NANYANG TECHNOLOGICAL UNIVERSITY SINGAPORE

Frontiers in Peptide Science and Drug Discovery

A Scientific Symposium in Honour of Professor James P. Tam

5 August 2022 | NTU School of Biological Sciences, Classroom 1

PROGRAMME & ABSTRACT BOOKLET

Co-organised by NTU School of Biological Sciences and NTU Institute of Advanced Studies

Content

Foreword

It is our great pleasure to welcome all participants of Frontiers in Peptide Science & Drug Discovery: A Scientific Symposium in Honour of Professor James P. Tam (FPSDDS). This symposium takes place on August 5th, 2022 at the School of Biological Sciences (SBS), Nanyang Technological University (NTU), Singapore.

Jointly organized by the School of Biological Sciences and NTU-Institute of Advanced Studies (NTU-IAS), FPSDDS is held to celebrate Professor James P. Tam's outstanding research works and lifetime achievements. Professor Tam has made great contributions to peptide and protein science in a broad range of research areas – such as the invention of peptide dendrimers as synthetic vaccines and protein quaternary structure mimetics, development of chemoselective peptide ligation methods and the discovery of peptide ligases, the discovery of ultra-stable cysteine-rich peptides from medicinal plants and their use as a therapeutic modality for disease treatment, and many more. Many of his contributions have become classic works with profound impacts on chemical biology and drug discovery, which won him various prestigious awards. Professor Tam is also well recognized as an accomplished educator and administrator. Several hundreds of undergraduate and post-graduate students, postdoctoral fellows, and visiting scientists have been trained in his laboratory throughout the years. Professor Tam is the Founding Dean of SBS, the Founding Director of the Biological Research Center and the Founding Director of the Double-Degree Program in Biomedical Science and Chinese Medicine at NTU.

Registered by about 100 academic scholars, researchers, industrialists and students, this one-day symposium consists of **11 talks** and **16 poster presentations**. It covers many exciting fields of peptide and protein research including chemistry, structure and function, and applications in medicine and materials science. We hope that this symposium would provide a platform for our attendees to present and learn about the current forefront of peptide/protein science and drug discovery, as well as to exchange ideas and establish research collaborations or business relations across both academia and industry.

We are grateful to having received strong organisational support from NTU-Institute of Advanced Studies, the School of Biological Sciences and Hengrui Medicine. We also sincerely thank all sponsors for their generous support towards the symposium.

We hope everyone will enjoy attending FPSDDS onsite or online and spend a wonderful day with us.

Symposium Chair
A/Prof LIU Chuan Fa
Associate Professor
School of Biological Sciences,
Nanyang Technological University, Singapore

Symposium Co-Chair
Dr ZHANG Lianshan
Senior Vice President and Global R&D President
Jiangsu Hengrui Medicine, China

Time (GMT +8)	Symposium Programme
08:00 - 08:50	Registration
	Opening Ceremony
09:00 - 09:05	Welcome Address by Symposium Chair **Assoc Prof LIU Chuan Fa** (Nanyang Technological University)
09:05 - 09:15	Opening Remarks by **Prof Simon REDFERN** (Dean, College of Science, Nanyang Technological University) & **Prof Lars NORDENSKIÖLD** (Chair, School of Biological Sciences, Nanyang Technological University)
09:15 - 09:25	Group Photo-Taking
	Session 1 Chair: Prof Shiroh FUTAKI (Kyoto University)
09:25 - 09:55	**Prof Kit LAM** (University of California, Davis) - online From Combinatorial Chemistry to Nanotheranostic Agents Against Cancer
09:55 - 10:25	**Prof Xiaoyuan (Shawn) CHEN** (National University of Singapore) Evan Blue-based Theranostics
10:25 - 10:55	Coffee Break
	Session 2 Chair: Prof Xiaoyuan (Shawn) CHEN (National University of Singapore)
10:55 - 11:25	**Prof Shiroh FUTAKI** (Kyoto University) Attenuated Membrane-lytic Peptides for Intracellular Delivery
11:25 - 11:55	**Prof Joshua MYLNE** (Curtin University) Biosynthesis, Evolution and Structural Diversity of the Cyclic Peptides Buried within Precursors for Daisy Seed Albumins
11:55 - 12:25	**Prof Kimberly KLINE** (University of Geneva) Targeting Both Host and Bacteria to Overcome Vancomycin Resistance in *Enterococcus faecalis*

Time (GMT +8)	Symposium Programme
12:25 - 14:00	Lunch cum Poster Presentation
	Session 3 Chair: Prof Joshua MYLNE (Curtin University)
14:00 - 14:30	**Prof LIU Lei** (Tsinghua University) - online Chemical Synthesis of Proteins using Hydrazide-based Chemistry
14:30 - 15:00	**Prof Hironobu HOJO** (Osaka University) Synthesis of Selenocysteine-containing Proteins and their Properties
15:00 - 15:30	**Prof Yoshio HAYASHI** (Tokyo University of Pharmacy and Life Sciences) A New Synthetic Chemistry of Cyclic Peptides for the Development of Mid-sized Peptide Drug
15:30 - 16:00	Coffee Break
	Session 4 Chair: Prof Hironobu HOJO (Osaka University)
16:00 - 16:30	**Prof WANG Xiaoliang** (Institute of Chinese Materia Medica) Development of Novel Drugs from Natural Products: L-3-n-Butylphalide for Ischemic Stroke and Dementia
16:30 - 17:00	**Prof Hirokazu TAMAMURA** (Tokyo Medical and Dental University) From HIV to SARS-CoV-2: Fusion Inhibitors Based on Dimerization of HR2 Region Peptides
17:00 - 17:30	**Prof Jean MARTINEZ** (University of Montpellier) - online Ghrelin Receptor Ligands, from Laboratory to Market
17:30 - 17:50	**Dr Chun-Lin Chen** (Founder & CEO of Medicilon) - online Bioanalytical Challenge & Strategy for Peptide Drugs
17:50 - 18:05	Medicilon Best Poster Award Ceremony & Closing Remarks by Symposium Co-Chair **Dr Lianshan ZHANG** (Jiangsu Hengrui Medicine)
19:15 - 21:00	Speakers' Dinner (by invitation only)

Speaker 1 (online)

From Combinatorial Chemistry to Nanotheranostic Agents Against Cancer

Kit S. Lam[1*]

[1]Department of Biochemistry and Molecular Medicine, UC Davis, USA
*kslam@ucdavis.edu

Biography

Prof Kit Lam was born and raised in Hong Kong. He is a physician-scientist and an expert in combinatorial chemistry, peptide chemistry, chemical biology, drug discovery and development, molecular imaging, nanotherapeutics, nanoimmunotherapy and medical oncology. He obtained his B.A. in Microbiology in 1975 at the University of Texas at Austin, his Ph.D. in Oncology in 1980 from McArdle Laboratory for Cancer Research, University of Wisconsin, and his M.D. in 1984 from Stanford University School of Medicine. He completed his Internal Medicine residency training and Medical Oncology Fellowship training at the University of Arizona. He is board certified in both Internal Medicine and Medical Oncology. He is currently Chair of the Department of Biochemistry and Molecular Medicine, University of California Davis School of Medicine, Professor of Hematology and Oncology, and a Fellow of the American College of Physicians. He has made a seminal scientific contribution through the development of the one-bead-one-compound (OBOC) approach to combinatorial chemistry. He has published over 350 peer-reviewed scientific publications and holds over 30 patents on inventions.

Abstract

The one-bead one-compound (OBOC) combinatorial chemistry method enables one to rapidly synthesize and screen large number of peptides or small molecules against various biological targets. Over the last two decades, OBOC method has evolved tremendously, in the areas of solid-support, chemical encoding, synthetic chemistry, and high-throughput biochemical and cell-based screening. Most recently, we have succeeded in using OBOC method to discover novel membrane active peptides, genetically encoded small illuminants (GESIs), and self-assembly peptides with supramolecular properties. We have also integrated cancer targeting peptides with self-assembly peptides to create novel cancer targeting transformable nanoplatform for cancer therapy. We were able to demonstrate that HER2-targeting nanoparticle could target HER2 positive tumors efficiently in live animals. At the tumor sites, the nanoparticle underwent receptor-mediated transformation into nano-fibrilles, leading to inhibition of HER2 dimerization, suppression of HER2 downstream signaling, tumor cell death and completion elimination of the tumors in xenograft models.

Speaker 2

Evans blue based Theranostics

Xiaoyuan (Shawn) Chen[1*]

[1]National University of Singapore
[*]chen.shawn@nus.edu.sg

Biography

Prof Xiaoyuan (Shawn) Chen received his Ph.D. in Chemistry from the University of Idaho (1999). After being a faculty at the University of Southern California, Stanford University and then Senior Investigator/Lab Chief at the National Institutes of Health, he is now Nasrat Muzayyin Professor in Medicine and Technology, Yong Loo Lin School of Medicine and Faculty of Engineering, National University of Singapore. His current research interests are mainly theranostics (radiotheranostics, nanotheranostics, immunotheranostics, magnetotheranostics, phototheranostics, etc.) that can be clinically translatable. He has published over 900 papers and numerous books (Total citations > 107,000, H index 170 based on Google scholar).

Abstract

Human serum albumin (HSA) is a heart-shaped protein with 3 homogeneous domains, each with 2 subdomains that own the same structural motifs. HSA contains 2 binding sites, one for carrying and delivering small aromatic molecules in blood circulation, the other for incorporating lipophilic carboxylate derivatives. This talk focuses on using Evans blue, a dye that binds reversibly to albumin, for multimodality imaging of blood pool and lymphatic system, and for modifying various peptides and drug molecules for cancer therapy. It appears that Evans blue conjugation prolongs circulation half-life and reduces renal clearance of molecules of interest, serving as an excellent (nano)platform for effective targeting and sustained drug release.

Speaker 3

Attenuated Membrane-lytic Peptides for Intracellular Delivery

Shiroh Futaki[1]

[1]Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan

Biography

Prof Shiroh Futaki obtained his Ph.D. in 1989 from Kyoto University, Japan. Following his appointment as a Research Associate and an Associate Professor at the University of Tokushima, he moved to Kyoto University in 1997. Meanwhile, he spent 16 months (1989-1991) in the USA as a Postdoctoral Associate in the Department of Biochemistry, Rockefeller University. He has been a Professor of Biochemistry at the Institute of Chemical Research, Kyoto University, since 2005.

