4th International Conference on Circular Proteins and Peptides Program

Wednesday 28th, November

17:00-18:00 Session 1: Opening remarks and Plenary Lecture
18:00-19:00 Welcome Drinks (King's Skyfront REI Hotel, 5F)
19:00-21:00 Dinner (King's Skyfront REI Hotel, Captain's Grill, 5F)

Thursday 29th, November

9:00-10:40 Session 2: Synthesis and Processing I
10:40-11:00 Morning Coffee Break
11:00-12:30 Session 3: Synthesis and Processing II
12:30-13:30 Lunch Break (PeptiDream)
13:30-14:00 CEM-sponsored talk
14:00-15:30 Session 4: "Hotspot" talks
16:00-18:00 Session 5: Technology
19:00-21:00 Dinner (King's Skyfront REI Hotel, Business Café and Lounge, 1F)
20:00-22:00 Poster session, (King's Skyfront REI Hotel, Business Café and Lounge, 1F)

Friday 30th, November

9:00-10:40 Session 6: Structure function
11:00-12:30 Session 7: Pharmacokinetics and design
12:30-13:30 Lunch Break (PeptiDream)
13:30-15:30 Session 8: Display selection
16:00-17:20 Session 9: Intracellular targeting
17:20-18:10 Session 10: Plenary Lecture
19:00-22:00 Dinner and Performance (TREX River Cafe)

Wednesday 28th, November

17:00-18:00 Session 1 Chair: Chris Hipolito

Chris Hipolito, University of Tsukuba, and Toby Passioura, University of Tokyo
Opening Remarks
Phil Dawson, The Scripps Research Institute
Peptide macrocycle conformational engineering using low molecular weight linkers

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O-1

Peptide macrocycle conformational engineering using low molecular weight linkers

Phil Dawson

The Scripps Research Institute

The manipulation of peptide structure through macrocyclization has become established as a powerful approach for the development of potent peptide based ligands and inhibitors. Bisalkylation of cysteine residues in peptides with dichloroacetone provides both macrocyclization and introduces a reactive ketone that can be used for subsequent functionalization through oxime formation. We have also developed a robust approach for the introduction of diyne linkers into peptides using ligand optimization to promote the Glaser reaction. The resulting rigid, linear 7 Å linkage is highly effective for linking macromolecules. In addition, when performed in an intramolecular context, diyne linkers can be utilized as tethers or as staples to stabilize alpha helical structures. The synthesis and application of diynes in peptides will be discussed.

Thursday 29th, November

9:00-10:40 Session 2: Synthesis and Processing I, Chairs: Phil Dawson and Max Cryle

Christina Schroeder, Institute for Molecular Bioscience, University of Queensland
Targeted delivery of cyclotides via conjugation to a nanobody
Rodney Perez, Kyushu University
Understanding the biosynthetic mechanism of the circular antimicrobial peptide, enterocin NKR-5-3B, from Enterococcus faecium
Rumit Maini, Merck & Co.
Ribosomal Synthesis of a Thioamide Bond
Lara Malins, Australian National University
A Versatile Approach to Peptide Macrocyclization and Late-Stage Modifications

11:00-12:30 Session 3: Synthesis and Processing II, Chairs: Yuki Goto and David Craik

Takeshi Zendo, Kyushu University
Characterization of circular bacteriocins produced by lactic acid bacteria
Shinya Kodani, Shizuoka University
Heterologous production of new lasso peptide using shuttle vector system
James Link, Princeton University
Making and Breaking Lasso Peptides
Max Cryle, Monash University
Understanding the glycopeptide antibiotic cyclisation cascade

13:30-14:00 Sponsored talk Chairs: Chris Hipolito

Jon Collins, CEM corporation
Highly Efficient Synthesis of N-methyl peptides

14:00-15:30 Session 4: "Hotspot" talks, Chairs: Christina Schroeder and Markus Muttenthaler

Mar Forner, Pompeu Fabra University
Peptide-based vaccines to treat foot-and-mouth disease: exploring their efficacy and molecular mode of action
Bronwyn Smithies, Institute for Molecular Bioscience, University of Queensland
Closing the ring: a new cyclisation position for the cyclic peptide MCoTI-II
Haiou Qu, Institute for Molecular Bioscience, The University of Queensland
Surveying a diverse set of monocots for cyclotides: are these cyclic peptides restricted to dicots?
Neha Kalmankar, National Centre for Biological Sciences and the University of Trans-Disciplinary Health Sciences and Technology
Isolation, Characterization and Conformational Analysis of Cyclotides, a Class of Macrocyclic Disulfide Bonded Plant Peptides
Timothy Craven, University of Washington
Design of Peptoid-peptide Macrocycles to Inhibit the β-catenin TCF Interaction in Prostate Cancer
Andrew White, University of Queensland
From sunflower to skin cancer: The development of melanocortin receptor agonists using sunflower trypsin inhibitor-1
Choi Li, University of Queensland
Design Of Potent And Selective Cathepsin G Inhibitors Based On The Sunflower Trypsin Inhibitor-1 Scaffold
Junqiao Du, Institute for Molecular Bioscience, University of Queensland
Discovery and Characterization of Novel Cyclic and Acyclic Trypsin Inhibitors from Momordica dioica

Gregoire Philippe, Institute for Molecular Bioscience, University of Queensland
Toxicity and mechanism of action of cyclic helix-loop-helix peptides
Benjamin Tombling, Institute for Molecular Bioscience, University of Queensland
Enhanced oral bioavailability and serum half-life of engineered cyclic peptides that target the neonatal Fc receptor
Wenyu Liu, University of Tokyo
Identification of Cyclotide-Like Human Factor XIIa Ligands Through mRNA-Display.

16:00-18:00 Session 5: Technology, Chairs: Johan Rosengren and Aline Dantes de Araujo

Ratmir Derda, University of Alberta
Novel genetically encoded cyclic and bicyclic architectures displayed on phage
Zhenling Cui, Institute for Molecular Bioscience, University of Queensland
Biosynthesis of macrocyclic peptides in sense codon reassigned in vitro translation system
Alexey Gurevich, St. Petersburg State University
Variable Identification and De Novo Sequencing of Cyclopeptides via Computational Processing of Tandem Mass Spectra
Yuki Goto, University of Tokyo
Tunable and selective covalent inhibitors based on macrocyclic peptides
Ali Tavassoli, University of Southampton
A platform for the generation and screening of cyclic peptide libraries of over a hundred million members.

20:00-22:00 Poster session, King's Skyfront REI Hotel

O-2

Targeted delivery of cyclotides via conjugation to a nanobody

Christina Schroeder

Institute for Molecular Bioscience, University of Queensland

TBA

O-3

Understanding the biosynthetic mechanism of the circular antimicrobial peptide, enterocin NKR-5-3B, from *Enterococcus faecium*

Rodney H. Perez[1, 2)], Haruki Sugino[1)], Takeshi Zendo[1)], and Kenji Sonomoto[1)]

[1)] Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Motooka 7-4-4, Fukuoka 819-0395, Japan
[2)] National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños, Los Baños, Laguna 4031, Philippines

Circular peptides have attracted considerable attention due to their remarkable stability brought about by the circular nature of their backbone structure arising from the covalent linkage of their terminal residues. Circular peptides and proteins are produced by many eukaryotic and prokaryotic organisms including a few select bacterial strains. Bioactive circular peptides of bacterial origin, known as circular bacteriocins, have been traditionally appreciated as a safe food preservative.
Enterocin NKR-5-3B (Ent53B) is a 64-residue novel circular bacteriocin produced by Enterococcus faecium NKR-5-3. It is synthesized from an 87-residue prepeptide that undergoes a series of enzymatic processing by its biosynthetic enzymes resulting in the removal of the leader peptide and covalent ligation of the N- and C-terminal residues to yield its mature circular form. To gain insights on the key region/residue that plays a role in Ent53B maturation, several mutations near the cleavage site on the prepeptide were introduced. Only mutations with helix structure-promoting hydrophobic residues at P'1 of the propeptide enabled the recombinant phenotype to produce the mature Ent53B derivatives. Whereas substitution of the Trp residue at P'64 with α-helix-disrupting residue blocks the production of the mature bacteriocin, but is less restrictive to non-hydrophobic residue substitution. These results suggest that the interaction of the biosynthetic enzymes and the precursor peptide could be a hydrophobic interaction driven process.
We also highlight the possible conformation-stabilizing role of the leader peptide to the precursor peptide during its interaction with the biosynthetic enzymes. Any truncations of the leader peptide interfered in the processing of the prepeptide. However, when propeptides of other known circular bacteriocins were cloned at the C-terminus of the leader peptide, the biosynthetic enzymes could succeed in yielding a mature bacteriocin. Taken together, these findings offer new perspectives in understanding the biosynthesis of this circular bacteriocin.
Keywords: lactic acid bacteria, bacteriocin, circular bacteriocin, bacteriocin biosynthesis

Corresponding author*

Takeshi Zendo
Laboratory of Microbial Technology, Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Motooka 7-4-4, Fukuoka, 819-0395, Japan
Tel and Fax: (+81) 92 802 4735
E-mail: zendo@agr.kyushu-u.ac.jp

O-4

Ribosomal Synthesis of a Thioamide Bond

Rumit Maini,[†] Ryo Takatsuji,[†] Hiroyuki Kimura,[†] Yuki Goto[†] and Hiroaki Suga[†,*]

[†]Department of Chemistry, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

It is well established that the ribosome can catalyze the formation of amide, *N*-methyl-amide and ester bonds during polypeptide synthesis. Here we demonstrate a strategy for the preparation of amino(thio)acyl-tRNA and its use in the ribosome-mediated catalysis of a thioamide bond in an mRNA dependent manner. Both, linear and thioether-cyclized peptides having one thioamide backbone were synthesized by the ribosome in a custom-made cell-free translation system in the combination with flexizymes. Moreover, ribosomal synthesis of an N-methyl-thioamide bond was shown for the first time. This method enables us to express a peptide library containing thioamide backbone at designated sites, possibly leading to such ligands against a protein target of interest.

O-5

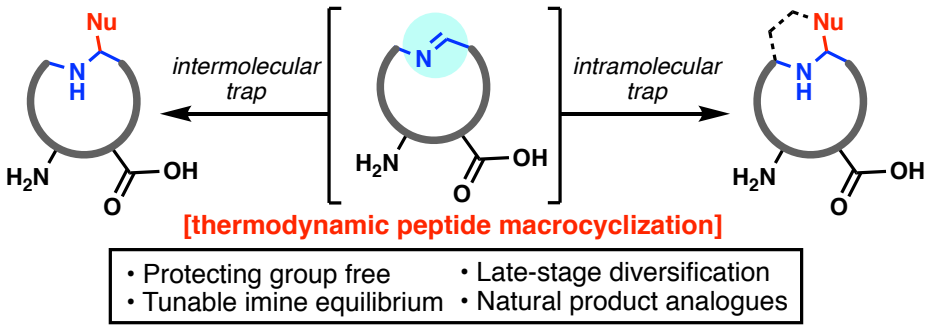
A Versatile Approach to Peptide Macrocyclization and Late-Stage Modifications

Lara Malins

Australian National University

Peptides have enormous therapeutic potential but traditionally suffer from proteolytic instability and poor bioavailability. Structural modifications, including macrocyclization and the incorporation of unnatural amino acids are key strategies for enhancing the drug-like properties of peptides.[1] Methods for the late-stage, divergent modification of native peptide substrates are therefore in exceedingly high demand in the pharmaceutical industry as a means to provide expedient access to novel drug leads.

In this presentation, our efforts toward the development of new strategies for peptide macrocyclization[2] and targeted amino acid functionalization will be detailed. Inspired by the biosynthesis of non-ribosomal peptide natural products, we explore the scope of a thermodynamic, imine-mediated head-to-tail macrocyclization strategy. The extension of this cyclization approach to peptide stapling—by exploiting the reactivity of native lysine residues with functionalized dialdehyde linkers—will also be discussed. The application of these tools in the total synthesis of bioactive peptide natural products and novel macrocyclic therapeutic leads will be highlighted.



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O-6

Characterization of circular bacteriocins produced by lactic acid bacteria

Takeshi Zendo[1], Rodney Honrada Perez[1,2], Kenji Sonomoto[1]

[1]Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Fukuoka, Japan
[2]National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños, Los Baños, Laguna, Philippines

A wide variety of bacteria including lactic acid bacteria (LAB) produce ribosomally synthesized antibacterial peptides known as bacteriocins. LAB bacteriocins generally inhibit the growth of closely related Gram-positive bacteria and are thought to contribute to the preservation of fermented foods. Since LAB are generally regarded as safe, their bacteriocins are presumed safe and have been utilized as food preservatives and for other antimicrobial applications. LAB bacteriocins are highly diverse in their bioactivities and structures. Among them, circular bacteriocins are characterized by their unique head-to-tail structure. Aside from the application of circular bacteriocins as antimicrobial agents, their cyclization mechanism presumably by a dedicated enzyme, is expected as a new peptide modification process. So far, we discovered three novel circular bacteriocins, lactocyclicin Q, leucocyclicin Q and enterocin NKR-5-3B produced by LAB strains isolated from fermented foods. We have characterized their biochemical properties and identified their biosynthetic gene clusters in the hope of understanding their biosynthetic mechanisms.

Lactocyclicin Q (LycQ) and Leucocyclicin Q (LcyQ) are both 61-residue circular bacteriocins, sharing very high sequence identity, and are produced by Lactococcus sp. QU 12 and Leuconostoc mesenteroides TK41041, respectively. Furthermore, their DNA sequence show that they are both synthesized as a 63-residue precursor containing 2 amino acid residue-leader peptide. Their minimum biosynthetic gene clusters, composed of the precursor gene and 5 biosynthetic genes involved in their cyclization, secretion and self-immunity, are also highly homologous.

