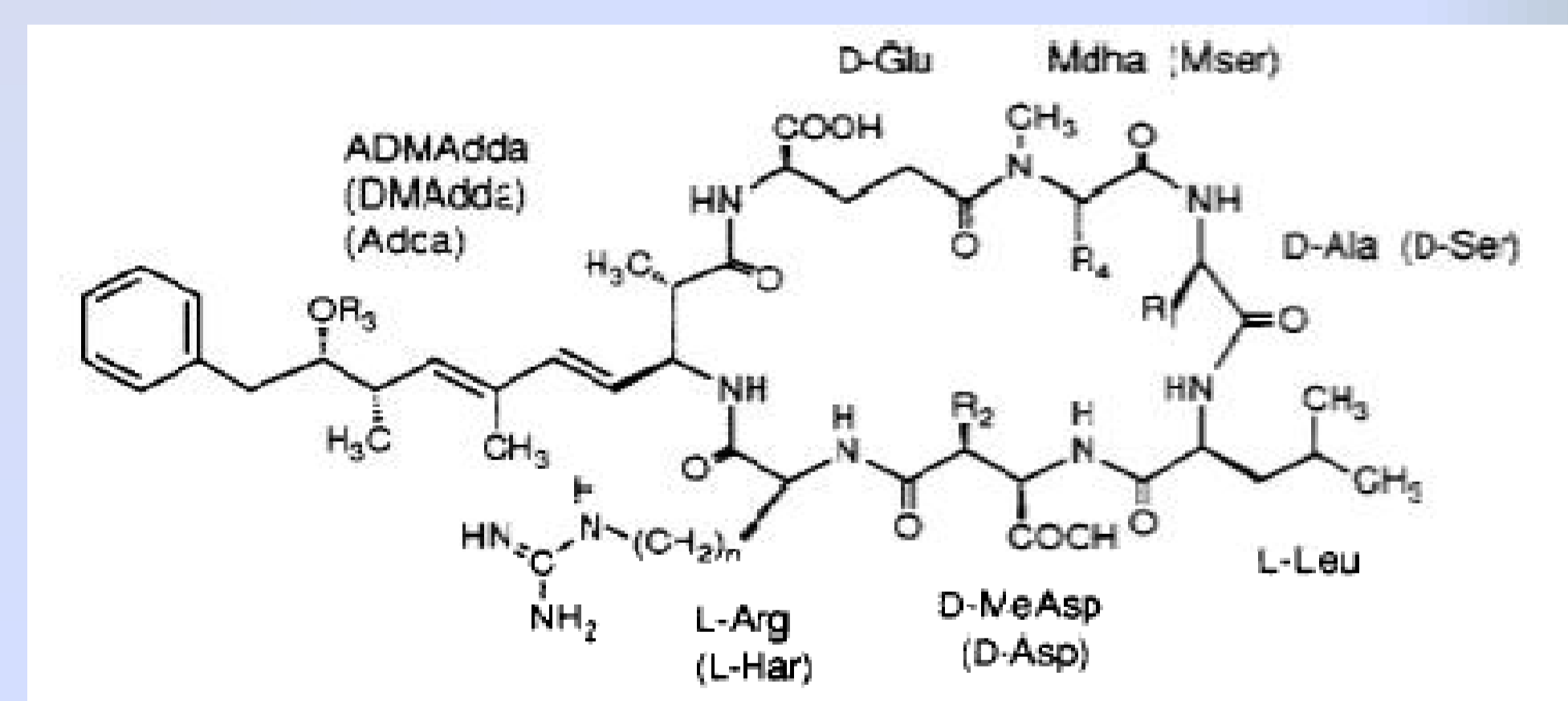
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Peptides produced by *Nostoc* 152



Nostophycin produced by *Nostoc* strain 152.

The mildly cytotoxic nostophycin contains the novel β -amino acid moiety, Ahoa, 3-amino-2,5-dihydroxy-8-phenyloctanoic acid, and seven amino acid ring with two proline residues.



Microcystins produced by *Nostoc* strain 152.

The protein phosphatase inhibiting hepatotoxic microcystins are characterized by the rare amino acid Adda, 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid, and seven amino acid ring with N-methyldehydroalanine.