Abstract

One of the major research interests of our group is to design peptides for intracellular delivery based on the understanding of the molecular interplay between peptides and membranes. Thus, the spider toxin-derived peptide, L17E, was developed and was found capable of delivering biomacromolecules into cells [1]. This peptide was obtained by substituting a hydrophobic leucine (Leu) to a negatively charged glutamic acid (Glu) to reduce hydrophobic interactions, thereby attenuating the membrane lytic activity on cell surfaces. L17E has achieved the efficient intracellular delivery of antibodies (IgGs) and other biofunctional molecules.

In our efforts to further enhance the delivery efficacy of L17E, Fc region binding peptide conjugated with L17E trimer [FcB(L17E)$_3$] was designed for IgG delivery into cells. Particle-like liquid droplets were generated by mixing Alexa Fluor 488 labeled IgG (Alexa488-IgG) with FcB(L17E)$_3$. Droplet contact with the cellular membrane led to spontaneous influx and distribution of Alexa488-IgG throughout cells in serum containing medium [2].

This presentation will introduce the impact of attenuated membrane-lytic peptides for intracellular delivery and chemical and biological studies.

References

1. Akishiba, M.; Takeuchi, T.; Kawaguchi, Y.; Sakamoto, K.; Yu, H.-H.; Nakase, I.; Takatani-Nakase, T.; Madani, F.; Gräslund, A.; Futaki, S. *Nat. Chem.* **2017**, *9*, 751-761
2. Iwata, T.; Hirose, H.; Sakamoto, K.; Hirai, Y.; Arafiles, J.V.V.; Akishiba, M.; Imanishi, M.; Futaki, S. *Angew. Chem. Int. Ed. Engl.* **2021**, *60*, 19804-19812

Speaker 4

Biosynthesis, Evolution and Structural Diversity of the Cyclic Peptides Buried within Precursors for Daisy Seed Albumins

Joshua S. Mylne[1,2*]

[1]Curtin University, Centre for Crop and Disease Management, Perth, Australia
[2]The University of Western Australia, School of Molecular Sciences, Perth, Australia
[*]josh.mylne@curtin.edu.au

Biography

Prof Joshua S. Mylne is a geneticist and biochemist who has worked broadly in plant genetic engineering (Ph.D. Botany, UQ 2002), developmental genetics and epigenetics at the UK's John Innes Centre (2001-2005) and peptide biochemistry in the IMB, a biomedical institute at UQ (2006-2012). He has held successive, national Australia Research Council QEII and Future Fellowships (2008-2016), was Australia's 2012 Goldacre Medal winner, a 2014 Feinberg Foundation Visiting Fellow to the Weizmann in Israel and was based at the University of Minnesota for 3 months as a 2018 Fulbright Professional Scholar. He founded his lab at the School of Molecular Sciences (mylne.org) in 2013, became tenured in 2017 and an Associate Professor in 2018. In 2021 he became a Professor and the Deputy Director at Curtin University's Centre for Crop and Disease Research. His lab has focussed on studies in protein evolution, peptide biosynthesis and structural enzymology, but currently (in close collaboration with synthetic organic chemists) pursues research programs in herbicide development and target discovery as well as *in planta* fungicide metabolism.

Abstract

A 14-residue circular peptide from sunflower seeds called SFTI-1 was discovered by Russian and British scientists in 1999 [1]. It's a potent inhibitor of trypsin and possible anti-feedant that's since been embraced as a scaffold and drug lead by the peptide community. Subsequent to its discovery, the most interesting aspect about SFTI-1 is its extraordinary biosynthesis; its sequence is buried inside a latent region of an otherwise dull seed storage albumin protein [2-4]. This strange dual biosynthesis has led to some interesting findings such as the mechanism for the ring-forming reactions [5-7] and its structural basis [8-10]. The biosynthesis of this family of peptides buried inside daisy seed albumin genes has been shown to have evolved stepwise over 45 million years [11-13]. A diverse and surprising range of ultra-stable peptide topologies with different physicochemical properties have evolved within sunflower relatives including tiny 5-residue rings, SFTI-like bi-cyclic peptides, a large hoop peptide and even a strange 28-residue ladder [14-19]. We have shown how easy this kind of 'hijacking' of protein hosts can be [20] and unearthed an unrelated family of helical hairpin peptides similarly buried and processed from within a latent region of seed vicilin precursors [21,22]. I will conclude with a historical segue to explain the genetic origins for the first plant cyclopeptide ever discovered; namely evolidine discovered in Australia back in the early 1950s[23].

References

1. Luckett, S.; et al. *J. Mol. Biol.* **1999**, *290*, 525-533
2. Mylne, J. S.; et al. *Nat. Chem. Biol.* **2011**, *7*, 257-259
3. Franke, B.; et al. *J. Biol. Chem.* **2017**, *292*, 12398-12411
4. Franke, B.; et al. *Nat. Prod. Rep.* **2018**, *35*, 137-146

5. Bernath-Levin, K.; et al. *Chem. Biol.* **2015**, *22*, 571-582
6. Mylne, J. S.; et al. *Plant Cell* **2012**, *24*, 2765-2778
7. James, A. M.; et al. *New Phytol.* **2018**, *2118*, 923-928
8. Haywood, J.; et al. *eLife* **2018**, 7, e32955
9. James, A. M.; et al. *Plant J.* **2019**, *98*, 988-999
10. Nonis, S. G.; et al. *Plant Cell*. **2021** 33, 2794-2811
11. Elliott, A. G.; et al. *Plant Cell* **2014**, *26*, 981-995
12. Jayasena, A. S.; et al. *Mol. Biol. Evol.* **2017**, *34*, 1505-1516
13. Fisher, M. F.; et al. *Plant Direct* **2018**, *2*, e00042
14. Elliott, A. G.; et al. *Amino Acids,* **2017**, 49, 103-116
15. Franke, B.; et al. *Biopolymers (Peptide Sci.)* **2016**, *106*, 806-817
16. Fisher, M. F.; et al. *J. Nat. Prod.* **2019**, *82*, 2152-2158.
17. Andersson, H. S.; et al. *RSC Chem. Biol*. **2021**, 2,1682-1691
18. Payne, C. D.; et al. *J. Nat. Prod.* **2021**, 84, 2914-2922
19. Payne, C. D.; et al. *Chem. Sci.* **2021**, 12:6670-6683
20. Pouvreau, B.; et al. *Plant Sci.* **2019**, *285*, 175-183
21. Zhang, J.; et al. *ACS Chem. Biol.* **2019**, *14*, 979-993
22. Payne, C.D.; et al. *J. Nat. Prod.* **2020,** 83, 3030-3040
23. Fisher, M. F.; et al. *J. Biol. Chem*. **2020**, 295, 14510-14521

Speaker 5

Targeting Both Host and Bacteria to Overcome Vancomycin Resistance in *Enterococcus faecalis*

Kimberly Kline[1*]

[1]University of Geneva, Switzerland
*kimberly.kline@unige.ch

Biography

Prof Kimberly Kline is a Professor at University of Geneva, Switzerland. Before that, she was a Professor of Microbiology at Nanyang Technological University in Singapore, and a Principal Investigator at the Singapore Centre for Environmental Life Sciences Engineering. Prior to coming to Singapore, Kimberly received an MPH in Biostatistics and Epidemiology and Ph.D. in Microbiology and Immunology from Northwestern University. Kimberly completed postdoctoral training at Washington University in St. Louis and at the Karolinska Institute in Stockholm Sweden.

Kimberly has received multiple awards for her contributions to the field of microbiology, including a NIH K99 Career Development Award in 2011, the Singapore National Research Foundation Fellowship in 2011, the ICAAC Young Investigator Award from the American Society of Microbiology in 2014, and the Nanyang Education Award in 2017.

Abstract

Among Enterococci, intrinsic and acquired resistance to antibiotics such as β-lactams and vancomycin critically limit treatment options for infection with these opportunistic pathogens. We have recently shown that *Enterococus faecalis* exists as both an extracellular pathogen and also replicates within a variety of mammalian cells, including macrophages, further complicates treatment of infections caused by this opportunistic pathogen. Antimicrobials that enhance the host immune response are emerging as alternative approaches, with the added advantage of overcoming bacterial resistance. Here, we investigate the antibiotic and immunological activity of an FDA-approved anticancer agent *in vitro* and *in vivo* against vancomycin resistant *Enterococcus faecalis* (VRE). *In vitro*, this drug is a potent antibiotic against Gram-positive bacteria through induction of reactive oxygen species and DNA damage. At sub-inhibitory concentrations, this drug synergizes with vancomycin and lowers the vancomycin concentration required to kill VRE by over 140-fold. This synergy is specific to vancomycin-resistant, but not susceptible, strains because vancomycin renders the resistant strains more permeable to this drug and thus drug-mediated DNA damage. In a murine wound infection model, treatment with this drug effectively reduced VRE bacterial numbers by 120-fold and with further reductions when combined with vancomycin. Wounds treated with this drug had significantly more macrophages and pro-inflammatory cytokines compared to untreated wounds. In addition, this drug augmented intracellular bacterial killing by both murine and human macrophages by upregulating the expression of lysosomal hydrolases. These results show that this drug is a potent antibiotic against Gram-positive bacteria, sensitizes VRE to vancomycin, enhances macrophage recruitment and intracellular bactericidal activity, and represent a promising dual bacterium- and host-targeted therapeutic for overcoming vancomycin resistance.

Speaker 6 - online

Chemical Synthesis of Proteins using Hydrazide-based Chemistry

Lei Liu[1*]

[1]Department of Chemistry / Centre for Synthetic & Systems Biology, Tsinghua University, China
*lliu@mail.tsinghua.edu.cn

Biography

Prof Lei Liu received B.S. from University of Science and Technology of China in 1999. He obtained Ph.D. in 2004 from Columbia University under the supervision of Prof Ronald Breslow. He went to Scripps Research Institute to conduct postdoc research in the laboratory of Prof Chi-Huey Wong from 2004 to 2007. He joined Tsinghua University since 2007 and has been working on chemical protein synthesis.

Abstract

Chemical protein synthesis enables the preparation of protein molecules that are difficult to obtain recombinantly. These proteins can be used as protein probes for cell biology, protein samples for structural biology, protein reagents for drug development, and protein catalysts for synthetic biology. We have been working on methods to enable more efficient chemical protein synthesis and have been developing particular method systems based on protein hydrazides as synthetic intermediates. We are hoping to solve the problem of making 100kD proteins from chemical synthesis. We are also actively applying chemically synthesized proteins to biochemistry studies and drug development.