Enterocin NKR-5-3B (Ent53B) is a 64-residue circular bacteriocin from Enterococcus faecium NKR-5-3. Its three-dimensional nuclear magnetic resonance solution structure was determined and showed four helical segments enclosing a compact hydrophobic core, which together with its circular backbone impart its high stability and structural integrity. It is synthesized as an 87-residue precursor and is processed by 4 proteins (EnkB1234) encoded on the biosynthetic gene cluster to yield active circular form.

O-7

Heterologous production of new lasso peptide using shuttle vector system

Shinya Kodani,[1,2,3] Hikaru Hemmi,[4] Yuto Miyake,[2] Issara Kaweewan,[3] and Hiroyuki Nakagawa[4,5]

[1]College of Agriculture, Academic Institute, Shizuoka University, Shizuoka 422-8529, Japan
[2]Graduate School of Integrated Science and Technology, Shizuoka University, Shizuoka 422-8529, Japan
[3]Graduate School of Science and Technology, Shizuoka University, Shizuoka 422-8529, Japan
[4]Food Research Institute, National Agriculture and Food Research Organization (NARO), Ibaraki 305-8642, Japan
[5]Advanced Analysis Center, National Agriculture and Food Research Organization (NARO), Ibaraki 305-8642, Japan

A lasso peptide is a unique peptide, normally 15-25 amino acids in length, which possesses the common motif of a knot structure in the molecule. Based on its biosynthetic system, it is classified with the ribosomally biosynthesized and post-translationally modified peptides (RiPPs). By genome-mining method, lasso peptide gene clusters are found to be distributed over proteobacteria and actinobacteria. To exploit the lasso peptide gene clusters, efficient method to perform heterologous production system is needed to obtain larger amount of new lasso peptide. We recently found production of a new lasso peptide, named subterisin, in a culture of *Sphingomonas subterranea* with high yield (Kuroha et al. Tetrahedron letters 2017). On the other hand, Hayashi and Kurusu reported the construction of stable shuttle vectors between *E. coli* and *Sphingomonas* species (Hayashi & Kurusu, Biosci Biotechnol Biochem, 2014). The GC content of genome of *S. subterranea* is high (approximately 63 %) compared to that of *Escherichia coli* (approximately 50 %), so there is an advantage to use *S. subterranea* as a host for expression of gene with high GC content. We proposed that expression of the lasso peptide biosynthetic genes with high GC content in *S. subterranea* could result in a large amount of production of the exogenous lasso peptide. Based on this speculation, we accomplished the construction of a shuttle vector between *E. coli* - *Sphingomonas* sp. and heterologous production of a new lasso peptide named brevunsin in *S. subterranea*. We present the heterologous production and structure determination of the new lasso peptide brevunsin.

O-8

Making and Breaking Lasso Peptides

James Link

Princeton University

This talk will introduce lasso peptides, a class of peptidic natural products that are cyclized by both a covalent bond and a mechanical bond resulting in a rotaxane structure. Recent efforts from my group and other in genome mining for lasso peptides has revealed thousands of examples of these peptides scattered through the genomes of diverse bacteria. Following background on the structure and function of lasso peptides, this talk will focus on recent breakthroughs in understanding the enzymes that catalyze the biosynthesis of lasso peptides. In addition, this talk will touch upon enzymes that catabolize lasso peptides and their potential biological function.

O-9

Understanding the glycopeptide antibiotic cyclisation cascade

Max Cryle

Monash University

The glycopeptide antibiotics (GPAs) are a structurally complex and medically important class of peptide natural products that include the clinical antibiotics vancomycin and teicoplanin. They contain a large number of non-proteinogenic amino acids and are produced by a linear non-ribosomal peptide synthetase (NRPS) machinery comprising seven modules. Furthermore, GPAs are extensively crosslinked late in their biosynthesis on the NRPS assembly line by the actions of a cascade of Cytochrome P450 enzymes, a process which contributes to the rigidity and structural complexity of these compounds. Due to the challenge of synthesising GPAs, biosynthesis remains the only means of accessing GPAs for clinical use, which makes understanding the biosynthesis of GPAs of key importance.

In this presentation, I will detail results from our studies (including recent unpublished investigations) into the NRPS machinery, the P450-cyclisation cascade and the interplay of these two important biosynthetic processes during GPA biosynthesis. This includes the characterisation of key enzymatic processes during NRPS-mediated peptide biosynthesis (chlorination, thioesterase activity and reconstitution of peptide synthesis) as well as the P450-mediated cyclisation cascade (substrate specificity of P450 enzymes and cascade reconstitution).

Overall, our results demonstrate how selectivity during GPA biosynthesis is mediated through the careful orchestration of critical modification steps and interactions between the peptide-producing NRPS machinery and trans-modifying enzymes.

O-10

Highly Efficient Synthesis of N-methyl peptides

Jonathan M. Collins

CEM Corporation

The synthesis of N-methyl peptides is difficult due to increased steric hindrance for coupling after incorporation of the N-methyl amino acid. Additionally, the presence of N-methyl groups can sometimes promote fragmentation during the acidic resin cleavage step with trifluoracetic acid. We demonstrate the ultra efficient synthesis of a series of difficult N-methyl peptides featuring multiple consecutive N-methylated amino acids. The process is based on the use of a one-pot coupling and deprotection step, an enhanced carbodiimide based coupling with microwave irradiation, and an optimized cleavage step. The result is extremely fast synthesis of N-methyl peptides in high purity and with absolutely minimal amounts of reagents. This process is also demonstrated on a range of other difficult sequences and synthesis scales.

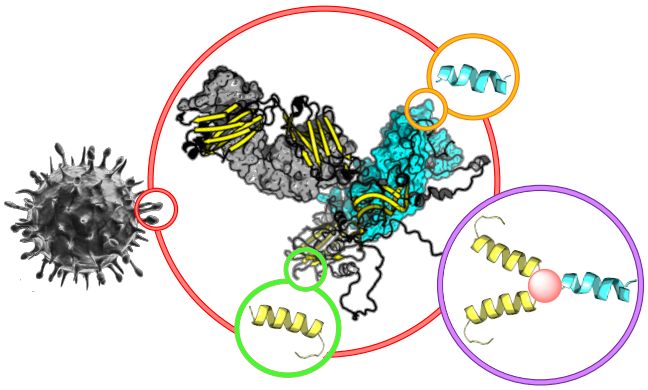
H1,P1

Peptide-based vaccines to treat foot-and-mouth disease: exploring their efficacy and molecular mode of action

Mar Forner,[1,3] Sira Defaus,[3] David James Craik,[1] Sónia Troeira Henriques,[1] David Andreu[2]

[1]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia
[2]Departament de Ciències Experimentals i de la Salut, Pompeu Fabra University, Barcelona, Spain

Foot-and-mouth disease virus (FMDV) is highly contagious and causes the livestock disease with most economic impact worldwide. Continuous emergence of fatal outbreaks shows the need for more effective strategies to eradicate endemic FMD, and to avoid outbreaks in free zones. The current strategies to treat FMDV are the classical vaccines with the inactivated virus and have serious limitations, such as, safety, marker nature, lack of cross-reactivity between serotypes and high costs in shipping and storing [1]. Peptide based vaccines are emerging as attractive candidates, as they can mimic epitopes in the absence of the infectious agent, they can differentiate infected from vaccinate animals (DIVA) and can be easily fine-tuned to be active against various viruses strains [2]. In 2016, we reported a panel of synthetic peptide-based vaccines displaying multiple antigen peptides (MAPs) in a single molecular platform that confers partial and full protection to immunized swine against FMDV challenge. Particularly, these dendrimeric constructions (B2T) combines two and one copies, respectively, of FMDV B- and T- cell peptide epitopes by highly efficient thiol-ene conjugation [3]. On the basis of the experimental results, we present a rational comparison between the most relevant B2T subunits in order to establish a structure-activity relation (SAR) by decipher the cellular uptake and their mode-of-action. To this end, synthetic strategy, internalization assays and toxicity studies within BHK-21 and PK-15 cell lines will be discussed.



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H2, P2

Closing the ring: a new cyclisation position for the cyclic peptide MCoTI-II

Bronwyn Smithies[1], Mark Jackson[1], Yen-Hua Huang[1], Edward Gilding[1], David J. Craik[1]

[1]Institute for Molecular Bioscience, 306 Carmody Rd (Building 80), The University of Queensland, QLD, Australia, 4072

Cyclotides are ribosomally-synthesised peptides that are naturally occurring in plants. Head-to-tail cyclisation of these peptides render their C- and N- terminal residues inaccessible to enzymatic degradation, while the presence of three disulfide bonds strengthens the structure further. This ultra-stable structure has been established as a scaffold to stabilise smaller therapeutic peptide epitopes that would otherwise be unstable. Cyclotides are amenable to solid phase peptide synthesis (SPPS) and native chemical ligation (NCL) which has enabled investigations into their structure, function and the engineering of new bioactive cyclotide analogues. The SPSS approach is limited in cases where inclusion of larger peptide epitopes approach the size limitations of SPPS. For greater yield and cost effectiveness, it is sensible to explore recombinant expression as a means of cyclotide-production. During chemical synthesis, the cyclotide backbone can be cyclised at almost any position using NCL, but plant-produced cyclotides are cyclised enzymatically at a precise position in an exposed region called loop 6. Incidentally, almost 75% of modified cyclotides with therapeutic potential interrupt this enzymatic cyclisation point in loop 6, meaning that recombinant expression and enzymatic cyclisation is not possible. To address this, we investigate the engineering of alternative enzymatic cyclisation sites within a cyclotide scaffold, thus retaining loop 6 for epitope grafting and recombinant-based cyclotide expression. Alternative points of cyclisation were rationally designed into MCoTI-II, a cyclotide commonly used as a scaffold for drug design by SPPS. We show that this engineered MCoTI-II precursor could be successfully processed into the mature cyclic product by the same enzymes found in cyclotide-producing plants. This achievement is a breakthrough in cyclotide production as it enables the implementation of stabilised and effective peptides as therapeutics biosynthesised at scale in recombinant systems.

H3,P3

Surveying a diverse set of monocots for cyclotides: are these cyclic peptides restricted to dicots?

Haiou Qu, Edward G Gilding, Mark A Jackson, Olivier J Cheneval, Kuok Yap, David J Craik

Institute for Molecular Bioscience, The University of Queensland

Cyclotides are a class of small peptides produced in plants with characteristic head-to-tail cyclized backbones, which are threaded by three intramolecular disulfide bonds. They were first discovered from the Rubiaceae (coffee family), and have since been documented in the Violaceae (violet family), Fabaceae (legume family), Solanaceae (potato family) and Cucurbitaceae (cucumber family). Cyclotides appear restricted to dicot plants, however some linear cyclotides, termed acyclotides, have been discovered from the Poaceae (grass family). To understand this selective distribution and the evolution of cyclotides, we searched monocot lineages for cyclotide-like gene sequences in RNA-seq data. Here, we report eight new cyclotide-like sequences uncovered from *Setaria italica*, *Setaria viridis*, and *Sorghum bicolor* and attempt to elucidate their expression in transcriptomes and proteomes. Of these new sequences, millet L1 (mL1) from *S. italica*, rice L1 (rL1) from *Oryza sativa* and panitide L1 (pL1) from *Panicum laxum* were expressed by a transient transformation system in *Nicotiana benthamania*. Successful expression of these peptides illustrates the possibility to produce heterologous cyclotide-like peptides *in planta*. Furthermore, the pL1 peptide was shown to be cyclizable by recombinant $OaAEP_{1b}$ *in vitro*, requiring only a few residue changes. These results provide support for the hypothesis that these peptides have topologies approaching that of known cyclotides from dicots. In addition, analysis of the promoter sequence of rL1 from rice shows that its expression may be relevant to plant defense, as is known for cyclotides from dicots.

Keywords: monocots, cyclotide-like genes, gene expression, cyclisation *in vitro*

H4,P4

Isolation, Characterization and Conformational Analysis of Cyclotides, a Class of Macrocyclic Disulfide Bonded Plant Peptides

Neha Kalmankar[1,2], Padmanabhan Balaram[1], Sowdhamini Ramanathan[1], Radhika Venkatesan[1]

[1]National Centre for Biological Sciences
[2]The University of Trans-Disciplinary Health Sciences and Technology

Cyclotides are a novel class of gene-encoded, disulfide-rich, macrocyclic peptides (26-37 residues) acting as defense mechanism in several plant species. However, the role of cyclotide expression in the purview of plant ecology remains poorly understood. In this study, we have purified and sequenced novel cyclotides from four different tissues (leaves, stems, pods & flowers) of an Indian medicinal plant Clitoria ternatea using RP-HPLC, MALDI and ESI-MS techniques. Using RNA-Seq data, we have identified 20 cyclotides precursor genes from the four tissues of C. ternatea. Results from both mass-spectrometry and transcriptome-mining suggest that several cyclotides differentially expressed across the tissues. The relative expression levels of the assorted peptides were also found to be different across the four tissues and revealed few novel structural arrangements.

Cyclotides can be divided into the Möbius and Bracelet subfamily, based on the presence or absence of a cis-proline residue in loop 5 of the polypeptide chain, respectively. Thus far, only a single crystal structure has been reported for a cyclotide (Möbius subfamily), while few NMR derived structures are available for the Bracelet subfamily. The paucity of high resolution structures calls for the development of improved computational methods for predicting disulfide-rich peptide structures and their interactions, using only the sequence information. On our sequenced peptides from Clitoria ternatea tissues, we employed the random conformation generation algorithm (RANMOD), which builds Ramachandran allowed conformations for peptides of defined length by assigning stereochemically accessible local conformations at each residue, in conjunction with the disulfide modelling algorithm MODIP to generate models of linear precursors with three disulfide bridges, followed by an energy minimization routine to select end to end cyclizable conformations. The three-dimensional models were found to be structurally compatible and similar to experimentally determined structures.