Speaker 7

Synthesis of Selenocysteine-containing Proteins and their Properties

Hironobu Hojo[1*]

[1]Institute for Protein Research Osaka University, Suita, Osaka, Japan
[*]hojo@protein.osaka-u.ac.jp

Biography

Prof Hironobu Hojo obtained his Ph.D. in organic chemistry from Osaka University, Japan in 1994, under the guidance of Prof Saburo Aimoto on the development of the chemical method for protein synthesis. After the completion of his Ph.D., he moved to the Osaka City University as lecturer and worked on the development of novel biomaterials. In 1998, he moved to Tokai University, Japan, as an Associate Professor. There, he started to develop a facile method for glycoprotein synthesis by collaboration with Prof Yoshiaki Nakahara. He was promoted to Professor of Tokai University in 2007. He moved to the present position, Professor of the Institute for Protein Research, Osaka University, in 2013 and is developing a chemical approach toward the understanding of protein and glycoprotein function.

Abstract

The substitution of disulfide bond to diselenide bond in peptide and protein is quite isomorphous in point of their structure and biological activity, which was demonstrated in the case of several peptides, such as endothelin [1] and conotoxin [2] by several researchers. One of the advantages of diselenide substitution is that it is more resistant to reduction. The above researches also showed that the diselenide cognates retained higher stability under reducing conditions as well as in blood plasma. In this presentation, I will talk about the synthesis of several diselenide cognates of natural proteins.

One is a selenoinsulin, in which one of the interchain disulfide bonds of the native insulin were substituted by diselenide bond [3-4]. We first synthesized A and B chain separately incorporating one selenocysteine (Sec) in each chain by the solid-phase method. Two chains were then subjected to the oxidative folding reaction to obtain selenoinsulin. The obtained selenoinsulin assumed quite similar structure with the native insulin and showed similar biological activity. On the other hand, it showed about 10 times higher stability to insulin-degrading enzymes, indicating that it would be useful as a long-lasting insulin drug.

I will then discuss about the synthesis of selenoferredoxin. Ferredoxin is an electron carrier protein, which transfers electrons from photo system I to various ferredoxin dependent enzymes in plant cells. The 2Fe2S cluster supported by four Cys residues are engaged in this process. To analyze the effect of Cys to Sec substitution, we synthesized selenoferredoxin, in which the four Cys residues were substituted with Sec, by the thioester method [5]. The result of the synthesis and its activity measurement will be shown.

Finally, the synthesis and its functional analysis of Sec-substituted epidermal growth factor will be discussed.

References

1. Pegoraro, S.; Fiori, S.; Rudolph-Böhner, S.; Watanabe, T.X., Moroder, L. *J. Mol. Biol*. **1998**, *284*, 779-792
2. Armishaw, C.J.; Daly, N.L.; Nevin, S.T.; Adams, D.J.; Craik, D.J.; Alewood, P.F. *J. Biol. Chem*. **2006**, *281*, 14136-14143
3. Arai, K.; Takei, T.; Okumura, M.; Watanabe, S.; Amagai, Y.; Asahina, Y.; Moroder, L.; Hojo, H.; Inaba, K.; Iwaoka, M.; *Angew. Chem. Int. Ed*. **2017**, *56*, 5522-5526
4. Weil-Ktorza, O.; Rege, N.; Lansky, S.; Shalev, D.E.; Shoham, G.; Weiss, M.A.; Metanis, N. *Chem. Eur. J.* **2019**, *25*, 8513-8521
5. Takei, T.; Ando, T.; Takao, T.; Ohnishi, Y.; Kurisu, G.; Iwaoka, M.; Hojo, H.; *Chem. Commun.,* **2020***, 56,* 14239-14242

Speaker 8

A New Synthetic Chemistry of Cyclic Peptides for the Development of Mid-sized Peptide Drugs

Akihiro Taguchi, Kiyotaka Kobayashi, Yan Cui, Hayate Shida, Chihiro Uchiyama, Akane Fukuda, Kyohei Muguruma, Sho Konno, Kentaro Takayama, Atsuhiko Taniguchi, **Yoshio Hayashi**[1*]

[1]Department of Medicinal Chemistry, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo 192-0392, Japan
*yhayashi@toyaku.ac.jp

Biography

Education: B.S.: Tokyo University of Pharmacy; graduated in March 1983. M.S.: Faculty of Pharmaceutical Science, Kyoto University, 1983-1985. Ph.D. Faculty of Pharmaceutical Science, Kyoto University in 1990, under the guidance of Emeritus Professors Haruaki Yajima and Nobutaka Fujii. Thesis title: “Basic research for the synthetic peptide vaccines and anti-viral agents”.

Research experience: 1986-1988: Researcher, Research and Development Center, Calpis Food Industry Co., Ltd. 1988-1991: Researcher, Life Science Research Center, Advanced Technology and Research Laboratories, Nippon Steel Corporation. 1991-1999: Senior Researcher, Life Science Research Center, Advanced Technology and Research Laboratories, Nippon Steel Corporation. 1999-2001: Lecturer, and 2001-2007: Associate Professor, Department of Medicinal Chemistry, Kyoto Pharmaceutical University (Prof Yoshiaki Kiso’s Lab.). 2007-present: Professor, Department of Medicinal Chemistry, Tokyo University of Pharmacy and Life Sciences.

Research Interests: Peptide Chemistry, Peptidomimetics and Medicinal Chemistry.
Award: The Pharmaceutical Society of Japan Award for Divisional Scientific Promotions '09.

Abstract

Development of the new chemistry in the synthesis of cyclic peptide is highly important for the mid-sized drug development. In the presentation, to efficiently construct cyclic peptides, I would like to introduce two topics based on the chemistry of 3-nitro-2-pyridinesulfenyl (Npys) group[1], i.e., new solid- or none-solid-supported 3-nitro-2-pyridinesulfenates (Npys-OR) as disulfide bond-forming agents[2] and solid-phase-assisted disulfide ligation method using an Npys-Cl resin[3,4], as well as another topic on the synthesis of microorganism derived cyclic depsipeptide MA026[5] which has a epithelial tight junction (TJ) opening activity.

In the first topic, our developed Npys-OMe realizes "on-resin" disulfide bond formation[6] for preparing cyclic peptides in combination with the treatment of 2-mercaptoethanol for the deprotection of tBu-thiol groups from two Cys residues in SPPS, which can be applied to the automated solid-phase protocol. Moreover, by the combination with iodine oxidation, more complicated disulfide peptides like "α-conotoxin" with four Cys residues can be successfully synthesized on resin.

In the second topic, by using Npys-Cl resin, we synthesized a disulfide peptide from two kinds of Cys-containing peptide fragments in a simple solid-phase assisted procedure, which can lead to the syntheses of 1) cyclic peptides via the subsequent intra-molecular amide bond formation and[3,7], 2) multi-connecting disulfide peptides via the repetitive disulfide ligation. Moreover, this chemistry is useful to construct a variety of disulfide conjugates. One of the typical examples is a conjugation between peptide and drug with a different solubility to the reaction solvent[4]. In the presentation, we will also discuss the development of a stable Npys-Cl surrogate, Npys-OPh(*p*F), to increase the efficiency of this technology[8,10].

In the final topic, we would like introduce structure correction of MA026[11], an N-terminal acylated cyclic depsipeptide with 14 amino acid residues from *Pseudomonas*, through the synthesis of MA026 derivatives and their HPLC, NMR and MS analyses. The synthesized cyclic depsipeptide with the corrected structure showed the same physicochemical property and TJ opening activity as natural MA026. The N-terminal modification and alanine scanning of the peptide revealed the important structural factors for the activity.

References

1. Rentier, C., Hayashi, Y., et al., J. Pept. Sci., 2017, 23, 496-504.
2. Taguchi, A., Hayashi, Y., et al., Chem. Eur. J., 2017, 23, 8262-8267.
3. Taguchi, A., Hayashi, Y., et al., Org. Biomol. Chem., 2015, 13, 3186-3189.
4. Muguruma, K., Hayashi, Y., et al., Angew. Chem. Int. Ed., 2018, 57, 2170-2173.
5. Sugawara, F., et al., J. Am. Chem. Soc., 2013, 135, 18949-18956.
6. Kobayashi, K., Hayashi, Y., et al., Eur. J. Org. Chem., 2021, 956-963.
7. Taguchi, A., Hayashi, Y., et al., J. Org. Chem., 2020, 85, 1495-1503.
8. Cui, Y., Hayashi, Y., et al., J. Pept. Sci., 2018, 24, e3070.
9. Cui, Y., Hayashi, Y., et al., Org. Biomol. Chem., 2020, 18, 7094-7097.
10. Cui, Y., Hayashi, Y., et al., Bull. Chem. Soc. Jpn., In press.
11. Uchiyama, C., Hayashi, Y., et al., Angew. Chem. Int. Ed., 2021, 60, 8792-8797.

Speaker 9

Development of Novel Drugs from Natural Products: L-3-n-Butylphalide for Ischemic Stroke and Dementia

Xiaoliang Wang[1*], Shaofeng Xu, Ling Wang, Nan Feng and Weiping Wang

[1]Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical Sciences, Beijing 100050, China
*wangxl@imm.ac.cn

Biography

Prof Xiaoliang Wang is a Professor of pharmacology at the Institute of Materia Medica (IMM), Chinese Academy of Medical Sciences & Peking Union Medical College, China and a Visiting Professor at Nanyang Technological University, Singapore. Prof Wang was the director of IMM from 1997 to 2010. He is also the director of the National Engineering Research Center for the Development of New Drugs.

Prof Wang is an executive member of Chinese Pharmacological Society and a member of Chinese Pharmaceutical Association. He is the Editor-in-Chief of ACTA Pharmaceutica Sinica (Chinese) and Associate Editor-in-Chief of Acta Pharmaceutica Sinica B (English). He obtained his Ph.D. from Universitaet Gesamthochschule Essen in Germany. Prof Wang's research is focused on electrophysiology and pharmacology of ion channels as well as development of channel modulators for disease treatment.

Abstract

L-3-n-Butylphalide (NBP) is a natural product originally extracted from the seeds of Apium graveolens Linn. It was developed as an anti-ischemic stroke agent and has been used in the clinic. However, due to the poor physicochemical properties and low bioavailability, its usage is limited. Recently, we have developed a prodrug of NBP, dl-PHPB or 2-(α-hydroxypentyl) benzoates.

At first, we investigated the pharmacokinetics of PHPB. We demonstrated that PHPB converted into NBP in plasma very fast and completely after oral administration. The AUC of NBP converted from PHPB was 3 folds higher than given NBP directly. However, the plasma-concentration curves and the AUCs of NBP were almost the same when given both compounds by *i.v.*, implying that the oral bioavailability of PHPB is significantly higher than NBP. It was also confirmed in phase I clinical trial.

The neuronal protection effects of PHPB in ischemic stroke were studied. PHPB showed potent protective effects at rat MCAO model and results showed brain infarct volume decreased clearly. One of the mechanisms is anti-ADP induced blood platelet aggregation. The target is P2Y1 receptor subtypes. It might activate PLC and enhance IP3, elevate the intracellular calcium level. P2Y1 might be a new target for inhibiting platelet aggregation.

Activating neurogenesis is another important mechanism of PHPB. By using Bromodeoxyuridine (BrdU) as a probe, we studied *in vivo* the neurogenesis after acute ischemic stroke. It was found that the neurons labeled with BrdU were still seen 8 weeks after MCAO operation, as opposed to only two weeks without PHPB treatment. Further study demonstrated that PHPB activated AKT-pAKT-pGSK3β system as well as PKA-pCREB, and finally induced BDNF production and release. This indicated that PHPB promoted brain tissue auto-repair after damage by ischemic attack. This role might also inhibit neuronal apoptosis.

As another new pharmacology direction, PHPB also shows great potential to treat dementia, including Alzheimer's diseases (AD) and vascular dementia (VD). It improved learning and memory capability in both AD and especially VD animal models. In acute or chronic brain ischemic animal model, PHPB might reduce animal mortality and meanwhile, it enhanced brain function as suggested by results from the Morris water maze test. Since 2018, the phase II and III studies were started for this indication. For treatment of AD, PHPB reduced Aβ plaque in APP/PS1 transgenic mice brain and in the same time PHPB could be effective against impairment of long-term potentiation (LTP) in APP/PS1 transgenic mice. PHPB reduced tau phosphorylation by inhibiting CDK-5 and GSK-3ß activities.

In summary, PHPB is a better drug than the original natural product BNP. It has better properties for use, and has potent neuronal protection, anti-platelet aggregation, neurogenesis and anti-dementia effects.

Key words: l-3-n-Butylphalide; 2-(α-hydroxypentyl) benzoates; PHPB; P2Y1 antagonist; ischemic stroke; dementia

Speaker 10

From HIV to SARS-CoV-2: Fusion Inhibitors Based on Dimerization of HR2 Region Peptides

Hirokazu Tamamura[1*]

[1]Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University (TMDU), Tokyo, Japan
*tamamura.mr@tmd.ac.jp

Biography

Prof Hirokazu Tamamura was graduated from Faculty of Pharmaceutical Sciences, Kyoto University in 1988 (Supervisor: Prof Haruaki Yajima), and his Ph.D. from Kyoto University in 1995. He became an Assistant Professor of Faculty of Pharmaceutical Sciences, Kyoto University in 1989 (Boss: Prof Nobutaka Fujii), and then a Lecturer of Graduate School of Pharmaceutical Sciences, Kyoto University in 1997. He became a Visiting Fellow, National Cancer Institute/NIH, USA in 1999-2000 (Lab. Medicinal Chemistry, Supervisor: Dr Victor E. Marquez). He became an Associate Professor of Graduate School of Pharmaceutical Sciences, Kyoto University in 2005, and then a Full Professor of Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University (TMDU) in 2005. His research fields extend to peptide & protein chemistry, chemical biology, medicinal chemistry and organic chemistry.

Abstract

The pandemic of COVID-19, which is caused by a positive-strand RNA virus SARS-CoV-2, has been continuing still in 2022. In June 2022, more than 500 million people have been infected with SARS-CoV-2 and more than 6 million people died of the virus-induced diseases. To date, more than 30 vaccines are approved and clinically used in the world to prevent SARS-CoV-2 infection and COVID-19 aggravation. Furthermore, some drugs have been developed and authorized including remdesivir, a repositioning inhibitor of RNA-dependent RNA polymerase (RdRp) from Ebola hemorrhagic fever, molnupiravir, a novel SARS-CoV-2 RdRp targeting inhibitor, and nirmatrelvir, an inhibitor of the main protease (M^{pro}) of SARS-CoV-2. In order to develop drugs with different mechanism of actions for increasing a repertory of drug choice, we focused on fusion inhibitors to inhibit the HR1–HR2 interaction, which plays an important role in membrane fusion step. There are many reports to develop fusion inhibitors based on the SARS-CoV-2 HR2 region. According to the classification of viruses, human immunodeficiency virus type-1 (HIV-1) and SARS-CoV-2 belong to positive-sense single-stranded RNA viruses. These viruses have similar fusion mechanism including the formation of 6-helix bundles (6HBs) by interaction of trimer of HR1 regions with corresponding three HR2 regions. In the past two decades, we have been developing anti-HIV-1 agents including fusion inhibitors, and found that the C-terminal dimer of HIV-1 HR2 region (C34) based peptides demonstrated two orders of magnitude higher potency than the parent monomer peptides [1]. Therefore, we envisioned that this dimerization strategy can be applicable to the development of novel SARS-CoV-2 fusion inhibitors. In this symposium I would like to introduce viral fusion inhibitors based on the dimerization strategy.

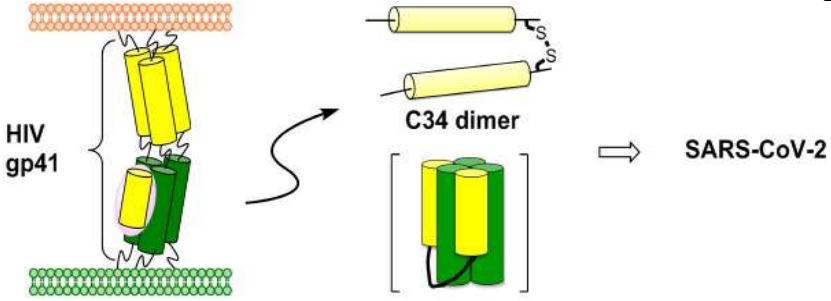


Fig. 1. The dimerization strategy of fusion inhibitors from HIV to SARS-CoV-2.

Reference

1. Kobayakawa, T.; Ebihara, K.; Honda, Y.; Fujino, M.; Nomura, W.; Yamamoto, N.; Murakami, T.; Tamamura, H. *ChemBioChem: Special Issue dedicated to the 10th IPS in Kyoto* **2019**, *20*, 2101-2108

Speaker 11 - online

Ghrelin Receptor Ligands, from Laboratory to Market

Jean Martinez[1*]

[1]Institut des Biomolécules Max Mousseron (IBMM), UMR 5247, CNRS - Université Montpellier - ENSCM, Faculté de Pharmacie, 15 avenue Charles Flahault, 34093 Montpellier Cedex 5, France
*Jean.martinez@umontpellier.fr

Biography

Prof Jean Martinez received both his Ph.D. in 1972 from the University of Montpellier 2, at the National School of Chemistry and his Dr Sciences Degree in 1976 from the same University, under the supervision of Prof F. Winternitz. The same year, he joined Dr E. Bricas group at Orsay, University of Paris Sud, as a post-doctorate fellow and in 1977 the laboratory of Prof M. Bodanszky at Case Western Reserve University in Cleveland, Ohio, USA, where he stayed till mid 1979.

Prof Martinez is a Full Professor at the University of Montpellier. In 2007, he created the « Institut des Biomolécules Max Mousseron » (IBMM), which he has been the Director until December 2014. He is actually Head of the department of Amino Acids, Peptides and Proteins at IBMM.

He served the University of Montpellier 1 as a Member of the Scientific Council for 8 years, and as Vice-President for 6 years (2009-2014).

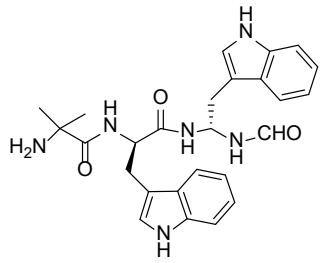
Prof Martinez is recognized for his important contributions, at the interface of chemistry and biology, to the development of methodology in organic and peptide synthesis, design and synthesis of various potent and selective neuropeptide analogues, and biomaterials containing biomolecules.

He has published over 900 original papers, 50 patents, numerous review articles, and he has been editor of several books. In 2012, he was accepted into the « Académie Nationale de Pharmacie », France, and in 2014 into the « Real Academia Nacional de Farmacia », Spain. In 2015, he has been nominated Docteur Honoris causa of the Jagellonian University of Kracow, Poland. He has received various prizes including the Leonidas Zervas Award (Barcelona, Spain 1990), the Silver Medal CNRS (Paris, France 1996), The Labbe Award (Paris, France 1996), The Mentzer Award (Caen, France 1998), the Max Bergmann Medal (Munster, Germany 2004), the Cathay Award (Shanghai, China 2006), the Akabori Award (Tokohama, Japan 2006), the Ehrlich Award (Lyon, France 2011), the Roche « Johannes Meinhofer Award » (Boulder Co, USA 2011), the Léon Velluz Award (Paris, France 2015), the Rudinger Award (Leipzig, Germany 2016). He is « Chevalier dans l'Ordre des Palmes Académiques » (2010), and Chevalier dans l'Ordre de la Légion d'Honneur (2011).

Abstract

The Ghrelin receptor (GHS-R1a for Growth Hormone Secretagogue Receptor type 1a) is a G protein-coupled receptor (GPCR) that mediates in particular ghrelin-induced growth hormone secretion, food intake, and reward-seeking behaviours. Because of its probable involvement in several physiological disorders such as obesity and drugs/alcohol addictions, GHS-R1a represents a major target for the development of therapeutic molecules.

We have developed a series of selective potent Ghrelin receptor ligands [1-5] including agonists, antagonists, partial agonists and inverse agonists. The design, synthesis and pharmacological evaluations of these compounds will be detailed and discussed. Among the ghrelin receptor agonists we have developed in our laboratory, compound JMV1843 showed particular interesting properties. The story of compound JMV1843, an orally active ghrelin agonist that is today commercialized (Macimorelin®, Macrilen®) [1,6] for the diagnosis of adult growth hormone deficiency (AGHD) will be presented.



JMV 1843: Aib-(D)Trp-*g*-(D)Trp-CHO

References

1. Guerlavais, D. B. et al. *J. Med. Chem.* **2003**, 14, 1191-1203
2. Demange et al., *J. Med. Chem.* **2007**, 50, 1939-1957
3. Moulin et al. *Amino Acids* **2013**, 44, 301-314
4. Damian et al. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, 112, 1601-1606
5. M'Kadmi et al. *J. Biol. Chem.* **2015**, 290, 27021-27039
6. Martinez, J.; Fehrentz, J-A. *Aust. J. Chem.* **2020**, 73, 334-337.