H5,P5

Design of Peptoid-peptide Macrocycles to Inhibit the β-catenin TCF Interaction in Prostate Cancer

Timothy Craven

University of Washington

New chemical inhibitors of protein-protein interactions are needed to propel advances in molecular pharmacology. Peptoids are peptidomimetic oligomers with the capability to inhibit protein-protein interactions by mimicking protein secondary structure motifs. I will report the in silico design of a macrocycle primarily composed of peptoid subunits that targets the β-catenin:TCF interaction. The β-catenin:TCF interaction plays a critical role in the Wnt signaling pathway which is over-activated in multiple cancers, including prostate cancer. Using the Rosetta suite of protein design algorithms, we evaluate how different macrocycle structures can bind a pocket on β-catenin that associates with TCF. The in silico designed macrocycles were screened in vitro using luciferase reporters to identify promising compounds. The most active macrocycle inhibits both Wnt and AR-signaling in prostate cancer cell lines, and markedly diminishes their proliferation. In vivo potential is demonstrated through a zebrafish model, in which Wnt signaling is potently inhibited.

H6,P6

From sunflower to skin cancer: The development of melanocortin receptor agonists using sunflower trypsin inhibitor-1

Andrew M. White,[1] Thomas Durek,[1] Christina I. Schroeder,[1] Quentin Kaas,[1] Peta J. Harvey,[1] Anita Dellsen,[3] Torben Österlund,[4] Niklas Larsson,[4] Minying Cai,[2] Victor J. Hruby,[2] Laurent Knerr,[5] Alleyn T. Plowright,[5] and David J. Craik[1]

[1]Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD 4072, Australia
[2]Department of Chemistry and Biochemistry, University of Arizona, Tucson, Arizona 85721, United States
[3]Mechanistic Biology & Profiling, Discovery Sciences, IMED Biotech Unit, AstraZeneca, Gothenburg 43183 Sweden
[4]Discovery Biology, Discovery Sciences, IMED Biotech Unit, AstraZeneca, Gothenburg 43183 Sweden
[5]Medicinal Chemistry, Cardiovascular and Metabolic Diseases, IMED Biotech Unit, AstraZeneca, Gothenburg 43183 Sweden

The melanocortin receptors are a family of five (MC1-5R) G-protein coupled receptors that control a wide variety of functions including appetite, energy homeostasis, skin pigmentation, and sexual function.[1,2] The pharmacology of these receptors has gained considerable attention for the treatment of disorders such as obesity, skin cancer, and sexual dysfunction and the first peptide based analogues are now emerging on the pharmaceutical market. The melanocortin receptors are regulated by the melanocyte stimulating hormones, all of which contain a conserved tetrapeptide activating motif (HFRW). To develop novel leads to target the melanocortin receptors we chose the cyclic peptide, sunflower trypsin inhibitor-1 (SFTI-1) as a scaffold to incorporate the HFRW epitope. Using this grafting approach, and a variety of optimization strategies we have engineered highly potent (EC_{50} <nM) and MC1R selective agonists that may hold potential application in the treatment of melanoma.[3]

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H7,P7

Design Of Potent And Selective Cathepsin G Inhibitors Based On The Sunflower Trypsin Inhibitor-1 Scaffold

C. Y. Li[1], S. de Veer[1], J. E. Swedberg[1], D. J. Craik[1]

[1]Institute for Molecular Bioscience, The University of Queensland, Brisbane QLD 4072, Australia

Serine proteases from immune cells are critical for the human immune system since these proteases have an important role in destroying invading pathogens. An imbalance between these proteases and their endogenous inhibitors contributes to the development of various chronic inflammatory diseases. Cathepsin G (CG) is a serine protease secreted by activated neutrophils. The overactivity of CG is involved in a range of chronic lung disorders, in particular chronic obstructive pulmonary disorder (COPD). There is a worldwide prevalence of 250 million people suffering from COPD. Development of potent and selective CG inhibitors could provide a novel therapeutic pathway. In this study, we characterized the substrate specificity (P4-P1) of CG by screening a non-combinatorial tetrapeptide substrate library. Substituting the preferred substrate sequences into sunflower trypsin inhibitor-1 (SFTI-1) produced the most potent CG inhibitor reported to date (K_i = 0.89 nM). To increase the specificity of the inhibitor, the P2' preference of CG was determined by screening a P2' diverse SFTI-based library. Substituting the most preferred P2' residue into the SFTI-1 scaffold in combination with the most preferred substrate sequence (P4-P2) and a non-proteinogenic P1 residue (4-guanidyl-L-phenylalanine) produced a potent CG inhibitor (K_i = 1.6 nM) (Figure 1). This inhibitor is the most selective compound reported for CG to date, with at least 360-fold selectivity over other serine proteases with similar substrate specificity. This compound is a promising lead for drug design targeting COPD.

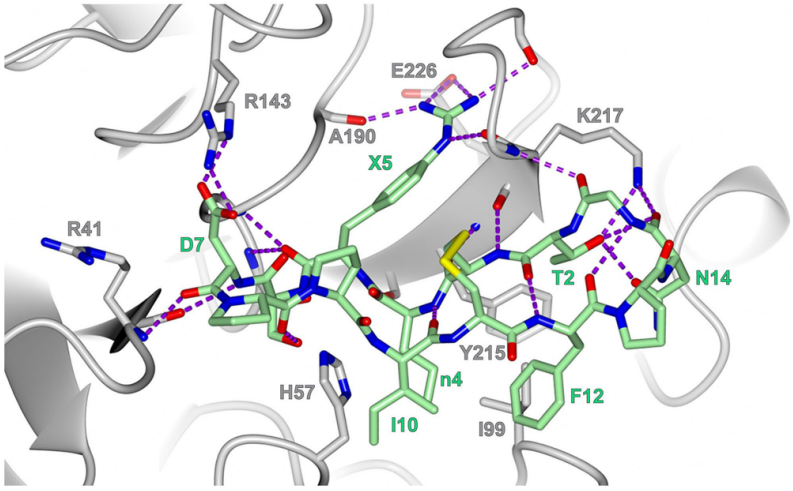

Figure 1. Stick model of SFTI-based inhibitor bound to CG.

H8,P8

Discovery and Characterization of Novel Cyclic and Acyclic Trypsin Inhibitors from Momordica dioica

Junqiao Du, Lai Yue Chan, Aaron G. Poth, David J. Craik*

Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, 4072, Australia

Momordica trypsin inhibitors (TIs) such as those isolated from the seeds of the gâc fruit, *Momordica cochinchinensis* (MCoTI-I and MCoTI-II), are widely used as scaffolds for drug design studies. To more effectively exploit these molecules in the development of therapeutics, there is a need for wider discovery of the natural sequence diversity among TIs from other species in the *Momordica* subfamily. In this work, we have identified the encoding gene and six TIs from the seeds of the spiny gourd, *Momordica dioica*, a climbing creeper from the Cucurbitaceae family, of which four are novel (Modi 1, Modi 3, Modi 5, Modi 6) and two (Modi 2, Modi 4) are known peptides (TI-14, TI-17) previously found in the wild bitter gourd, *Momordica subangulata*. Modi 6 is an acyclic peptide featuring a pyrrolidone carboxylic acid modification, whereas the remaining five TIs are cyclic. All Modi peptides display similar overall structures (based on NMR) and trypsin inhibitory activities compared to native MCoTI-II. No toxicity was observed for Modi peptides when tested against cancer and insect cells. Both cyclic and acyclic Modi peptides were exceptionally stable over 24 h in human serum, suggesting these peptides might be excellent candidates as scaffolds for epitope stabilization in drug design studies. The biosynthetic origin of the six peptides as tandem repeats on a single encoding gene offers an efficient production route, with nature employing a dual strategy to stabilize the peptides: either head-to-tail cyclisation for the first five peptides or N-pyrolation for the final (acyclic) peptide.

H9,P9

Toxicity and mechanism of action of cyclic helix-loop-helix peptides

Gregoire Philippe[1], Diana Gaspar[2], Yen-Hua Huang[1], Joachim Weidmann[1], Nicole Lawrence[1], Johannes Koehbach[1], Caibin Sheng[3], Alexander Loewer[3], Miguel ARB Castanho[2], David Craik[1], Sónia Henriques[1]

[1]Institute for Molecular Bioscience, the University of Queensland, QLD 4072, Australia
[2]Faculdade de Medicina da Universidade de Lisboa, 1649-028 Lisboa, Portugal
[3]Technical University Darmstadt, 64287 Darmstadt, Germany

Inhibition of intracellular protein-protein interactions (PPIs) involved in cancer pathways is a valid strategy to develop more specific anticancer drugs. In particular, inhibition of MDMX:p53 and MDM2:p53 interactions are of great interest due to their involvement in different cancers, including breast and skin cancer. The transcription factor p53 is a tumor suppressor that induces apoptosis of damaged cells. In healthy cells, p53 activity is regulated and inhibited by the proteins MDM2 and MDMX, but some cancer cells overexpress MDM2 and/or MDMX and inactivate p53, which facilitate tumor development. Cyclic helix-loop-helix peptides carrying cell-penetrating properties on one helix, and a mimic of the p53 binding site on the second have recently been developed. Intramolecular interactions create a helical structure, which increase the affinity of the p53-mimic for inhibitors MDM2 and MDMX. This peptide also demonstrated outstanding cell-penetrating properties. In the current study, we were interested in characterising its mechanism of action. Our results suggest the peptide does not interact strongly with the membrane but triggers toxicity independent of p53-pathway. The peptide showed identical toxicity against a broad range of cells including healthy and p53-mutant cells. Using p53-reporter cells and time-lapse microscopy we observed an increase in the p53 levels after incubation with the peptide but the cells did not die from apoptosis as expected. Atomic force microscopy studies suggest disruption of the cell membrane or necrosis. These results will help improve this scaffold to decrease its unwanted toxicity to healthy cells and improve its intracellular activity.

H10,P10

Enhanced oral bioavailability and serum half-life of engineered cyclic peptides that target the neonatal Fc receptor

Benjamin J. Tombling, Finja König, David J. Craik, Conan K. Wang*

Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland 4072, Australia

Currently, the therapeutic potential of peptides is limited by their poor oral bioavailability and short circulating plasma half-life. The neonatal Fc receptor (FcRn) is responsible for the long serum half-life of immunoglobulin G and albumin, and as a result has attracted pharmaceutical interest for improving the pharmacokinetic properties of protein therapeutics. Phage-display derived peptide epitopes of FcRn were shown to improve the pharmacokinetic profile of a fusion protein; however, these peptide epitopes are not well suited to survive the harsh environment of the gastrointestinal tract. Therefore, we used molecular grafting to incorporate an FcRn-binding peptide epitope onto the plant-based cyclic peptides sunflower-trypsin inhibitor-1 and kalata B1, which are macrocyclic peptide scaffolds shown to be intrinsically stable and tolerable to sequence modification, with the aim of stabilizing the bioactive epitope. Using this approach, we designed novel cyclic peptides that retained the structure and stability of the parent scaffold. The engineered cyclic peptides could be used as next-generation macrocyclic disulfide-rich scaffolds for biotechnological applications.

H11,P11

Identification of Cyclotide-Like Human Factor XIIa Ligands Through mRNA-Display.

Wenyu Liu[1], Joakim Swedberg[2], Toby Passioura[1], David Craik[2] and Hiroaki Suga[1]

[1]Department of Chemistry, Graduate School of Science, the University of Tokyo
[2]Institute for Molecular Bioscience, the University of Queensland.

Momordica cochinchinensis trypsin inhibitor-II (MCoTI-II) is a cyclotide (macrocyclic peptide) that exhibits potent inhibitory activity against trypsin proteases. MCoTI-II possesses 3 pairs of disulfide bonds in a cystine-knot conformation and is head-to-tail backbone cyclic, making it an attractive candidate for drug development due to its proteolytic stability and potential oral availability. A number of trypsin-like proteases are involved in disease states, and it is therefore an intriguing possibility to develop drug candidates that target these proteases by taking advantage of the MCoTI-II cystine-knot scaffold.

In this work, an MCoTI-II-based peptide library containing 12 randomized amino acids in Loops 1 and 5 was designed, synthesized and screened by mRNA display against the human trypsin-like protease, coagulation factor XIIa (FXIIa), using a selection process that affords very high library diversity (more than 10^{12} compounds). Several of the most abundant peptides following selection were synthesized by standard solid phase techniques and FXIIa binding affinity measurements revealed high affinity, with dissociation constants (K_D) as low as 33 nM, indicating potential inhibitory activity of the selected peptides towards FXIIa. This successful application of an MCoTI-II-based peptide library provides a novel route for developing drug candidates based on cystine-knot scaffolds.

Friday 30th, November

9:00-10:40 Session 6: Structure function Chairs: Akane Kawamura and Meritxell Teixido

Johan Rosengren
A novel macrocyclic peptide from the common zinnia adopts a stable but chameleonic clam-shaped structure

Parissa Hosseinzadeh, University of Washington
Computational tools to design selective cyclic peptide-based inhibitors

Simon De Veer, Institute for Molecular Bioscience, University of Queensland
Exploring the links between structure and function in sunflower trypsin inhibitor-1 guides the design of new inhibitor variants

Aline Dantes de Araujo, Institute for Molecular Bioscience, University of Queensland
Targeting Intracellular Protein-Protein Interactions with Covalent Peptide Inhibitors

11:00-12:30 Session 7: Pharmacokinetics and design Chairs: Scott Lokey and Christian Hakenberger

Markus Muttenthaler
Chemical strategies to tune potency, selectivity and stability of neuropeptides.

Conan Wang, Institute for Molecular Bioscience, University of Queensland
Smarter, Not Harder: Time Hacks for Structure Determination of Cyclic Peptides

Nasir Bashiruddin
Targeting Plexin-B1 with Macrocyclic Peptides

Meritxell Teixido
Minichlorotoxins, a New Family of Blood-Brain Barrier Peptide Shuttles

13:30-15:30 Session 8: Display selection Chairs: Ali Tavassoli and Toby Passioura

Akane Kawamura, University of Oxford
TBC (cyclic peptide JMJD2 and/or PHD inhibitors)

Joseph Rogers, Vertex Pharmaceuticals
Nonproteinogenic optimization of linear and cyclic peptides

Takayuki Katoh
Translation of consecutive D-amino acids

Yizhen Yin, University of Tokyo
Construction of macrocyclic peptide libraries by integrating with boronated amino acids and selenocysteine.

Kunio Matsumoto, Kanazawa University
Macrocyclic peptides targeting HGF and MET

16:00-17:20 Session 9: Intracellular targeting Chairs: Chris Hipolito

Scott Lokey, University of California, Santa Cruz
A Roadmap for Designing and Filtering Large, Encoded Libraries of Cyclic Peptides for Cell Permeability.

Crystal Huang, Institute for Molecular Bioscience, University of Queensland
Targeting the oligomerization interfaces of BCR-ABL1 kinase with macrocyclic peptides: An alternative strategy to combat chronic myeloid leukemia

Christian Hackenberger, FMP Berlin
Round it up: Cyclic peptide-conjugates for intracellular protein delivery

17:20-18:10 Session 10 Chairs: Toby Passioura

David Craik, Institute for Molecular Bioscience, University of Queensland
The past, present and future of plant-derived cyclic peptides

O-11

Novel genetically encoded cyclic and bicyclic architectures displayed on phage

Ratmir Derda

University of Alberta

The talk will describe genetically-encoded (GE) platform for discovery of macrocyclic and macrobicyclic peptides synthesized by aqueous late-stage functionalization of readily-available libraries of peptides displayed on phage. The structure of these chemical post-translational modifications can be encoded in the genome of phage using silent encoding technology. The resulting phage displayed libraries of "unnatural" macro(bi)cyclic peptides could be used to target either proteins or cells and tissues; the latter targets are difficult to address with DNA/RNA or bead-based libraries. Expanded chemical space offers value-added properties such as stability to aggressive protease environment and incorporation of unnatural chemotypes that are known to increase bioavailability.

O-12

Biosynthesis of macrocyclic peptides in sense codon reassigned in vitro translation system

Zhenling Cui[1], Yue Wu[1], Sergey Mureev[1,*] and Kirill Alexandrov[1,2,*]

[1]Institute for Molecular Bioscience, The University of Queensland, St Lucia, QLD 4072, Australia
[2]Australian Institute for Bioengeneering and Nanotechnology, The University of Queensland, St Lucia, QLD 4072, Australia

Sense codon reassignment to unnatural amino acids (uAAs) represents a powerful approach for introducing novel chemical into polypeptides. The main obstacle to this approach is competition between the native isoacceptor tRNA(s) and orthogonal tRNA(s) for the reassigned codon. While several chromatographic and enzymatic procedures for selective deactivation of tRNA isoacceptors in cell-free translation systems exist, they are complex and not scalable. We designed a set of tRNA antisense oligonucleotides that could efficiently and selectively sequester native $tRNA^{Ser}GCU$ directly in translation-competent *Escherichia coli* S30 lysate. Addition of such oligonucleotides to the lysate abrogate its translational activity and liberate the AGU/AGC codons. Expression of eGFP protein from the template harboring a single reassignable AGU codon in $tRNA^{Ser}GCU$-depleted *E. coli* lysate allowed its homogeneous modification with *n*-propargyl-L-lysine (Prk) or *p*-azido-L-phenylalanine (AzF). This strategy is generic, as demonstrated by sequestration of $tRNA^{Arg}CCU$ isoacceptor. Furthermore, combination of two specific antisense oligonucleotides enables sequestration of both $tRNA^{Ser}GCU$ and $tRNA^{Arg}CCU$ simultaneously and liberates two sense codons for reassignment. Using this approach we demonstrate simultaneously installation of Prk and AzF into a peptide sequence in a one-pot reaction via reassignment of AGU and AGG codons. These two uAAs formed 1, 4-disubstituted 1, 2, 3-triazole bond via copper-catalyzed click chemistry. This approach represents a new direction in genetic code reassignment based on S30 cell free translation system with numerous practical applications including generating macrocyclic peptide library with expanded chemical diversity for peptide drug discovery.

O-13

Variable Identification and De Novo Sequencing of Cyclopeptides via Computational Processing of Tandem Mass Spectra

Alexey Gurevich[1], Bahar Behsaz[2], Alla Mikheenko[1], Alexander Shlemov[1], Anton Korobeynikov[1,3], Hosein Mohimani[4], Pavel A. Pevzner[1,5]

[1] Center for Algorithmic Biotechnology, St. Petersburg State University, St. Petersburg, Russia
[2] Bioinformatics and Systems Biology Program, University of California, San Diego, La Jolla, CA, USA
[3] Department of Mathematics and Mechanics, St. Petersburg State University, St. Petersburg, Russia
[4] Department of Computational Biology, Carnegie Mellon University, Pittsburgh, PA, USA
[5] Department of Computer Science and Engineering, University of California, San Diego, La Jolla, CA, USA

Tools availability: http://cab.spbu.ru/software/dereplicator/

Identification of tandem mass spectra derived from peptidic natural products (PNPs) and especially cyclopeptides is more difficult than traditional linear peptide identification in proteomics. In addition to a nonlinear structure, these compounds also contain nonstandard amino acids and complex modifications that makes the use of conventional proteomics software impractical. Dereplicator[1] was one of the first high-throughput PNP identification approaches capable of searching millions of spectra against compounds in a chemical library. However, Dereplicator is strictly limited to finding known peptides and it fails to identify their novel modified/mutated variants if they are absent in a database (so-called *variable identification*).

Variable identification is crucial for PNP discovery because the current chemical databases are small and dominated by the most abundant representatives of PNP families while more rare peptide variants are sometimes more clinically effective. To address this problem, we developed VarQuest[2], an extension of Dereplicator. Since the computational space for variable PNP identification is several orders of magnitude larger than for standard identification, VarQuest searches are performed in two stages. First, a set of feasible spectra and structure matches is constructed. Second, this filtered set is rigorously scored taking into account all possible modifications.

Identification of known PNPs and their unknown variants using Dereplicator and VarQuest leads to the discovery of completely novel peptides in the remaining tandem mass spectra. To speed up the discovery process, we developed CycloNovo[3], a tool for *de-novo* cyclopeptide sequencing. This approach is based on the de Bruijn graph concept, the workhorse of modern genome sequencing algorithms[4].

The developed tools were benchmarked on millions of high-resolution spectra from GNPS[5]. VarQuest increased the number of identified cyclopeptides five times comparing to Dereplicator results (1508 vs 309). Furthermore, CycloNovo discovered 512 unique putatitive cyclopeptides, out of which only 67 were known before.

References:

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O-14

Development of tunable and selective protein modifiers based on macrocyclic peptides

Goto, Yuki; Ozawa, Naoya; Suga, Hiroaki

Department of Chemistry, Graduate School of Science, The University of Tokyo

Molecules that covalently bind to a native protein of interest are useful for detection and regulation of the protein. Such protein modifiers can be developed by embedding a reactive group into a non-covalent ligand scaffold. When the molecule is associated with the protein, the reactive group is positioned near an appropriate amino acid on the protein surface and covalent bond formation is induced by proximity effect. This strategy has been applied to various protein-ligand pairs including enzymes with their small molecule ligands, proteins forming heterodimers, and proteins with their cognate antibodies. The key to successful development of selective modifiers includes high selectivity of the ligand, and appropriate reactivity and proper orientation of the reactive group, achievement of which often requires intense efforts. Moreover, three-dimensional structures of the protein-ligand complex are often required to determine the appropriate site for embedding the reactive group, which limits the versatility of this strategy.

Macrocyclic peptides could be ideal as the non-covalent ligand scaffold for selective covalent modifiers, because their large surface areas and diverse structures allow for high selectivity to various target proteins. Previously, our group has developed a methodology for facile construction of a library of trillions of macrocyclic peptides containing various non-natural amino acids by combining in vitro translation system with genetic code reprogramming technique. Our group has also demonstrated that peptides selectively binding to a given target protein can be readily discovered from the library by means of in vitro selection.

We here report a new methodology for facile discovery of peptide-based selective covalent modifiers with desired reactivity. An mRNA-displayed library of macrocyclic peptides bearing a tunable reactive group was constructed and screened by in vitro selection, resulting in covalent modifiers of the designated target protein. The efficiency of the covalent bond formation could be readily modulated by rational modification of the reactive group without loss of the selectivity.

O-15

A platform for the generation and screening of cyclic peptide libraries of over a hundred million members.

Ali Tavassoli

University of Southampton

SICLOPPS is an intracellular method for the generation of cyclic peptide libraries of over a hundred million members. We have interfaced SICLOPPS libraries with a variety of cell-based assays for the identification of inhibitors of a variety of protein-protein interactions, including several transcription factor (e.g. HIF-1). Here, we detail a newly developed ultra-high throughput screening platform that allows the generation of SICLOPPS cyclic peptide libraries in femtolitre-sized microfluidic droplets. This newly developed platform holds several advantages over existing methods, including the kilohertz speeds with which over 10^10 independent reaction vessels may be generated per millilitre of emulsion, the ability to run functional assays (as opposed to binding assays traditionally employed by other technologies), the ability to encode multiple non-natural amino acids into the library, the ability to interface this library with existing in vitro assays, as well as the potential to significantly reduce reagents used in high-throughput screening.

O-16

A novel macrocyclic peptide from the common zinnia adopts a stable but chameleonic clam-shaped structure

K. Johan Rosengren[1], Bastian Franke[1], Mark F. Fisher[2], Jingjing Zhang[2], Achala S. Jayasena[2], Richard J. Clark[1], Joshua S. Mylne[2]

[1]The University of Queensland, School of Biomedical Sciences, Brisbane, QLD 4072, Australia [2]The University of Western Australia, School of Chemistry and Biochemistry & ARC Centre of Excellence in Plant Energy Biology, Crawley, WA 6009, Australia

Macrocyclic peptides with a head-to-tail cyclized backbone are intriguing natural products with interesting properties. A family of macrocyclic peptides named PawS-Derived Peptides (PDPs) is produced from precursors of seed storage albumins in species of the sunflower family. The prototypic member is the potent sunflower trypsin inhibitor-1 (SFTI-1). Here we report an unusually large PDP found in the seeds of *Zinnia elegans*. PDP-23 is twice the size of most PDPs and the first macrocyclic peptide known to possess two disulfide bonds. PDP-23 was identified by *de novo* transcriptomics and confirmed at the protein level by liquid chromatography-mass spectrometry. The peptide was produced by chemical synthesis and two-dimensional solution NMR spectroscopy was used to elucidate its structural features. The PDP-23 macrocycle adopts a unique conformation in which two *β*-hairpins, each stabilized by one of the disulfide bonds, fold on top of each other enclosing a hydrophobic core. The clam-shaped tertiary structure is remarkably stable and does not substantially unfold at near-boiling temperatures. However, it is not restrained by the disulfides allowing the peptide to open and adopt a different structure upon interactions with membranes, suggesting it might have advantages over more rigid scaffolds and represent a versatile template for drug development.

O-17

Computational tools to design selective cyclic peptide-based inhibitors

Parisa Hosseinzadeh, David Baker

University of Washington

Peptide macrocycles are attractive alternatives to small molecules and antibodies as next generation of therapeutics. However, their use has been limited in part due to the limited power of designing them in the conformation we need. Library-based approaches are powerful but can't sample the entire space available to macrocycles, especially when D-amino acids and unnatural amino acids are also introduced. In this work, I present the computational method we developed to robustly design structured macrocycles, as well as the resulting library of 200 stable ordered macrocycles in the range of 7-10 residues. I will then present my latest work on developing computational tools to design macrocycles as inhibitors of enzymes or protein-protein interactions. In particular, I will talk about my latest results in computational design of a histone deacetylase 2 inhibitor with IC50 of 9 nM. This macrocycle was obtained from a selection of 40 other macrocycles from three rounds of design, proving the power of the design approach. I then talk about future directions in terms of improving selectivity and developing general approaches for similar inhibitor design problems.

O-18

Exploring the links between structure and function in sunflower trypsin inhibitor-1 guides the design of new inhibitor variants

Simon de Veer[1], Joakim Swedberg[1], Choi Yi Li[1], Sixin Tian[1], Jonathan Harris[2], David Craik[1]

[1]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia
[2]Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia

Plant seeds are a rich source of protease inhibitors that contribute to protecting seeds from enzymatic digestion and aid seed dispersal. One of the smallest seed-derived protease inhibitors is a 14-amino acid cyclic peptide found in sunflower seeds, sunflower trypsin inhibitor-1 (SFTI-1), which shows potent activity against trypsin. Using SFTI-variants with different modifications, we have explored the links between structure and function that convey the potency and target selectivity of SFTI-1. These insights are important as trypsin is a prototypic member of a large family of serine proteases that share a common fold. These enzymes have evolved to carry out diverse biological functions that are prominent in homeostasis and disease. Accordingly, SFTI-1 has excellent credentials as a design scaffold for engineering protease inhibitors that have potential pharmaceutical applications. Guided by our findings on SFTI-1, we have developed several approaches for optimizing different residues in the SFTI-scaffold in order to generate inhibitors for diverse serine protease targets. In recent work, we have applied these strategies to mesotrypsin, neutrophil serine proteases and members of the kallikrein-related peptidase family, which have led to the production of new inhibitor variants for enzymes with trypsin-like, chymotrypsin-like and elastase-like specificity. These findings provide new insights into protease inhibitor design and demonstrate the remarkable versatility of SFTI-1 as an engineering scaffold, defying its relatively simple architecture.