Poster Abstract 1

Expanding the Substrate Specificity of a Peptide Asparaginyl Ligase

Chua Niying[1,2], El Sahili Abbas[1,2], Julien Lescar[1,2*] and James P. Tam[1]

[1]School of Biological Sciences, Nanyang Technological University, Singapore
[2]NTU Institute of Structural Biology, Nanyang Technological University, Singapore

Abstract

The development of peptide ligases would have various beneficial applications in academic research, therapeutic applications and bioproduction. Previously, peptide-bond formations were accomplished using enthalpic reagents and other expensive biologicals. The introduction of peptide ligase to catalyze peptide-bond formation between the terminus of a target protein or peptide has allow a steady progress in understanding the enzymatic and cellular functions via site-specific protein modifications [1]–[4]. These peptide ligases could potentially be employed for downstream applications such as producing drug-conjugated antibodies [5] and therapeutic cyclic proteins [6]. So far there are few well-elucidated ligases such as Sortase A [7] and subtiligases [8] but in recent years the discovery from cyclotides producing plants ligases named Peptide Asparaginyl ligases (PALs) [9]–[11] have expandedthe catalog of available ligases. These PALs are derived from asparagine endopeptidases (AEPs) and recognizes short N/GL containing motifs for transpeptidation. Changing the substrate specificities of PALs would broaden the current list of enzymes. To do so, we use directed-evolution techniques to generate a library of PALs for orthogonal ligation. In this study, we showcase a cell-surface display system that is employed in establishing an efficient high-throughput screening method for the directed-evolution of PALs mutants with different specificities.

References

1. C. N. Bedbrook *et al.*, “Genetically Encoded Spy Peptide Fusion System to Detect Plasma Membrane-Localized Proteins In Vivo,” *Chem. Biol.,* vol. 22, no. 8, pp. 1108–1121, 2015.
2. G. K. T. Nguyen, Y. Cao, W. Wang, C. F. Liu, and J. P. Tam, “Site-Specific N-Terminal Labeling of Peptides and Proteins using Butelase 1 and Thiodepsipeptide,” pp. 15694–15698, 2015.
3. C. Rao *et al*., “Enzymatic Engineering of Live Bacterial Cell Surfaces Using Butelase 1,” *Angew. Chemie Int. Ed.*, vol. 56, no. 27, pp. 7822–7825, 2017.
4. J. P. Tam, J. Xu, and K. D. Eom, “Methods and strategies of peptide ligation,” *Biopolym. - Pept. Sci. Sect*., vol. 60, no. 3, pp. 194–205, 2001.
5. G. Falck and K. M. Müller, “Enzyme-Based Labeling Strategies for Antibody–Drug Conjugates and Antibody Mimetics,” *Antibodies*, vol. 7, no. 1, p. 4, 2018.
6. X. Hemu, Y. Qiu, G. K. T. Nguyen, and J. P. Tam, “Total Synthesis of Circular Bacteriocins by Butelase 1,” pp. 1–4, 2016.
7. H. Mao, S. A. Hart, A. Schink, and B. A. Pollok, “Sortase-Mediated Protein Ligation: A New Method for Protein Engineering,” *J. Am. Chem. Soc.*, vol. 126, no. 9, pp. 2670–2671, 2004.
8. T. K. Chang, D. Y. Jackson, J. P. Burnier, and J. A. Wells, “Subtiligase: a tool for semisynthesis of proteins.,” *Proc. Natl. Acad. Sci.*, vol. 91, no. 26, pp. 12544–12548, 2006.
9. J. P. Tam, S. Loo, A. Kam, L. X. Pan, A. E. Jansson, and G. K. T. Nguyen, “Butelase 1: A Versatile Ligase for Peptide and Protein Macrocyclization,” *J. Am. Chem. Soc.*, vol. 137, no. 49, pp. 15398–15401, 2015.
10. R. Yang, Y. H. Wong, G. K. T. Nguyen, J. P. Tam, J. Lescar, and B. Wu, “Engineering a Catalytically Efficient Recombinant Protein Ligase,” *J. Am. Chem. Soc.*, vol. 139, no. 15, pp. 5351–5358, 2017.
11. X. Hemu et al., “Structural determinants for peptide-bond formation by asparaginyl ligases,” *Proc. Natl. Acad. Sci.* U. S. A., pp. 1–10, 2019

Poster Abstract 2

α-astratide aM1, an insulin-mimetic from *A. membranaceus*, restores glucose homeostasis and improves insulin sensitivity in insulin-resistant

Bamaprasad DUTTA[1], Wei Song SEETOH[1], Samuel Wen Hui LAI[1], and James P TAM[1*]

[1]School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551
*jptam@ntu.edu.sg

Abstract

In recent years, our lab discovered plenty of bioactive cysteine-rich peptides (CRPs) from different plant origins, which show a wide range of bioactivity. Cysteine-rich peptides are a class of potential orally active peptides coveted for their hyper-stability against proteolytic, heat, and pH degradation. They are characterized by a molecular weight of 2-6 kDa, with sequences comprising 6-10 cysteine residues forming strong intramolecular disulfide bonds. Recently, our laboratory has discovered a plant-derived hyperstable peptide from *Astragalus membranaceus*. However, very little information is available about this recently-discovered astratide. Here we report a novel cysteine, glycine, and proline-rich α-astratide aM1 from the *A. membranaceus* root, restoring glucose homeostasis in both wildtype and insulin-resistant mutant-based *in-vitro* diabetes models. We also reported that α-astratide aM1 increases both hepatic and peripheral glucose uptake through activating PI3K/Akt-signaling-mediated glucose uptake pathway in cell-based in vitro models. aM1 is equally effective against IR and improves insulin sensitivity in insulin-resistant myotubes and adipocytes. aM1 further restores lipid homeostasis, reduces IMLs (Intramyocellular lipids) accumulation, and prevents lipid-induced insulin resistance in muscle cells. Overall, our data indicate that α-astratide aM1 is an insulin-mimetic peptide that restores glucose homeostasis and insulin sensitivity and improves insulin-resistant, which may provide therapeutic benefits as an orally active insulin substitute.

Acknowledgment

This research was partly supported by Nanyang Technological University Internal Funding -Synzymes and Natural Products (SYNC) and the AcRF Tier 3 funding (MOE2016-T3-1-003).

Poster Abstract 3

β-Gingkotide is a Adaptogenic and Neuroprotective Hyperdisulfide Peptides from *Ginkgo biloba*

Bamaprasad DUTTA[1], Teffarina Hui Wen TAY[1] and James P TAM[1*]

[1]School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551
[*]jptam@ntu.edu.sg

Abstract

Plants produce disulfide-rich peptides, also known as cysteine-rich peptides (CRPs), as part of their host-defense mechanism against environmental hazards, including climates, microbes, and insects. Most CRPs contains 15-25% of cysteine per molecule or about one cysteine per 4 to 7 amino acid residues. Recently, we discovered the small, hyperdisulfide peptide β-gingkotide β-gB1 from ginkgo nuts. β-gingkotide β-gB1 is a highly unusual molecule containing >30% cysteine per molecule or one cysteine per 3 amino acid residues. However, very little is known about this recently-discovered family of molecules. Here we report the biophysical and functional characterizations of β-gingkotide β-gB1 from *Ginkgo biloba*. Our findings reveal that β-gB1 is highly stable against thermal, acid, and proteasemediated degradation. Data mining also revealed that the β-gB1 loop 2 contains the canonical LIR-motif crucial for selective autophagy. Cell-based assays and western blot analysis showed that β-gB1 is a cell-penetrating peptide, able to maintain cellular homeostasis through induced autophagosome formation and protects neuron cells by targeting intracellular proteins from amyloid beta-mediated neurotoxicity. β-gingkotide is the first in the class of an LIR- motif-containing peptide natural product. Together, our results suggest that the plant-derived β-gingkotide is an LIR-Motif containing peptide capable of producing adaptogenic and neuroprotective effects in cell models.

Acknowledgment

This research was supported in part by Nanyang Technological University Internal Funding-Synzymes and Natural Products (SYNC) and the AcRF Tier 3 funding (MOE2016-T3-1-003)

Poster Abstract 4

Identification of Asparaginyl Ligases from Plant Legumains by Substrate-binding Gly

Xinya Hemu, James P. Tam*

[1]School of Biological Sciences, Nanyang Technological University

Peptide asparaginyl ligases (PALs) are the very rare ligase-type plant legumains catalysing Asn/Asp(Asx)-specific peptide bond formation. Represented by butelase-1, PALs are highly active enzymes with 10^5-10^6 $M^{-1}s^{-1}$ catalytic efficiency and broad substrate tolerance to the Asx-adjacent recognition signals and the incoming nucleophile. Therefore they are versatile chemoenzymatic tools for peptide and protein engineering. PALs share high sequence identity and structural similarity to the canonical protease-type legumains, asparaginyl endopeptidases (AEPs), and thus challenging to be distinguished from sequence or structures. Our previous works have identified amino acid variants flanking the S1 cysteinyl catalytic site that could control the directionality of a PAL or an AEP. In this work, we refined our findings on the molecular basis of ligase activities by building and analysing a large dataset of 1.5 thousand legumain sequences. Bioinformatics and functional study of selected legumains revealed that three conserved Gly in S2, S1', and S2' substrate-binding pockets of plant legumains are the most important ligase activity determinants (LADs). In PALs they have different amino acid compositions, which could affect substrate binding and the stability of S-acyl intermediate to steer the ligase activity of plant legumains. These functional-determining Gly sites are the keys to distinguish PALs from AEPs, which led to successful identification of 18 PALs. Our work shows that the LAD hypothesis is a general and promising strategy to discover new PALs as a distinct subtype of plant legumains.

Poster Abstract 5

Bioinspired Peptide Materials and Peptide Materials for Therapeutics Delivery

S. H. Hiew and A. Miserez

School of Material Science and Engineering, Nanyang Technological University (NTU), Singapore 639798.

Abstract

Sucker ring teeth (SRT) of the Humboldt squid (*Dosidicus gigas*) are unusual hard tissues that have inspired the design of several protein-based and peptide-based materials for potential biomedical applications [1]. The teeth are natural "biotools" that are hard and robust, non-mineralized and fully proteinaceous, comprising of proteins named "suckerins" with high sequence modularity.

With previous work unraveling the molecular-scale interactions, self-assembly mechanisms, the smart selection of protein sequences that drive the hierarchical assembly of natural SRT material in a unique fashion [1], we now adapt the modular peptide sequences to fabricate useful materials for real-world solutions.

This presentation highlights the study of peptide materials inspired by SRT peptides, including the molecular-scale interactions, self-assembly mechanism, material properties and exploration of their potential as therapeutics delivery vehicles. The unique self-assembly property of a short SRT-bioinspired peptide, GV8 [2], enables a simple one-pot encapsulation of therapeutics under mild aqueous conditions and the delivery of complex growth factors such as stem cell secretome. Delivery of these complex regenerative therapeutic candidates have been faced with multiple challenges that impede their application and GV8 hydrogels demonstrate the capability to address these issues, hence a promising candidate for wound-healing applications [3,4].

The boundless lessons we learn from a single structural protein can equip us with a plethora of possibilities toward engineering new peptide-based biomimetic materials, from a detailed study of SRT-bioinspired peptides, GX8. GX8 peptides are found in many structural proteins in nature too, and here we present first-hand the intriguing self-assembled materials from GX8 [5].

References

1. Shu Hui Hiew and Ali Miserez*, Squid Sucker Ring Teeth: Multiscale Structure-Property Relationships, Sequencing and Protein Engineering of a Thermoplastic Biopolymer. *ACS Biomaterials Science & Engineering*, **2017**, 3, 680-693.
2. Shu Hui Hiew, Harini Mohanram, Lulu Ning, Jingjing Guo, Antoni Sánchez-Ferrer, Xiangyan Shi, Konstantin Pervushin, Yuguang Mu, Raffaele Mezzenga, Ali Miserez*, Short Peptide Hydrogel with High Stiffness Induced by 3_{10}-Helices to β-Sheet Transition in Water. *Advanced Science*, **2019**, 6(21), 1901173.
3. Shu Hui Hiew*, Jun Kit Wang, Kenrick Koh, Haibo Yang, Abbas Bacha, Junquan Lin, Yun Sheng Yip, Marcus Ivan Gerard Vos, Liyan Chen, Radoslaw M Sobota, Nguan Soon Tan, Chor Yong Tay*, Ali Miserez*, Bioinspired short peptide hydrogel for versatile encapsulation and controlled release of growth factor therapeutics. *Acta Biomaterialia*, **2021**, 136, 111-123.
4. Ali Miserez, Shu Hui Hiew, Chor Yong Tay, Jun Kit Wang, Hydrogel-forming peptides, and methods of use thereof. US Patent App. 17/364,153, **2022**.
5. Shu Hui Hiew*, Yang Lu, Atul Parikh, Mu Yuguang*, Ali Miserez*, Structural regulation of short peptides via single amino acid alteration from conserved sequences of structural proteins in nature, **2022**, to be submitted.

Poster Abstract 6

Amino acid scanning revealed a potent analog of a hyperstable non-canonical plant-derived EGFR agonist

Antony Kam[1], Shining Loo[1], Wang Xiaoliang[1.2], James P Tam[1*]

[1]School of Biological Sciences, Nanyang Technological University, Singapore
[2]Institute of Materia Medica, Chinese Academy of Medical Sciences, China
[*]jptam@ntu.edu.sg

Abstract

Plant-derived bleogen pB1 is the first hyperstable non-canonical epidermal growth factor receptor (EGFR) agonist from *Pereskia bleo*, a popular Southeast Asian cactus plant used for treating gastritis, hemorrhoids, and ulcers. Bleogen pB1 is a low affinity EGFR agonist but more proteolytic stable than EGF. Bleogen pB1 displays no sequence homology or structural similarity to any canonical EGFR ligands, except the presence of a pentapeptide YXGXK motif in its loop 4. Here, using focused positional Ala- and D-amino acid scans at the pentapeptide motif, we performed structure-activity relationship studies on bleogen pB1 for EGF-like activities. Ala-scan revealed that the pentapeptide motif in bleogen pB1 is crucial for its EGF-like activities. Surprisingly, we identified two analogs with improved EGFR binding affinity using D-amino acid scan, with [K29k]pB1 having the highest affinity. The high-affinity pB1 analog, [K29k]pB1, has a 60-fold-improved EGFR affinity and mitogenicity. Additionally, we show that the potency of [K29k]pB1 and EGF is comparable in a diabetic mouse wound-healing model. Like bleogen pB1, [K29k]pB1 is also hyperstable, being >100-fold more stable than EGF against proteolytic degradation. Overall, our discovery of a non-canonical proteolytic-resistant EGFR agonist scaffold could open new avenues for developing wound healing and skin regeneration therapeutics and biomaterials.

Acknowledgement

This research was supported in part by the Competitive Research Grant by National Research Foundation in Singapore (NRF-CRP8–2011-05), Nanyang Technological University Internal Funding–Synzyme and Natural Products (SYNC), and the AcRF Tier 3 funding (MOE2016-T3–1-003). S.L. and A.K. are recipient of the Mistletoe Research Fellowship.

References

Loo, S., Kam, A., Li, B.B., Feng, N., Wang, X. and Tam, J.P., 2021. Discovery of Hyperstable Noncanonical Plant-Derived Epidermal Growth Factor Receptor Agonist and Analogs. *Journal of Medicinal Chemistry*, *64*(11), pp.7746-7759.

Poster Abstract 7

Characterization of an Unusual Lysine Side Chain-to-Tail Ligation by Peptide Asparaginyl Ligases

Ching Koon LIM[1], James P. TAM[1]*

[1]School of Biological Sciences, Nanyang Technological University, Singapore
*jptam@ntu.edu.sg

Abstract

Asparaginyl endopeptidases (AEPs), also known as legumains, are Asn/Asp (Asx)-specific proteases that are capable of catalyzing proteolysis under physiological conditions. Peptide asparaginyl ligases (PALs) belong to a subset of AEPs that possess high level of ligation activity. Owing to their high specificity and ligation efficiency without the need for ATP and cofactors, PALs are highly versatile tools for a wide array of biochemical and biotechnological applications. Head-to-tail cyclization and intermolecular ligation of peptides are the primary ligation mode of PALs, but it was recently discovered that peptide can still undergo cyclization via ligation between lysine side chain and Asn if an unfavoured free N-terminal nucleophile is present. Herein, we reported the characterization of PAL-mediated lysine side chain-to-tail ligation and its potential applications. The efficiency of this secondary ligation mode appears to be dependent on several factors such as pH condition, presence of incoming peptide, types of PALs, and proximity between N- and C-termini. It has also been shown to be applicable to the design of monocyclic and bicyclic peptides, thus laying a foundation for the design of highly stable multi-cyclic peptide biotherapeutics.

Poster Abstract 8

Dual Native Chemical Ligation (dNCL) for Easy Access to *N*-Glycopeptides and *N*-Glycoproteins

Junjie Desmond Lin[1], **Chen-Long Li**[1], Xue-Wei Liu[1*]

[1]Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, 21 Nanyang Link, Singapore 637371, Singapore
*xuewei@ntu.edu.sg

Abstract

Nowadays, glycoproteins are widely found and used as therapeutic proteins on the market or in clinical study. However, the major problem in current pharmaceutical use of biologically expressed glycoproteins is their natural heterogeneity in both glycosylation site and glycan sequence, which leads to a problem in the corresponding structure-activity relationship. As a consequence, the chemical synthesis of glycopeptides and glycoproteins in ultrapure and well-defined forms has been highly motivated, considering the great demand of new vaccines, diagnostics and therapeutics.

Native chemical ligation (NCL) that can link two peptides, is widely employed in the synthesis of *N*-glycopeptides and *N*-glycoproteins. However, the Cys at *N*-terminal of peptide sequence is definitely required for the traditional NCL, which highly limits tailor-made synthetic routes of targeted peptides or proteins. In order to obtain *N*-glycopeptides or *N*-glycoproteins, stepwise chemical synthesis is introduced, in which N-glycoside Asn in solid phase peptide synthesis (SPPS) and Cys residues in NCL are essential. In this method, the biggest challenge is the potential degradations or side reactions of glycosides in later SPPS or NCL. To avoid the utilization of Cys, Wong group designed a modified thiol-containing sugar to conduct a NCL reaction.[1] Although it overcomes the requirement for Cys residue near ligation site, the further desulfurization step is required, which leads to orthogonal protection of other Cys residues. Moreover, it's still necessary to firstly synthesize the *N*-glycoside Asn used in SPPS. Inspired by Wong's work, we previously developed a practical approach to *N*-glycopeptides via the auxiliary-mediated dual NCL at aspartic acid.[2] In our design, the *N*-linked glycosyl auxiliary and the thioester side chain of an *N*-terminal aspartate oligopeptide are keys to success. Whereas, the difficulty of the thioester side chain synthesis greatly blocked applications of our approach. Therefore, it's urgent to develop new, practical, and efficient method to access *N*-glycopeptides and *N*-glycoproteins.

Recently, we have designed a new bifunctional template to obtain *N*-glycopeptides or *N*-glycoproteins at asparagine via native chemical ligation and traceless Staudinger ligation (Figure 1)[3]. The protocol we proposed is firstly to introduce a removable bifunctional template **2** to the peptide **1** at aspartic acid, which then can afford auxiliary-appended peptide **3** by cleavage off from resin. After that, a native chemical ligation between **3** and thioester peptide **4** will be employed to give a longer peptide product **5**. Finally, through Staudinger reaction of **5** and glycosyl azide **6**, *N*-glycopeptide **7** can be synthesized, in which the template is removed without the influence on peptides at the same time. Moreover, the auxiliary-attached aspartic acid will be converted to asparagine in this method.

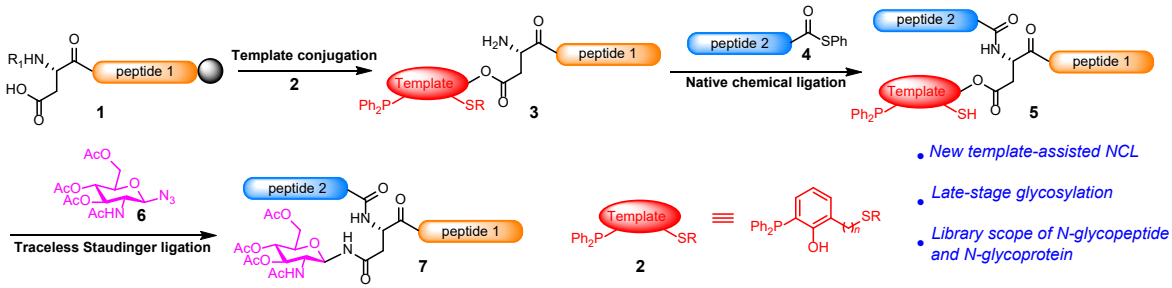


Figure 1. New dNCL method

References

1. Brik, A.; Ficht, S.; Yang, Y.-Y.; Wong, C.-H. *J. Am. Chem. Soc.* **2006**, *128*, 15026.
2. Chai, H.; Le, K. M. H.; Vu, M. D.; Pasunooti, K. K.; Liu, C.-F.; Liu, X.-W. *Angew. Chem., Int. Ed.* **2016**, *55*, 10363.
3. Lin, J. D.; Li, C.-L.; Liu, X.-W. *manuscript in preparation*.