O-19

Targeting Intracellular Protein-Protein Interactions with Covalent Peptide Inhibitors

Aline Dantas de Araujo[1], Junxian Lim[1], Andrew C. Good[2], Renato T. Skerlj[2] and David P. Fairlie[1]

[1]Division of Chemistry and Structural Biology, ARC Centre of Excellence in Advanced Molecular Imaging, Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD 4072, Australia
[2]Noliva Therapeutics, Newton, MA 02465, USA

Stapled peptides are a fast growing class of bioactive helical peptides modelled after the alfa-helix domain of proteins involved in intracellular protein-protein interactions (PPIs). Their potential to penetrate cells and bind tightly to shallow PPI interfaces has opening up new opportunities to pharmacologically modulate a range of "undruggable" intracellular biological targets, exceptionally those implicated in oncogenesis. Here, we aim to improve the potency of stapled peptides by applying the concept of covalent inhibition. Covalent targeting has long been exploited to increase therapeutic efficacy of some small organic drugs, with some successes making a major impact on human health (omeprazole, cloridogrel and aspirin are blockbuster examples).

To demonstrate the feasibility of the covalent approach applied to stapled peptides acting on intracellular PPIs, we incorporated an acrylamide warhead into a hydrocarbon-stapled peptide ligand BIM to target the oncogenic protein Bcl2A1. We demonstrated that the warhead-bearing stapled peptide was capable of entering live cells and binding covalently to cytosolic endogenous Bcl2A1. By rational design, we developed a covalent peptide inhibitor that was shorter and bonded faster and more selectively than the parent compound. This innovative approach demonstrates the potential to selectively silence a PPI inside cells with stapled peptide inhibitors, allowing longer duration of action, non-competition with other endogenous ligands and avoiding peptide clearance once bound to target protein.

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O-20

Chemical strategies to tune potency, selectivity and stability of neuropeptides

Markus Muttenthaler[1,2]

[1] Institute of Biological Chemistry, University of Vienna, Vienna, Austria
[2] Institute for Molecular Bioscience, University of Queensland, Brisbane Australia

Peptides have emerged as a therapeutically and commercially important class of drugs with the advantage of great specificity, potency and low toxicity profiles. They have become the drug of choice for the treatment of numerous diseases, such as diabetes, HIV, hepatitis and cancer with >150 peptides undergoing clinical trials. Peptides are furthermore invaluable research tools helping to dissect the physiological functions of many human receptors and elucidating the biological mechanisms underlying diseases.

Our lab is particularly interested in disulfide bond containing human neuropeptides that regulate a variety of important physiological functions including pain, appetite, social behaviour and inflammation. Taking advantage of the ancient and well-conserved character of these neuropeptides, we explore natural sources and innovative chemistry to develop advanced probes and therapeutic leads to study these signalling systems. Here we will present a variety of chemical strategies that allows us to tune potency, selectivity and stability of these intriguing class of molecules and discuss their potential therapeutic applications.

O-21

Smarter, Not Harder: Time Hacks for Structure Determination of Cyclic Peptides

Conan K Wang, Gordon J King, Suelam D Ramalho, Susan E Northfield, Paola O Ojeda, Anne C Conibear, Mariana C Ramos, Steph Chaousis, Sónia T Henriques, Yen-Hua Huang, Vanderlan S Bolzani, David J Craik

The Institute for Molecular Bioscience, The University of Queensland

The structures of cyclic peptides provide invaluable information that aid in understanding function and facilitating design. They are frequently solved by either X-Ray crystallography or NMR spectroscopy, however these techniques have been classically resource intensive. Here, I will go through approaches that potentially streamline the process of structure determination, namely the use of racemic mixtures in X-Ray crystallography and chemical shifts in NMR spectroscopy. In crystallography, racemic mixtures can be used to enable facile crystal growth, overcoming one of the major bottlenecks of the technique. We have demonstrated this approach on prototypic members from five different classes of cyclic peptides, obtaining high-resolution crystal structures (0.99–1.85Å).[1,2] More recently, we have explored new approaches to NMR-based structure determination, and found that accurate structures of cyclic disulfide-rich peptides can be obtained from chemical shift information, circumventing the traditional need for an exhaustive set of restraints. We demonstrated this on the five cyclic peptides used in racemic crystallography studies1,2 as well as on >100 disulfide-rich peptides whose structures were extracted from the PDB and chemical shifts from BMRB. The structures determined could be further refined to high resolution by the incorporation of sparse distance restraints or homology information. The approaches discussed herein appear to have broad utility in the structural biology of cyclic peptides.

References

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O-22

Targeting Plexin-B1 with Macrocyclic Peptides

Nasir K. Bashiruddin[1], Yukiko Matsunaga[2], Yan Wu[3], Mikihito Hayashi[4], Masanobu Nagano[3], Tomoki Nakashima[4], Junichi Takagi[2], Hiroaki Suga[3]

[1] PeptiDream Inc.
[2] Osaka Univeristy
[3] The University of Tokyo
[4] Tokyo Medical and Dental University

Bone homeostasis involves a variety of signals which balance bone formation and resorption. The interaction of Semaphorin4D with the transmembrane protein Plexin-B1 on osteoblast cells results in the inhibition of bone formation and, therefore, mice lacking expression of either protein have increased bone density making this interaction a possible target for reversing the effects of bone loss in diseases such as osteoporosis and multiple myeloma. A selection campaign using the RaPID system to select for macrocyclic peptide inhibitors of PlexinB1 resulted in the discovery PB1m6 which showed potent inhibitory activity against the Semaphorin4D and PlexinB1 interaction. While the PlexinB1 N-terminal domain shows 86% sequence identity between the human and mouse homologs, PB1m6 was only able to bind and inhibit the human homolog that it was selected for which hindered validation of this peptide in downstream mouse models. Affinity maturation of PB1m6 against the mouse homolog resulted in a peptide, mP6-9, which showed improved binding to both mouse and human PlexinB1. Subsequent analysis of this peptide showed inhibition of mouse Semaphorin4D-PlexinB1 signaling as well as increased bone density in an osteoporosis mouse model and these results will be presented.

O-23

Minichlorotoxins, a New Family of Blood-Brain Barrier Peptide Shuttles

Meritxell Teixido

IRB Barcelona

Brain delivery is one of the major challenges in drug development because of the high number of patients suffering from central nervous diseases (CNS) and the low efficiency of treatments available. Although the blood–brain barrier (BBB) prevents most drugs from reaching their targets, BBB-shuttle peptides offer great promise to safely overcome this formidable obstacle. Peptides which are experiencing a golden era are receiving growing attention because of their lower cost, reduced immunogenicity, and higher chemical versatility than traditional Trojan horse antibodies to be used as BBB-shuttles, as we have recently reviewed.[1-3]

Over the last years, we have reported the use of BBB-shuttles inspired in peptides found in venoms. We have minimized apamin, a neurotoxin from bee venom, by reducing its complexity, toxicity and immunogenicity, while preserving brain targeting, active transport, and protease-resistance leading to MiniAp-4. [4]

In this communication, a recently discovered new family of peptides able to cross the BBB, MiniChlorotoxins (MiniCTXs) will be presented. They are derived from Chlorotoxin (CTX), a disulphide-rich stable peptide derived from the venom of the Israeli scorpion *Leirus quinquestriatus*, which is able to enter the brain and bind specifically to tumor tissue. MiniCTXs are the result of the research performed to decipher the minimal part of CTX that maintains transport capacity and but abolishing its toxicity. Presenting unpublished results on their potential application in the field of delivery of macromolecules and/or nanoparticles as promising future therapies for CNS disorders that require crossing the BBB.

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O-24

Enzyme-substrate complex capture by cyclic peptides for the HIF prolyl hydroxylases

Akane Kawamura

University of Oxford

Prolyl hydroxylases (PHDs) catalyses the hydroxylation of Hypoxia Inducible Factor (HIF1) transcription factor in an oxygen dependent manner, which signals for degradation via the proteasomes. PHDs are thus key regulators in the cellular hypoxic response and are current therapeutic target (e.g. treatment for anaemia in chronic kidney disease, ischemic diseases). While HIF1 is a well-established substrate for PHDs, PHDs have been reported to associate with, and/or, regulate other proteins. Validation of these interactions have been challenged by reproducibility issues with antibodies. Motivated by the recent successes in the development of highly potent and selective binding inhibitors against enzymes / proteins using RaPID-based macrocyclic peptide selection, we explored whether high affinity probes for investigating protein-protein interactions of the PHDs could be generated using this platform.

Biophysical analyses of hit sequences using biolayer interferometry revealed that these 14-mer cyclic peptides selected against PHD2 were highly potent binders (K_D < nM) with long dissociative half-lives ($t_{1/2}$ > 1hr), and comparable to that of antibodies. Using ^{1}H-^{15}N-HSQC NMR and crystallography, the binding site was mapped to a previously unobserved region of PHD2, in the opposite face to the active site. The HIF1 substrate binding and PHD2 enzyme activity were both unperturbed on binding of the cyclic peptides, demonstrating the peptides to be non-competitive binders. The hit peptide was further developed as an affinity probe by addition of a biotinylated linker sequence. The probe was able to capture PHDs, as well as its substrate HIF1, at endogenous levels from cell lysates. PHD2 enzyme activity was also preserved during capture, demonstrating its future development potential as a cellular probe for investigating protein-protein interactions.

O-25

Nonproteinogenic optimization of linear and cyclic peptides

Joseph Rogers[1,2], Toby Passioura[1], Hiroaki Suga[1]

[1]Department of Chemistry, Graduate School of Science, The University of Tokyo, Tokyo, Japan
[2]Current Affiliation: Vertex Pharmaceuticals

The 20 proteinogenic amino acids have physicochemical properties that allow peptides and proteins to fold and bind. However, there are numerous unnatural, nonproteinogenic amino acids that may be equally good, or even better, at folding and binding. Exploration of these alternative peptide building blocks has been limited by slow, one-at-a-time synthesis and testing. We describe how, in a single experiment, multiple nonproteinogenic amino acids can be trialed at all positions in a peptide sequence, with thousands of modifications tested in parallel. This permits detailed analysis of how chemical structure relates to function and allows for systematic comparisons of proteinogenic and nonproteinogenic chemistry. Such analysis can guide the improvement of drug-candidate peptides, including the therapeutically promising class of cyclic peptides.

O-26

Logical engineering of tRNA for incorporation of D- and β-amino acids into macrocyclic peptides

Takayuki Katoh[1], Sayaka Imanishi[1], Yizhen Yin[1], Mituhiro Yamada[1], Yoshihiko Iwane[1], Hiroaki Suga[1]

[1]Univ. of Tokyo, Japan

Genetic code manipulation methodologies such as genetic code reprogramming enable incorporation of various nonproteinogenic amino acids into peptides. However, incorporation of D- and β-amino acids are extremely inefficient, and therefore consecutive incorporation of these amino acids had not been accomplished previously. In order to solve this issue, here we logically engineered D-arm and T-stem of tRNAs for enhancing binding affinity to EF-P and EF-Tu, respectively. The combination of EF-P with the new tRNA, refered to as $tRNA^{Pro1E2}$, pre-charged with various D- and β-amino acids enabled incorporation of up to ten consecutive D- or β-amino acids into model peptides. Furthermore, synthesis of macrocyclic peptides containing consecutive D- or β-amino acids closed by a thioether bond was also demonstrated.

Then, we applied this translation system for synthesizing macrocyclic peptide libraries containing 5 or 7 different kinds of D-amino acids, and carried out in vitro selection against human EGFR. Macrocyclic peptides obtained by the selection have up to 6 D-amino acids including consecutive ones, and exhibited not only binding ability with nM-range K_D values but also extremely high peptidase resistance.

O-27

Construction of macrocyclic peptide libraries by integrating with boronated amino acids and selenocysteine.

Yizhen Yin[1], Nobuaki Ochi[2], Nagio Takigawa[2], Hiroaki Suga[1,*]

[1]Department of Chemistry, School of Science, the University of Tokyo, Tokyo 113-0033, Japan.
[2]Department of General Internal Medicine 4, Kawasaki Medical School, 2-6-1 Nakasange, Kita-ku, Okayama, 700-8505, Japan.

Boron has been widely employed in life sciences for discovering particular peptide ligands with novel biological activities. Boronic acids integrating peptide or antibody libraries have potentially paved a new way for screening the candidates having boronic acids against ribonucleotide acid (RNA), sugars, glycoproteins, etc. However, there is no report regarding the development of a library of peptides incorporating carborane, which is of particular interest in medicinal chemistry. On the one hand, carborane affords the molecules with improved hydrophobicity, stability to metabolic degradation, and non-classical interactions with target proteins. On the other hand, carborane can also be applied in boron neutron capture therapy (BNCT), which is a binary and promising therapeutic technique for selectively eliminating cancers through the highly energetic $4He^{2+}$ and $7Li^{3+}$ particles arising from the thermal neutron capture by boron-10 (^{10}B). We here report the ribosomal expression of highly diverse thioether-macrocyclic peptide libraries bearing L-boronophenylalanine (L-BPA) and L-carboranylalanine (L-CBA) under the genetic code reprogramming, which provided us with a rapid and versatile way to identify the bioactive boronated peptide ligands against a cancer biomarker, human epidermal growth factor receptor (hEGFR).