Poster Abstract 9

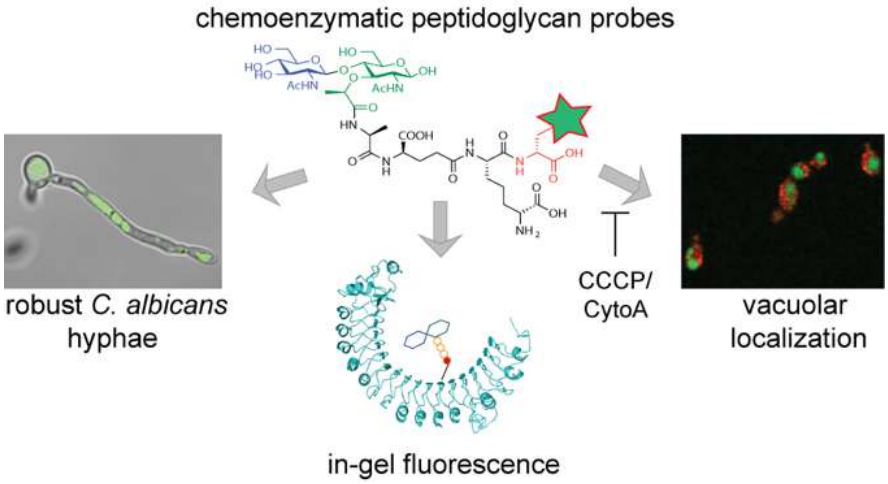
Chemoenzymatic probes reveal peptidoglycan recognition and uptake mechanisms in Candida albicans

Lanxin Li[1], Yuan Qiao[1]*

[1]Division of Chemistry and Biological Chemistry (CBC), School of Physical and Mathematical Sciences (SPMS), Nanyang Technological University, 21 Nanyang Link, Singapore S637371
*Corresponding author: yuan.qiao@ntu.edu.sg

Abstract

Candida albicans, the major fungal pathogen in humans, is under the strong influence of bacterial peptidoglycan fragments to undergo the yeast-to-hyphae transition, a key virulent step in *C. albicans* pathogenesis and infections. However, due to the synthetic difficulties of obtaining peptidoglycan fragments for biological studies, mechanistic details of how *C. albicans* recognizes and uptakes these peptidoglycan fragments have not been well-elucidated. Notably, previous works have solely focused on the synthetic peptidoglycan ligand, muramyl dipeptide (MDP), despite its poor hyphal-inducing activity in *C. albicans*. In this work, we isolated and purified natural peptidoglycan fragments via enzymatic degradation of bacteria cell wall sacculi, and chemoenzymatically installed a series of functional D-amino acids into the natural muropeptide, creating peptidoglycan probes that bear photoaffinity, bio-orthogonal, or fluorescent functionality. Using these chemoenzymatic peptidoglycan probes, we established that natural peptidoglycan fragments, which are potent hyphal-inducers, interact with the *C. albicans* Cyr1 sensor protein in the in-gel fluorescence assay as well as in *in vitro* pull-down studies. Moreover, we established that bacterial peptidoglycan probes enter *C. albicans* cells via an energy-dependent endocytic process.



Poster Abstract 10

Discovery of a cactus-derived hyperstable non-canonical EGFR agonist

Shining Loo[1], Antony Kam[1], Wang Xiaoliang[1,2], James P Tam[1*]

[1]School of Biological Sciences, Nanyang Technological University, Singapore
[2] Institute of Materia Medica, Chinese Academy of Medical Sciences, China
[*] jptam@ntu.edu.sg

Abstract

Plant-derived cysteine-rich peptides with molecular masses of 2-6 kDa represent an expanding class of peptidyl-type natural products with diverse functions and high proteolytic stability. Previously, we identified a family of anti-fungal CRPs, designated bleogens, from *Pereskia bleo*, a popular Southeast Asian cactus plant used for treating gastritis, hemorrhoids, and ulcers. Here we report the discovery of the first plant-derived and non-canonical epidermal growth factor receptor (EGFR) agonist, the 36-residue bleogen pB1 from *Pereskia bleo*. *In silico* modeling showed that bleogen pB1 could interact with the ectodomain of EGFR. Using a suite of chemical, biochemical, cellular, and animal experiments which include incisor eruption and wound-healing mouse models, we show that bleogen pB1 is a low-affinity EGFR agonist and has functional properties like EGF. Different to all known mammalian-derived EGFR agonists, the plant-derived bleogen pB1 has a different cysteine motif which produces a cystine-knot disulfide connectivity, resulting in a different, but far more, compact knotted-structure than the canonical non-cystine-knot EGF-like domain. Consequently, bleogen pB1 is hyperstable, being >100-fold more stable than EGF against proteolytic degradation.

Overall, our discovery of bleogen pB1 provides the first evidence for the ethnomedicinal uses of *Pereskia bleo* and could open new avenues for developing novel wound healing and skin regeneration therapeutics.

Acknowledgement

This research was supported in part by the Competitive Research Grant by National Research Foundation in Singapore (NRF-CRP8–2011-05), Nanyang Technological University Internal Funding–Synzyme and Natural Products (SYNC), and the AcRF Tier 3 funding (MOE2016-T3–1-003). S.L. and A.K. are recipient of the Mistletoe Research Fellowship.

References

Loo, S., Kam, A., Li, B.B., Feng, N., Wang, X. and Tam, J.P., 2021. Discovery of Hyperstable Noncanonical Plant-Derived Epidermal Growth Factor Receptor Agonist and Analogs. *Journal of Medicinal Chemistry*, *64*(11), pp.7746-7759.

Poster Abstract 11

Discovery and characterization of hyperdisulfided peptides from Schisandra chinensis

Fan Tang[1], Antony Kam[1], Shining Loo[1], Ka H. Wang[1], Stephanie V. Tay[1], Jiayi Huang[1], James P. Tam[1]*

[1]School of Biological Sciences, Nanyang Technological University, Singapore
*JPTam@ntu.edu.sg

Abstract

Hyperdisulfided peptides are characterized by their high cysteine content, compact structure, and super stability, properties that are desirable for designing orally-active peptidyl drugs. However, as a family, they are underexplored and rarely found in plants. Here, we report the identification and characterization of a novel hyperdisulfided peptide, wuweizitide-1 (wZ1), from Schisandra chinensis which is a common medicinal plant to treat liver ailments in traditional medicines. Combining proteomics, transcriptomics, and structural analysis, we showed that wZ1 is an 18-residue peptide with six cysteine residues and biosynthetically derived from an 86-residue, 3-domain precursor. NMR spectroscopy revealed that the three disulfide bonds of wZ1 are arranged as a cystine knot. This scaffold together with its small size and highly disulfide-crosslinked structure renders wZ1 superstable and >100-fold more stable than its S-alkylated linear form against proteolytic degradations, conditions simulating enzymatic degradations in the gastrointestinal tracts. Sequence analysis showed that wZ1 has a novel cysteine motif (CC-C-C-CC) representing a new cysteine-rich peptide family in plants However, this cysteine motif shares similarities with the M superfamily of conotoxins. Together, our results suggest that the hyperdisulfided wZ1 could be a useful scaffold for drug design.

Poster Abstract 12

HIV-1 fusion inhibitors targeting the membrane-proximal external region of Env spike

Xiao Tianshu*[1,2], Gary Frey*[1,3], Qingshan Fu[4], Christy L Lavine[5], David A Scott[6], Michael S Seaman[5], James J Chou[4], Bing Chen[1]

[1]Division of Molecular Medicine, Boston Children's Hospital, and Department of Pediatrics, Harvard Medical School, Boston, MA, USA.
[2]School of Biological Sciences, Nanyang Technological University, Singapore
[3]ICCB-Longwood Screening Facility, Harvard Medical School, Boston, MA, USA.
[4]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA.
[5]Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Boston, MA, USA.
[6]Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA, USA.

Abstract

Combination antiretroviral therapy (cART) has transformed HIV-1 infection, once a fatal illness, into a manageable chronic condition. Drug resistance, severe side effects and treatment noncompliance bring challenges to the cART implementation in clinical settings and indicate the need for additional molecular targets. Here we have identified several small-molecule fusion inhibitors, guided by a neutralizing antibody, against an extensively studied vaccine target- the membrane proximal external region (MPER) of HIV-1 envelope (Env) spike. These compounds specifically inhibit the Env-mediated membrane fusion by blocking CD4-induced conformational changes. An NMR structure of one compound complexed with a trimeric MPER construct reveals that the compound partially inserts into a hydrophobic pocket formed exclusively by the MPER residues, thereby stabilizing its prefusion conformation. These results suggest that the MPER is a potential therapeutic target for developing fusion inhibitors and that strategies employing an antibody-guided search for novel therapeutics may be applied to other human diseases.

Poster Abstract 13

A highly stable Asx-specific splicing enzyme from *Momordica cochinchinensis*

Janet To[1], Heng Tai Liew[1], Xiaohong Zhang[1], Xinya Hemu[1], Ning-Yu Chan[1], Aida Serra[2,3], Siu Kwan Sze[1], Chuan-Fa Liu[1], James P. Tam[1]*

[1]School of Biological Sciences, Nanyang Technological University, Singapore
[2]IMDEA Food Research Institute, Madrid, Spain
[3]Proteored – Instituto de Salud Carlos III (ISCIII), Madrid, Spain
*JPTam@ntu.edu.sg

Abstract

Legumains, also known as asparaginyl endopeptidases (AEPs) or vacuolar processing enzymes (VPEs), are cysteine proteases which cleave peptide bonds after Asn/Asp (Asx) residues. A specific class of legumains known as peptide asparaginyl ligases (PALs) can perform backbone cyclization involving an Asx residue at the ligation site. Owing to their site-specific protein ligation efficiency, PALs are versatile biotechnological tools for precision biomanufacturing of new drugs and biologics. In plants, certain legumains also have ligase activity that catalyzes the biosynthesis of Asx-containing cyclic peptides. An example is the biosynthesis of MCoTI-I/II, a squash family-derived cyclic trypsin inhibitor, which involves splicing to remove the N-terminal prodomain and then N-to-C terminal cyclization of the mature domain. To identify plant legumains responsible for the maturation of these cyclic peptides, and to expand our search for Asx-specific PALs, we have conducted a large-scale screening study to discover novel PALs from diverse plant species. Our discovery efforts led to the isolation and characterization of an unusual legumain, McPAL1, from *Momordica cochinchinensis*. Functional studies revealed that recombinantly expressed McPAL1 displays a pH-dependent, trimodal enzymatic profile. McPAL1 can cut, join, and splice peptide precursors into active biologics. At pH 4-6, McPAL1 selectively catalyzed Asp-ligation and Asn-cleavage, but at pH 6.5-8, Asn-ligation predominated. With peptide substrates containing N-terminal Asn and C-terminal Asp, such as is found in precursors of MCoTI-I/II, McPAL1 mediates proteolysis at the Asn site and then ligation at the Asp site at pH 5-6. Furthermore, McPAL1 is an unusually stable legumain that is tolerant of heat and high pH. Together, our results support that McPAL1 is a splicing legumain at acidic pH that can mediate biosynthesis of MCoTI-I/II. We purport that the high thermal and pH stability of McPAL1 could have applications for protein engineering.