O-28

Macrocyclic peptides targeting HGF and MET

Kunio Matsumoto

Cancer Research Institute, Nano-Life Science Institute, Kanazawa University
Kakuma, Kanazawa 920-1192, Japan

Hepatocyte growth factor (HGF) is bioactive protein composed of 697 amino acids. HGF binds to and activates the MET transmembrane receptor. HGF-induced activation of the MET receptor drives mitogenesis (cell proliferation), motogenesis (cell migration), morphogenesis (3-D tubulogenesis), and cell survival, thereby participates in regeneration/reconstruction of damaged tissues, including the liver and nervous tissue. Clinical trials using recombinant HGF protein are ongoing for treatment of spinal cord injury and amyotrophic lateral sclerosis. In cancer tissues, aberrant activation of MET receptor participates in malignant progression of cancer such as drug resistance and invasion-metastasis.

Using RaPID (Random Peptide Integrated Discovery) system, an innovative drug discovery platform, we obtained macrocyclic peptides that specifically bind to HGF or MET with 1 - 10 nM K_d values. The cross-linking of MET-binding macrocyclic peptides, thereby capable of bivalent display of MET-binding peptides, conferred them an ability to selectively activate MET [1-3]. These artificial MET-ligands induced dimerization and activation of MET receptor, and exhibited mitogenic, motogenic, and morphogenic activities that are comparable to native ligand HGF. Our approach for generating bivalent macrocyclic peptides as non-protein ligands for cell surface receptors may be useful for development of non-native ligands with a broad range of applications.

HGF is secreted extracellularly as biologically inactive single-chain HGF (scHGF). Protease-medicated processing into two-chain HGF (tcHGF) is critical step for activation of HGF. In normal tissues, HGF exists as inactive scHGF, while tcHGF is generated in restricted tissues such as cancer tissues. The selective detection and inhibition of tcHGF are important for cancer diagnosis and therapeutics. By RaPID system, HGF-inhibitory macrocyclic peptide-8 (HiP-8) was obtained. HiP-8 binds to HGF and inhibits HGF-MET association. HiP-8 exhibits superior selectivity and chemical properties potentially applicable for diagnosis and therapeutics.

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O-29

A Roadmap for Designing and Filtering Large, Encoded Libraries of Cyclic Peptides for Cell Permeability.

Scott Lokey

University of California, Santa Cruz

Cyclic peptides have undergone a renaissance in medicinal chemistry, as studies into structure-property relationships have revealed that passive cell permeability can be designed into synthetic cyclic peptide scaffolds when conformational factors are considered. This renaissance has been fueled, in part, by the development of DNA-encoded and mRNA display library technologies, in which vast numbers of cyclic peptides can be synthesized and screened against any target of interest. While these technologies have yielded highly potent leads against a variety of challenging drug targets, their ability to deliver compounds with activity in cells has been impeded by insufficient membrane permeability among their members. My group has been interested in the physico-chemical properties that underlie passive membrane permeability in cyclic peptides, and we have determined a set of rules that may help to bias large, encoded libraries toward cell permeability. We have developed methods for identifying scaffolds with high membrane permeability, and for predicting side chain combinations that preserve the permeability of the core scaffold. These methods provide a roadmap for directing the synthesis and screening of cyclic peptide libraries toward intracellular targets, with the prospect of identifying leads that are either highly permeable or that can be readily optimized into permeable compounds without compromising biochemical potency.

O-30

Targeting the oligomerization interfaces of BCR-ABL1 kinase with macrocyclic peptides: An alternative strategy to combat chronic myeloid leukemia

Yen-Hua Huang,[†] Toby Passioura,[‡] Hayden Peacock,[†,‡] Sónia Henriques, [†] Conan K. Wang,[†] Quentin Kaas,[†] Hiroaki Suga,[‡] and David J. Craik[*,†]

[†] Institute for Molecular Bioscience, The University of Queensland, Brisbane Queensland 4072, Australia
[‡] Department of Chemistry, Graduate School of Science, The University of Tokyo, 7-3-1 Bunkyo-ku, Tokyo 113-0033, Japan

The oncogenic tyrosine kinase BCR-ABL1 encoded by Philadelphia chromosome (Ph) is the causative agent of around 95% of chronic myeloid leukemia (CML) and 25% of adult acute lymphoblastic leukemia (Ph^+ ALL). Current treatments for CML patients rely on tyrosine kinase inhibitors targeting ATP-binding site. Nevertheless, 20−30% of patients manifest drug resistance associated with point mutations within the drug-binding site of BCR-ABL1. To overcome the drug resistance, discovery of inhibitors targeting the coiled-coil region involved in the activation of BCR-ABL1 kinase could be an alternative approach to generate drug leads with a durable therapeutic response and to prevent or delay the emergence of resistance. In the current study, the coiled-coil domain BCR^{1-72} was synthesised chemically and screened against a thioether-macrocyclic peptide library generated using a cutting-edge technique named RaPID (Random nonstandard Peptides Integrated Discovery). Out of 14 lead molecules discovered by the RaPID system, six peptides showed significant binding affinities to the BCR^{1-72} in surface plasmon resonance and three of them further displayed growth inhibitory effects on K562 cells in the low micromolar concentration range but neglectable toxicity against breast cancer cell line (MCF-7) and human *peripheral blood mononuclear cells*. Taken together, the macrocyclic peptides discovered using the RaPID system exhibited inhibitory activity towards the CML blast crisis cell line but not to the non-malignant human cells, indicating that they are promising candidates for therapeutic development.

O-31

Round it up: Cyclic peptide-conjugates for intracellular protein delivery

Christian Hackenberger

Humboldt Universität zu Berlin and Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP)
Robert-Rössle-Str. 10, 13125 Berlin, Germany

www.fmp-berlin.info/hackenbe

Our lab aims to identify new bioorthogonal reactions for the synthesis and modification of functional peptides and proteins. We apply these highly selective organic reactions to study functional consequences of naturally occurring posttranslational protein modifications (PTMs) as well as to generate novel peptide- and protein-conjugates for pharmaceutical and medicinal applications.
In this presentation I will focus on the chemical modification of functional proteins as well as their cellular delivery. Here, we employ cyclic cell penetrating peptides (cCPPs) to transport a functional full length protein to the cytosol of living cells as recently demonstrated by the direct delivery of GFP-conjugates.[1] For protein modification we use a combined approach of intein expression as well as recently developed bioorthogonal reactions and enzymatic ligations, for instance the so-called Tub-tag labeling.[2] This concept is finally applied to generate cell-permeable nanobodies, i.e. small antigen binding proteins that remain active within the reductive milieu inside living cells, to interfere with intracellular targets.[3]

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O-32

The past, present and future of plant-derived cyclic peptides

David J Craik

Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD 4072, Australia

Our work focuses on the discovery of cyclic peptides in plants and their applications in drug design and agriculture. We have a particular interest in a family of proteins called cyclotides, which comprise ~30 amino acids and incorporate three disulfide bonds arranged in a cystine knot topology, which makes them exceptionally stable.[1] Cyclotides occur in all plants from the Violaceae family and in certain plants from the Rubiaceae, Cucurbitaceae, Solanaceae and Fabaceae, where they are present as host defence agents against insects and nematodes.[2] A single plant may contain dozens to hundreds of cyclotides expressed in a wide range of tissues, including leaf, flower, stem and roots. Their stability and compact structure makes cyclotides an attractive protein framework onto which bioactive peptide epitopes can be grafted to stabilise them.[3] Because plants produce cyclotides in large quantities (up to 2g/kg plant weight) we are using crop plants as expression systems for the production of pharmaceutically active cyclotides. This presentation will give an overview on the discovery, biosynthesis and applications of cyclotides and also describe the use of transgenic plants as vehicles for the production of cyclotide-based drug leads for cancer, cardiovascular disease and pain.[4]

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POSTERS Continued

P-12

Re-engineering disulfide bonds: a report on the synthesis, structure, and activity of a selenocyclotide

Peta J Harvey, Olivier Cheneval, Sónia Troeira Henriques, David J Craik

The Institute of Molecular Bioscience, University of Queensland, QLD, Australia 4072

In addition to their circular backbone, cyclotides contain a network of cross-linking disulfide bonds arranged in a cysteine knot topology that imparts remarkable stability. This knotted arrangement, in which a disulfide bond penetrates an embedded ring, formed by two additional disulfide bonds and their connecting backbone segments, gives rise to a scaffold that can be used to graft active peptides or to engineer peptide-based therapies.

Recent work from our laboratory has focused on the significance of membrane interactions in the mechanism of how cyclotides exert their diverse biological effects. Since the cyclic cysteine knot (CCK) topology is critical for stability, it was thought that the conservative replacement of a disulfide bond with a diselenide bond may shed some light on the role of these cross linkages in membrane binding.

In this work, we discuss the synthesis, NMR analysis and structural characterization of a selenocysteine analogue of kalataB1, the prototypic cyclotide. In 5,19U-kB1, a single disulfide bond between residues 5 and 19 was replaced with a diselenide bond. An evaluation of its three-dimensional structure along with bio-activity data and membrane binding ability will be presented. On the basis of this experimental data, we suggest that further investigations of disulfide mimetics in the context of the cyclotide scaffold may enhance its potential for drug delivery and development.

P-13

Comparison of circulating INSL3 and INSL3-C-peptide levels in the male and female serum

Jaw-Kang Chang, Xing-Hai Yao, Tian Qing, Rong-Ming Lyu

Phoenix Pharmaceuticals, Inc.
Burlingame, CA 94010, United States

Comparison of circulating INSL3 and INSL3-C-peptide levels in the male and female serum
Introduction: Insulin-C-peptide is produced from insulin precursor as a by-product of insulin, and is co-secreted into the blood stream. While insulin is quickly metabolized in the liver (t1/2=6 mins), C-peptide is metabolized in the kidney, which results in C-peptide having a longer half-life in circulation (t1/2=30 mins). Because of this, Insulin-C-peptide in serum is considered to be a better maker for the activity of functional pancreatic beta cells. Because INSL3 is an important hormone corresponding to the reproductive life span1, the aim of this study is to compare the circulating levels of INSL3 and INSL3-C-peptide in human serum.

Methods & Results: By immunizing rabbits with target INSL3 and INSL3-C-peptides, we produced antibodies that were specific against INSL3 and its C-peptide. Immunohistochemistry in the placenta or prostate showed a similar staining pattern when the tissues were applied with these two antibodies.. The circulating levels of INSL3 and INSL3-C-peptide were also measured in different groups of individuals by using Radioimmunoassay (RIA) and Fluorescent Enzyme-Immunoassay (FEIA). The preliminary results are shown below:

Serum Targets	INSL3 Levels (pg/ml)		INSL3-C-peptide Level (pg/ml)	
	RIA measurement	FEIA measurement	RIA measurement	FEIA measurement
Male (N=5)	444± 103	620± 145	460± 32	564± 192
Female (N=5)	86± 12	37 ± 5	124± 9	270± 58

Conclusion & Discussion: This study showed that serum levels of INSL3 and INSL3-C-peptide are similar in males. However, tINSL3-C-peptide levels were higher thanINSL3 in serum from women. The results indicate that INSL3-C-peptide might be more resistant to protease degradation or metabolism in the organs, in comparison to INSL3.. The serum levels of INSL3 and INSL3-C-peptide showed gender-dependent regulation, and is similar to the circulating serum of adiponectin, leptin, and vaspin. Currently, similar studies have been conducted to compare the serum levels of INSL4 and INSL4 C-peptide.

P-14

Lariat Based Cyclic Peptide Design for Targeting GPCRs

Kristine K. Deibler[1,2], David Baker[1,2]

[1]University of Washington
[2]Institute of Protein Design

G protein-coupled receptors (GPCRs) are the largest family of cell surface receptors and are important human drug targets. Of the 826 human GPCRs, 118 of them recognize endogenous peptide or protein ligands. Many of the GPCRs that remain unexplored as therapeutic targets are peptide- or protein-binding receptors. In these cases, identification of binders has been more challenging due to the difficulties involved in developing small-molecule binders based on the endogenous ligand, which is a major obstacle for discovery of novel GPCR drugs. Here in we discuss progress towards targeting select GPCRs via de novo peptide design and optimization.

P-15

Trends in Cyclotide Research

Meng-Wei Kan and David J. Craik

Institute for Molecular Bioscience, The University of Queensland, Brisbane QLD 4072, Australia

Cyclotides are bioactive peptides from plants that have exceptional stability owing to their cyclic peptide backbone and knotted arrangement of three conserved disulfide bonds (**Figure 1**). This peptide family was first defined in 1999, although a few individual members had been identified in independent studies in earlier decades in the course of pharmaceutical screening or ethnobotanical studies. The structures, bioactivities and applications of cyclotides have been extensively reviewed. This presentation provides an analysis of trends in cyclotide literature over the last three decades and aims to be a useful resource for following the development of the cyclotide field and for introducing new researchers to the complete literature of the field.[1]

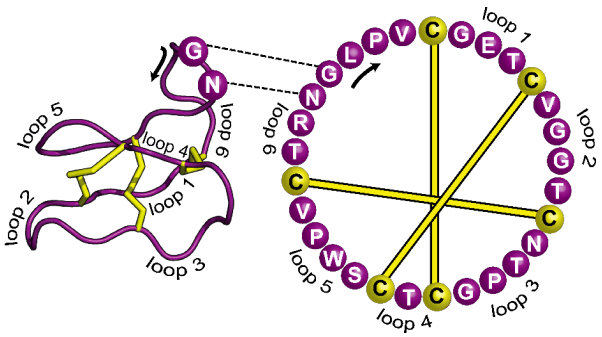

Figure 1: Sequence and structure of the prototypical cyclotide kalata B1 (PDBID: 1NB1), showing the labelling of backbone loops and the cystine knot motif.

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P-16

FIT-based screening allows to uncover a new method for peptide constraining

K. Decoene[1], T. Passioura[2], W. Vannecke[1], Hiroaki Suga[2] and A. Madder[1]

[1] Organic and Biomimetic Chemistry Research group, Department of Organic and Macromolecular Chemistry, Ghent University, Ghent
[2] Department of Chemistry, Graduate School of Science, The University of Tokyo,7-3-1 Bunkyo-ku, Tokyo 113 0033, Japan

In this work, nonstandard peptides bearing a furan moiety were expressed via the flexible in vitro translation (FIT) system1 in a screening experiment. The objective was to identify nucleophilic residues which can react with an oxidized furan moiety2. The peptide template used in this work contains a furylalanine and another canonical nucleophilic residue. These peptides were treated with N-bromosuccinimide (NBS) to oxidize the furan moiety resulting in a reactive keto-enal intermediate prone to react with nucleophiles. Several peptides selected following the screening were synthesized on SPPS and analyzed further. Our data demonstrates that the amine side chain from lysine reacts with the oxidized furan and forms an imine. After a reduction done in a one-pot fashion the resulting amine can react further to form a pyrrole via aromatization and loss of water. We here provide structural evidence (NMR) for the formation of the pyrrole unit as cyclisation motif and explore the generality of this new peptide constraining methodology.

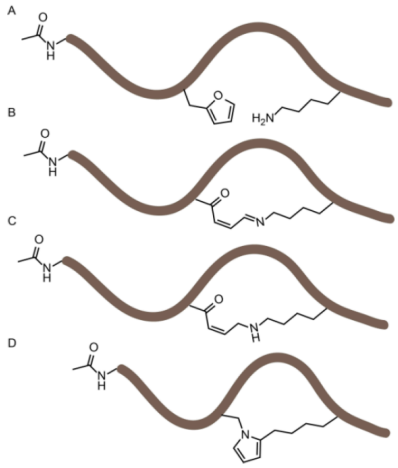

Figure 1. Schematic of the peptide constraining strategy proposed in this work. A. Peptide template bearing a furan moiety and lysine residue. B. Resulting imine after selective furan oxidation. C. The amine formed after imine reduction. D. final constrained peptide with a pyrrole unit.

P-17

In vitro cell-free platform for production of peptides with intramolecular bonds and unnatural amino acids

Yue Wu[1], Zhenling Cui[1], Sergey Mureev[1], Kirill Alexandrov[2]

[1]Institute for Molecular Bioscience
[2]Institute for Molecular Bioscience and Australian Institute for Bioengineering and Nanotechnology

The field of peptide science is steadily growing alongside with the peptide drug's market reaching $25 billion this year owing its tremendous progression to the potential and significance of peptides as candidates for the development of novel therapeutics. Cyclic peptides combine few important qualities for making a perfect drug, such as small size, cell permeability, oral bioavailability and large surface area. Despite the growing number of commercial partnerships for each strategy, the most commonly used solid phase synthesis, constraining and screening of small libraries of native toxin variants is costly and time consuming. To this end, we set out to develop a robust and efficient cell-free translation platform for high-throughput and cost-effective cyclic peptide synthesis and purification. The platform builds on cell-free translation system supplemented with affinity-clamp (AC) coated beads, which are responsible for co-translational capturing immobilization and display of any translated peptide carrying the respective C-terminal tag. Such platform enables protection from proteolysis during translation reaction, deep purification and controlling the chemical environment for systematic cyclization and/or peptide modification. As a proof of principle, several cyclic peptides were successfully expressed, purified, oxidized, eluted and backbone-cyclized. Besides the listed advantages, the platform is compatible with non-natural amino acid incorporation and can be tailored to the requirements of random peptide library display.

P-18

Are Cyclotides Libido Enhancers? Discovery and Characterization of Novel Cyclotides from *Hybanthus enneaspermus*

Qingdan Du, Lai Yue Chan, Yen-Hua Huang, Quentin Kaas, Edward Gilding, David J. Craik

Institute for Molecular Bioscience, The University of Queensland, Brisbane, Qld 4072, Australia

Cyclotides are a large family of plant-derived peptides, which are characterized by a head-to-tail cyclic backbone, a cystine knot motif with three disulfide bonds and typically ~30 amino acid residues. Due to their unique structure, cyclotides are exceptionally stable to proteolytic degradation and are tolerant to amino acid residue substitutions. Their stability and cell penetrating properties make them ideal scaffolds for grafting a wide range of bioactive epitopes. In this study, ten novel cyclotides (Hyens1-5 and 7-11) and one known cyclotide (cycloviolacin O2; Hyen6) were isolated from *Hybanthus enneaspermus*, a herb from the Violaceae family that has been commonly used by the Indian community as a natural libido enhancer. The sequences of these novel cyclotides were characterized using tandem mass spectroscopy and were further confirmed by NMR spectroscopy. Results showed that these newly discovered cyclotides have similar secondary structures to cycloviolacin O2 and they exhibited potent cytotoxic activity against cancer cells, including PC3 (prostate cancer cells) and MCF7 (breast cancer cells). To determine whether these cyclotides contribute to the libido enhancer activity, they will be evaluated in a rat erectile dysfunction model in future. Overall, this study will broaden the horizon of the bioactivity of cyclotides, expand the knowledge on cyclotides sequence diversity and can offer more scaffold options for grafting studies.

P-19

The insecticidal effect of Sero-X on Helicoverpa larvae

Majbrit Frøsig-Jørgensen, David J. Craik

Institute for Molecular Bioscience, University of Queensland

Crop pests represent a major problem for the farming industry and until recently the problem has been controlled with chemical pesticides which can be highly toxic and not target specific. Cyclotides, which are peptides found in plants, have the advantage of being target specific and eco-friendly. Sero-X is the first bio-insecticide discovered and developed in Australia in a collaboration between Innovate Ag and Professor David Craik (UQ). It is based on cyclotide extracts found in Clitoria ternatea (butterfly pea) that protect cotton from amongst others Helicoverpa larvae.

My work will focus on setting up a feeding assay to determine the insecticidal properties of Sero-X towards Helicoverpa larvae. I wish to establish the LC50 value and quantitatively determine the rate of resistance. Lastly, I would like to investigate if there are any genetic changes that could explain this.

This work will help to optimize the use of Sero-X in the farming industry.

P-20

Chemical and ribosomal synthesis of topologically defined multicyclic peptides primed by selenoether formation

Yizhen Yin[1], Qianran Fei[2], Weidong Liu[2], Hiroaki Suga[1,*], and Chuanliu Wu[2,*]

[1]Department of Chemistry, Graduate School of Science, The University of Tokyo, Tokyo, 113-0033, Japan.
[2]Department of Chemistry, College of Chemistry and Chemical Engineering, The MOE Key Laboratory of Spectrochemical Analysis and Instrumentation, Xiamen University, Xiamen, 361005, P.R. China.

Bicyclic and tricyclic peptides are emerging as promising candidates for the development of protein binders with high affinities and new therapeutics of high potency. However, convenient and efficient strategies that can generate topologically controlled bicyclic and tricyclic peptide scaffolds from fully-unprotected peptides are still much in demand, particularly for those amenable to the design of biosynthetic libraries. In this work, we report a reliable strategy for the chemical and ribosomal synthesis of topologically controlled bicyclic and tricyclic peptides. Our strategy relies on the prior selenoether formation between selenocysteine (Sec) and halogenated residues in peptides followed by disulfide or thioether cyclization under mild conditions, giving a set of desirable bicyclic and tricyclic peptides with controlled topology. This work thus lays foundation for developing topologically controlled multicyclic peptides in future for screening bioactive molecules.

P-21

Synthesis, conformational analysis and antibacterial activity of amphiphilic diphenylacetylene-based peptidomimetics

Hayden Peacock[a,b], Sónia Troeira Henriques[b], Aurélie H. Benfield[b], James Luccarelli[c], Alysha G.Elliott[b], Jinghui Luo[d], David Craik[b], Andrew D. Hamilton[e]

a) Present address: Department of Chemistry, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
b) Institute for Molecular Bioscience, The University of Queensland, St Lucia QLD 4072, Australia
c) Dana-Farber Cancer Institute, Longwood Center 3217, Boston MA 02215, USA
d) Present address: Paul Scherrer Institut, Forschungsstrasse 111, 5232 Villigen, Switzerland
e) Department of Chemistry, New York University, New York, New York 10003, USA

Compounds that disrupt the bacterial cell membrane are attractive next-generation antibiotics. Here we report the design, synthesis, conformational analysis and bioactivity of a new class of membrane-active compounds based on a diphenylacetylene scaffold. In these compounds, a central diphenylacetylene moiety is surrounded by eight amino acids in a C2-symmetrical, facially-amphiphilic arrangement. Within a small set of analogues, we observed a positive correlation between amphiphilicity, degree of structuring, efficiency to partition into negatively-charged membranes and antibacterial activity. The most amphiphilic compound has a membrane-disruptive mode of action, adopts an unusual cation-π-stabilised conformation, and displays bioactivity that is selective for bacterial species. These results may inform the development of highly selective membrane-disruptive compounds for therapeutic applications.

P-22

One-pot Regioselective Disulfide/thioether Formation of Cell-free Translated Peptides

Yichao Huang and Hiroaki Suga

Department of Chemistry, Graduate School of Science, The University of Tokyo

Email: hycjimmy@chem.s.u-tokyo.ac.jp

Small cysteine-rich peptides have gained increasing attention as a class of constrained peptides of therapeutical interest[1]. However, few examples have been reported that use molecular display technologies to create cysteine-rich peptide libraries for binding-based selections. In the past decade, mRNA-display-based RaPID (Random non-standard Peptide Integrated Discovery) system[2] which consists of a subsystem called FIT (Flexible *In vitro* Translation)[3], has proved powerful to discover desirable nM-range-affinity binders from *in vitro* translated peptide libraries. In this preliminary study, we reprogrammed protected cysteines[4] into the FIT sytem to control the topology of cell-free expressed bicyclic stapled peptides. These cysteine-rich bicyclic peptides can be useted as new scaffolds for the library design in the RaPID system.

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P-23

Ribosomal incorporation of *N*-substituted amino acids into peptides

Naoya Kawakami, Toby Passioura and Hiroaki Suga.

Department of Chemistry, Graduate School of Science, the University of Tokyo

To date, many peptidomimetics have been developed, including *N*-methylated peptides and *N*-substituted glycines (so-called “peptoids”). Many studies have demonstrated that *N*-methylation or *N*-substitution (of glycine) confers more attractive properties as therapeutic agents, including higher cell membrane permeability and greater peptidase resistance. However, there are few reports of peptides containing non-methyl *N*-substituted amino acids bearing an ✔-side chain. In this study, we tested the incorporation of *N*-substituted Ala, Ser, ✔-aminobutyric acid, Leu and Phe into model peptides through *in vitro* ribosomal translation. The incorporation of 18 *N*-substituted amino acids (out of 22 tested) was observed by MALDI-TOF-MS. Based on the incorporation compatibility of these amino acids, we conclude that side chains rather than *N*-substituents have the greater influence on ribosomal incorporation.

P-24

RaPID Selection of Macrocyclic Peptide Inhibitors of Membrane-Type 1 Matrix Metalloproteinase

Otero Ramirez, M. E.[a]; Passioura, T.[a]; Hoshino, D.[b]; Koshikawa, N.[b] and Suga, H[a].

[a]Department of Chemistry, Graduate School of Science, The University of Tokyo, Japan
[b]Division of Cancer Cell Research, Kanagawa Cancer Center Research Institute, Japan

Most cellular processes required for cell survival and development depend, in different ways, on interactions with the extracellular matrix (ECM). This is composed of a varied array of macromolecules that are carefully regulated to provide specific biochemical and physical properties that can control cellular behavior[1]. Among these, Matrix Metalloproteinases (MMPs) carry out proteolysis for the activation or suppression of a diverse of substrates that can, in turn, manage a complex series of signal transduction processes. Within this large and structurally similar family, Membrane-Type 1 MMP (MT1-MMP) is remarkably known for its high expression in neoplastic cells, and has been found to be deeply involved in their expansion and invasion through its interaction with a large variety of substrates, making it an attractive target for therapeutic development[2-3]. Nonetheless, currently available inhibitors exhibit low specificity or adverse side effects *in vivo*, making its controlled inhibition a challenging task.

Through the use of the RaPID (Random non-standard Peptide Integrated Discovery) system[4], we have been able to develop and identify thioether-cyclized macrocyclic peptide binders and potential inhibitors of MT1-MMP (sequences not disclosed), which show remarkably strong binding kinetics with some binding constants (K_D) in the single-digit nanomolar or subnanomolar range. Additionally, several of the identified peptides were able to strongly inhibit neoplastic cell migration and invasion processes, even exceeding the potency of a well-characterized small molecule inhibitor known as MMI-270 (Figure 1). Surprisingly, this activity could not be completely correlated with direct proteolytic inhibition *in vitro*, suggesting MT1-MMP may play a role in proteolysis-independent mechanisms to inhibit migration, serve as a potential basis in the development of cancer therapeutic drugs with a novel mechanism of action.

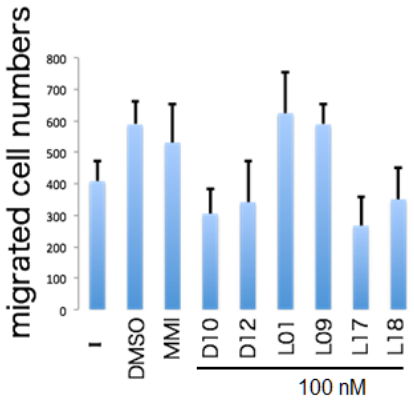

Figure 1. Inhibition of cellular migration by peptides selected against MT1-MMP.

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P-25

Incorporation of D-amino acids in the translation system using EF-Tu variants

Hiroyuki Kimura, Takayuki Katoh and Hiroaki Suga

Department of Chemistry, Graduate School of Science, the University of Tokyo

The translation system has a great specificity of substrate amino acids in order to maintain the quality of peptides and proteins. The elongation factor thermal unstable, EF-Tu, is one of the most important factor, which recognizes amino acyl-tRNA structure and contribute the quality maintenance of peptides. To incorporate non-canonical amino acids into peptides in the translation system, the specificity of EF-Tu has been modulated by mutating its amino acid binding pocket. Previously, some EF-Tu variants have been reported and several non-canonical amino acids were successfully incorporated into peptides by using these variants [Ref1, 2]. However, the tolerance of EF-Tu towards D-amino acids are not well studied.
In this work, the D-amino acids tolerance of EF-Tu variants were studied by gel mobility shift assay. This assay revealed that one of EF-Tu variant, Glu215Ala, which was originally generated for the purpose of incorporating bulky pyrenyl-alanine, showed enhanced tolerance towards D-prolyl-tRNA or other D-amino acyl-tRNA. Finally, D-proline was incorporated into a peptide with increased purity by using this EF-Tu variant, compared with the wild type EF-Tu. This research will lead to the understanding of structure activity relationships between EF-Tu and amino acyl-tRNA, and to the development of enhanced translation system which is able to incorporate non-canonical amino acids efficiently.
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P-26

Discovery of Macrocyclic Peptides Inhibiting β-galactosidase Toward Development of a Peptide Drug Internalized to Lysosome

Kyohei Miyairi, Yuki Goto, Hiroaki Suga

Department of Chemistry, Graduate School of Science, the University of Tokyo

Macrocyclic peptides, which can potentially exhibit strong target binding and proteolytic stability, have been expected as promising drug candidates. Such peptides however remain a problem in their membrane permeability, being a huge obstacle for targeting intracellular proteins. Thus, a certain means for internalizing them into cells is required for practical peptide therapy.

Here, we propose a rational strategy to send macrocyclic peptide inhibitors into cells by conjugation with another macrocyclic peptide ligand targeting a membrane receptor. Our laboratory has developed a platform technology named RaPID system to discover macrocyclic peptide ligands against proteins of interest including enzymes and receptors. In this strategy, a RaPID-derived macrocyclic peptide inhibiting a lysosomal enzyme is linked with another RaPID-derived macrocyclic peptide binding to a membrane receptor. Such heterodimeric peptides would interact with the membrane receptor and induce receptor-mediated endocytosis, and thereby, could be localized at intracellular vesicles such as lysosome where the peptide would inhibit the target enzyme.

To demonstrate this strategy, beta-galactosidase, a glycoside hydrolase localized in lysosome, was chosen as a model intracellular target to be inhibited by such heterodimers. This work focuses on discovery of macrocyclic peptides that inhibit beta-galactosidase by means of the RaPID system. Furthermore, heterodimers consisting of the beta-galactosidase inhibitors and receptor-binding peptides previously discovered were synthesized. We expect that a further cell-based assay utilizing these heterodimers can be a proof-of-concept for a novel intracellular peptide delivery method.

P-27

Display Selection Of Low-Polarity Cyclic Peptide Ligands Expressed Under A Radically Reprogrammed Genetic Code

Toby Passioura, Wenyu Liu, Daniel Dunkelmann, Takashi Higuchi, and Hiroaki Suga.

Department of Chemistry, Graduate School of Science, The University of Tokyo, 7-3-1, Hongo, Tokyo 113-0033.

ABSTRACT: Bioactive naturally occurring macrocyclic peptides often exhibit a strong bias for hydrophobic residues, a feature that appears critical for their biological activities. Recent advances in *in vitro* display technologies have made possible the identification of potent macrocyclic peptide ligands to protein targets of interest. However, such approaches have so far been restricted to using libraries composed of peptides containing mixtures of hydrophobic and hydrophilic/charged amino acids encoded by relatively canonical (*i.e.* comprised primarily of the 20 proteinogenic amino acids) genetic codes. In the present study, we have demonstrated ribosomal expression of highly exotic macrocyclic peptides under a radically reprogrammed, relatively hydrophobic, genetic code, comprising 12 proteinogenic and 11 non-proteinogenic amino acids. Screening of this library for affinity to the interleukin-6 receptor (IL6R) as a case study successfully identified exotic macrocyclic peptide ligands with high affinity, validating the feasibility of this approach for the discovery of relatively hydrophobic macrocyclic peptide ligands with similarity to natural products.

P-28

Ribosomally synthesized foldamer-containing peptides

Christos Tsiamantas,[a] Sunbum Kwon,[b] Joseph M. Rogers,[a] Simon J. Dawson,[b] Pradeep K. Mandal,[b] Hiroaki Suga,[a] Ivan Huc[b]

[a] Department of Chemistry, Graduate School of Science, The University of Tokyo, Tokyo, Japan,
[b] Department of Pharmacy, Ludwig-Maximilians-Universität, München, Germany

hsuga@chem.s.u-tokyo.ac.jp, ivan.huc@cup.lmu.de

In vitro, flexible tRNA aminoacylation ribozymes, known as flexizymes, have enabled charging of non-canonical amino acids onto tRNA molecules.[1] Subsequent addition to a solution containing the essential components of an *in vitro* translation system enables the assembly of mRNA-encoded peptides, equipped with various building blocks. D-amino acids, N-alkyl amino acids, fluorescent/ biotin labeled amino acids and exotic peptides among others have been successfully incorporated.[2] Coupling of the above technology with mRNA display has yielded numerous scaffolds manifesting enormous potential in drug discovery and imaging.[3]
This presentation will focus on recent advances on the incorporation of aromatic oligoamide foldamers into peptides, taking advantage of nature's translational machinery. It was recently shown that oligoamides may successfully initiate the translation of foldamer-peptide hybrid molecules.[4] Recent progress not only includes an expanded scope of substrates for initiation, but also show that foldamers may be included into appendages in peptide elongation. Our findings pave the way to introducing foldamers in mRNA display, with a whole new range of attributes to be discovered.

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P-29

Comprehensive elucidation of effect of nascent peptide sequences and EF-G concentration on incorporation of prolines in translation

Kenya Tajima[1], Takayuki Katoh[1, 2], Hiroaki Suga[1]

[1]Graduate School of Science, The University of Tokyo
[2]JST PRESTO

In the canonical translation, ribosome along with protein factors and tRNAs polymerizes amino acids into a full-length peptide encoded in an mRNA sequence, by repeating peptide bond formation, translocation and accommodation in this order. Proline, the only secondary proteinogenic amino acid, is less compatible to the active site of ribosome, making its peptide bond formation slow. Their slow incorporation into nascent peptides causes ribosome stalling on the mRNA and inhibits polypeptide elongation.

During the elongation event, the interaction between the nascent peptide on the peptidyl-tRNA and the ribosome exit tunnel alters the active site conformation, which controls the rate of peptide bond formation in the sequence-dependent manner. In addition, translocation catalyzed by elongation factor G (EF-G) could precede the slow peptide bond formation of prolines. As a result, the peptidyl-prolyl-tRNA could drop off from a ribosome in the above stalling, which can restart the translation from the middle of the mRNA and synthesize a truncated C-terminal peptide.

Here, we elucidated the effects of nascent peptide sequences and EF-G concentration on the drop-off of peptidyl-prolyl-tRNA. We attempted consecutive incorporation of three prolines into randomized nascent peptide sequences, as Xaa_n-Pro-Pro-Pro (Xaa: any proteinogenic amino acids, n = 1-3, Pro: proline), under different EF-G concentrations. By utilizing mRNA display, nascent peptides successfully incorporating three prolines without the drop-off were conjugated with corresponding mRNAs, reverse-transcribed and selectively recovered as cDNAs. Sequencing results indicated a correlation between their incorporation efficiency and the polarity of nascent peptides. Detailed results will be discussed in the poster presentation.

Delegate list

Name	email	Name	email
Christopher Hipolito	hipolito@md.tsukuba.ac.jp	Masataka Fumoto	masataka.fumoto@shionogi.co.jp
Toby Passioura	toby@chem.s.u-tokyo.ac.jp	Thomas Durek	t.durek@imb.uq.edu.au
Masanobu Nagano	iccpp2018@gmail.com	Suzuki Yuta	yuta.suzuki@mochida.co.jp
Nohara Goto	s1511799@u.tsukuba.ac.jp	Shinya Kodani	kodani.shinya@shizuoka.ac.jp
Hiroaki Suga	hsuga@chem.s.u-tokyo.ac.jp	James Link	ajlink@princeton.edu
Philip Dawson	dawson@scripps.edu	Zhenling Cui	z.cui@uq.edu.au
David Craik	d.craik@imb.uq.edu.au	Mar Forner	mar.forner@upf.edu
Lara Malins	lara.malins@anu.edu.au	Bronwyn Smithies	b.smithies@imb.uq.edu.au
Ratmir Derda	ratmir@ualberta.ca	Haiou Qu	h.qu@imb.uq.edu.au
Max Cryle	max.cryle@monash.edu	Neha Kalmankar	nehavk@ncbs.res.in
Johan Rosengren	j.rosengren@uq.edu.au	Timothy Craven	twcraven@uw.edu
Aline Dantas de Araujo	a.dantasdearaujo@imb.uq.edu.au	Andrew White	a.white7@uq.edu.au
Scott Lokey	slokey@ucsc.edu	Choi Li	choi.li@uq.net.au
Markus Muttenthaler	markus.muttenthaler@univie.ac.at	Junqiao Du	j.du@imb.uq.edu.au
Tina Schroeder	c.schroeder@imb.uq.edu.au	Gregoire Philippe	g.philippe@uq.edu.au
Akane Kawamura	akane.kawamura@chem.ox.ac.uk	Klaas Decoene	klaas.decoene@ugent.be
Kunio Matsumoto	kmatsu@staff.kanazawa-u.ac.jp	Yue Wu	yue.wu@uq.edu.au
Meritxell Teixido	meritxell.teixido@irbbarcelona.org	Ben Tombling	b.tombling@uq.edu.au
Takeshi Zendo	zendo@agr.kyushu-u.ac.jp	Qingdan Du	qingdan.du@imb.uq.edu.au
Christian Hackenberger	Hackenbe@fmp-berlin.de	Fabian Rehm	f.rehm@imb.uq.edu.au
Joseph Rogers	josephrogers1000@gmail.com	Majbrit Frosig-Jorgensen	m.froesig@imb.uq.edu.au
Rumit Maini	rumit.maini@merck.com	Yuki Goto	y-goto@chem.s.u-tokyo.ac.jp
Ali Tavassoli	A.Tavassoli@soton.ac.uk	Takayuki Katoh	katoh@chem.s.u-tokyo.ac.jp
Jon Collins	jon.collins@cem.com	Yizhen Yin	yizhenyin@chem.s.u-tokyo.ac.jp
Conan Wang	conanw@gmail.com	Richard Obexer	robexer@chem.s.u-tokyo.ac.jp
Aleksey Gurevich	alexeigurevich@gmail.com	Hayden Peacock	peacock@chem.s.u-tokyo.ac.jp
Rodney Perez	rodneyperez1984@gmail.com	Jimmy Huang	hycjimmy@chem.s.u-tokyo.ac.jp
Parisa Hosseinzadeh	parisah@uw.edu	Rong Gao	gaorong@chem.s.u-tokyo.ac.jp
Yen-Hua Crystal Huang	y.huang@imb.uq.edu.au	Mitsuhiro Yamada	mkuriyama@chem.s.u-tokyo.ac.jp
Simon de Veer	s.deveer@imb.uq.edu.au	Alex Vinogradov	a_vin@chem.s.u-tokyo.ac.jp
Peta Harvey	peta.harvey@imb.uq.edu.au	Wenyu Liu	wyliu@chem.s.u-tokyo.ac.jp
Jaw-Kang Chang	jawkangchang@yahoo.com	Naoya Kawakami	k.naoya@chem.s.u-tokyo.ac.jp
Kristine Deibler	deiblerk@uw.edu	Manuel Otero-Ramirez	motero@chem.s.u-tokyo.ac.jp
Annie Kan	a.kan@uq.edu.au	Hiroyuki Kimura	hkimura@chem.s.u-tokyo.ac.jp
Ito, Yuji	yito@sci.kagoshima-u.ac.jp	Kyohei Miyairi	miyairi@chem.s.u-tokyo.ac.jp
Cedric Rentier	cedric.rentier@biotage.com	Hirotaka Nishimura	hnishimura@chem.s.u-tokyo.ac.jp
Satoshi Mashiko	satoshi.mashiko@bachem.com	Christos Tsiamantas	christos.tsiamantas@chem.s.u-tokyo.ac.jp
Rie Hirokane	hirokane.rie00@chugai-pharm.co.jp	Wei Lu	wei_lu@chem.s.u-tokyo.ac.jp
Bhavesh Premdjee	bhpr@novonordisk.com	Zhao Jinxuan	zhaojx@chem.s.u-tokyo.ac.jp
Fumio Kumakura	Fumio.Kumakura@biotage.com	Edison Zhang	yczhang@chem.s.u-tokyo.ac.jp
Christian Cunningham	cunningham.christian@gene.com	Yamato Komatsu	ykomatsu@chem.s.u-tokyo.ac.jp
Tomomi Yoshitomi	yoshitomi.tomomi.bx@daiichisankyo.co.jp	Renier van Neer	renier@chem.s.u-tokyo.ac.jp
Kishan Agarwala	k-agarwala@hhc.eisai.co.jp	Thomas Ploemacher	tploemacher@chem.s.u-tokyo.ac.jp
Shunsuke Oishi	oishi@itbm.nagoya-u.ac.jp	Marina Kawai	mkawai@chem.s.u-tokyo.ac.jp
Kenichiro Ito	kenichiro_ito@ajinomoto.com	Koki Shimbara	k-shimba@chem.s.u-tokyo.ac.jp
Hiroki Ozasa	hiroki.ozasa@shionogi.co.jp	Kenya Tajima	ktajima@chem.s.u-tokyo.ac.jp