Reference

1. Liew, H. T.*; To, J.*; Zhang, X.; Hemu, X.; Chan, N. Y.; Serra, A.; Sze, S. K.; Liu, C. F.; Tam, J. P. *J. Biol. Chem.* **2021,** 297, 101325.

Poster Abstract 14

The design of inhibitors targeting bacterial tRNA methyltransferases

Yee Hwa Wong[1,2], Wenhe Zhong[3], Juthamas Jaroensuk[4], Peter C. Dedon[3,5*], Julien Lescar[1,2*]

[1]School of Biological Sciences, Nanyang Technological University, Singapore
[2]NTU Institute of Structural Biology, Nanyang Technological University, Singapore
[3]Singapore-MIT Alliance for Research and Technology Antimicrobial Resistance and Infectious Disease Interdisciplinary Research Group, Singapore
[4]Applied Biological Sciences Program, Chulabhorn Graduate Institute, Chulabhorn Royal Academy, Bangkok, Thailand
[5]Department of Biological Engineering, Massachusetts Institute of technology, Cambridge, MA, USA
[*]Peter C. Dedon: pcdedon@mit.edu
[*]Julien Lescar: julien@ntu.edu.sg

Abstract

Antimicrobial resistance (AMR) is a global health and development threat. Antibiotics are becoming increasingly ineffective as drug-resistance spreads globally leading to more difficult to treat infections and death. According to the World Health Organization (WHO) list of global priority pathogens (GPP), *Pseudomonas aeruginosa* is one of the critical pathogens that need our attention belonging to the ESKAPE list. We targeted the tRNA (m^1G37) methyltransferase TrmD that is conserved and essential in most bacterial species, and yet, differs structurally from its human Trm5 homolog, making it a prime antibacterial drug target. Crystal structures of TrmD from *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* in complex with AZ51 revealed conformational changes unique to the Gram-negative bacterial TrmD. Elaborating on a thienopyrimidinone scaffold, we prepared and analyzed a series of TrmD inhibitors, which revealed a novel SAM-competitive, active site Tyr-flipping inhibition mechanism that distinguished Gram-negative TrmDs from Gram-positive and mycobacterial counterparts. Several of these compounds showed nanomolar TrmD inhibition, tRNA competitive binding, and micromolar antimicrobial activity against Gram-positive bacteria and, in some instances, Gram negatives and mycobacteria. The strong Structure-activity relationship (SAR) for TrmD inhibition by thienopyrimidinone compounds established here provides a foundation for pursuing antibacterial SAR.

References

1. Juthamas Jaroensuk*, Yee Hwa Wong*, et al. RNA. 2019. Crystal structure and catalytic mechanism of the essential m1G37 tRNA methyltransferase TrmD from Pseudomonas aeruginosa. DOI: 10.1261/rna.066746.118.
2. Wenhe Zhong, Kalyan Pasunooti, Seetharamsing Balamkundu, **Yee Hwa Wong**, et al. *Journal of Medicinal Chemistry.* 2019. Thienopyrimidinone Derivatives That Inhibit Bacterial tRNA(Guanine37-N1)-Methyltransferase (TrmD) by Restructuringthe Active Site with a Tyrosine-Flipping Mechanism. DOI: 10.1021/acs.jmedchem.9b00582

Poster Abstract 15

Nγ-Hydroxyasparagine: A Multifunctional Unnatural Amino Acid That is a Good P1 Substrate of Asparaginyl Peptide Ligases

Yiyin Xia[1], Janet To[1], Ning-Yu Chan[1], Side Hu[1], Heng Tai Liew[1], Seetharamsing Balamkundu[1], Xiaohong Zhang[1], Julien Lescar[1], Surajit Bhattacharjya[2], James P. Tam[1]* and Chuan-Fa Liu[1]*

[1]School of Biological Sciences, Nanyang Technological University 60 Nanyang Drive, Singapore 637551
[2]Singapore-MIT Alliance for Research and Technology Singapore 138602
*jptam@ntu.edu.sg
cfliu@ntu.edu.sg

Abstract

Peptidyl asparaginyl ligases (PALs) are powerful tools for peptide macrocyclization. Herein, we report that a derivative of Asn, namely Nγ-hydroxyasparagine or Asn- (OH), is an unnatural P1 substrate of PALs. By Asn(OH)-mediated cyclization, we prepared cyclic peptides as new matrix metalloproteinase 2 (MMP2) inhibitors displaying the hydroxamic acid moiety of Asn(OH) as the key pharmaco- phore. The most potent cyclic peptide (Ki = 2.8 $\pm$ 0.5 nM) was built on the hyperstable tetracyclic scaffold of *rhesus* theta defensin-1. The Asn(OH) residue in the cyclized peptides can also be readily oxidized to Asp. By this approach, we synthesized several bioactive Asp-containing cyclic peptides (MCoTI-II, kB2, SFTI, and integrin-targeting RGD peptides) that are otherwise difficult targets for PAL-catalyzed cyclization owing to unfavorable kinetics of the P1-Asp substrates. This study demonstrates that substrate engineering is a useful strategy to expand the application of PAL ligation in the synthesis of therapeutic cyclic peptides.

References

Xia, Y.; To, J.; Chan, N.-Y.; Hu, S.; Liew, H. T.; Balamkundu, S.; Zhang, X.; Lescar, L.; Bhattacharjya, S.; Tam, J. P.; Liu, C.-F. *Angew. Chem. Int. Ed.* **2021**, 60, 22207–22211.

Poster Abstract 16

The improvement of isolation/expression yield of Butelase-1 and their biochemical properties

Xiaohong Zhang[1], Xinya Hemu[1], Nguyen K. T. Giang[2], , Janet To[1], Chuan-Fa Liu[1], James P. Tam[1*]

[1]School of Biological Sciences, Nanyang Technological University, 637551 Singapore
[2]WIL@NUS Corporate Lab, MD6 Centre for Translational Medicine, Wilmar International Limited, National University of Singapore, 117599 Singapore
*jptam@ntu.edu.sg

Abstract

Butelase-1, an asparaginyl endopeptidase or legumain, is the prototypical and fastest known Asn/Aspspecific peptide ligase. It is highly useful for engineering and macrocyclization of peptides and proteins. However, certain biochemical properties and applications of naturally occurring and recombinant butelase-1 remain unexplored. Here we report methods to increase the yield of natural and bacterial expressed recombinant butelase-1. First, the yield of natural butelase-1 was increased 3-fold to 15 mg/kg by determining its highest distribution which is found in young tissues, such as shoots. The yield of recombinantly-produced soluble butelase-1 was improved by promoting cytoplasmic disulfide folding, codon changes, and truncation of the N-terminal pro-domain. Natural and recombinant butelase-1 displayed similar ligase activity, physical stability, and salt tolerance. Furthermore, the processing and glycosylation sites of natural and recombinant butelase-1 were determined by proteomic analysis. Storage conditions for both forms of butelase-1, frozen or lyophilized, were also optimized.

References

1. Nguyen, G.K., Wang, S., Qiu, Y., Hemu, X., Lian, Y., and Tam, J.P. (2014). Butelase 1 is an Asx-specific ligase enabling peptide macrocyclization and synthesis. Nat Chem Biol 10(9), 732-738. doi: 10.1038/nchembio.1586.
2. Nguyen, G.K.T., Qiu, Y., Cao, Y., Hemu, X., Liu, C.-F., and Tam, J.P. (2016). Butelase-mediated cyclization and ligation of peptides and proteins. Nature Protocols 11(10), 1977-1988. doi: 10.1038/nprot.2016.118.

Medicilon

Reliable CRO with Integrated Preclinical R&D Services

As one of the top preclinical CROs in China, Medicilon offers fully integrated drug R&D services for the global healthcare community. Our services across drug discovery, pharmaceutical reaserch and preclinical research are uniquely designed to help clients develop their R&D programs from the initial ideas to the IND filing stage.

Hit »» Lead Optimization »» Preclinical Candidate »» IND Filing

18+	Years of experience in new drug R&D
1500+	Active clients worldwide
2440+	Newdrug discovery scientists and service personnel
74000+	Square meters of Laboratory floor space
221	Projects successfully approved with FDA, NMPA, EMA, and TGA

COLLABORATIVE PARTNERS

Shanghai Medicilon Inc.

Address: No.585 Chuanda Road, Pudong New Area, Shanghai (Headquarters) Website: www.medicilon.com

Tel:+86 (21) 5859-1500 Fax: +86 (21) 5859-6369 Email: Marketing@medicilon.com.cn

Antibody Drug Discovery Roadmap

GenScript
Make Research Easy

1 Target Discovery

Therapeutic targets are identified and characterized through different approaches to demonstrate how their modulation affects disease states, thus revealing their direct involvement in pathogenesis.

- Precise Synthetic Oligo Pools
- CRISPR gRNA Library
- Lentivirus Packaging
- CellPower™ Assay Cell Line

2 Lead Generation

Validated antigen targets are used to generate therapeutic antibody candidates by eliciting an immune response through antigenic presentation.

- Antigen Synthesis
- Transgenic Model by GenExact™ ssDNA
- MonoRab™-B Cell Cloning
- Beacon-Single B Cell Sorting
- Antibody Sequencing (Sanger & NGS)

3 Lead Identification & Optimization

Different approaches may be leveraged to modify therapeutic antibody candidates to increase their effectiveness and affinity and reduce immunogenicity.

- Ab Genes to Vectors
- CRISPR Mediated Cell Engineering
- Precision Mutant Library
- TurboCHO™ High Throughput/Express
- CellPower™ Assay Cell Line

4 Preclinical Stage

Once lead candidates are identified, preclinical tests evaluate their safety. Preclinical trials are conducted on non-human subjects to study the efficacy, toxicity, and pharmacokinetics of therapeutic antibodies.

- Anti-idiotype Antibody Discovery
- TurboCHO™ High Performance
- Custom ELISA Kits - PK/ADA/HCP

164, Kallang Way, #06-12, Singapore 349248
ap.marketing@genscript.com
www.genscript.com

GenScript
Make Research Easy

thermo scientific

Advance your Quantitation

With next-level productivity and performance

Laboratory needs for productivity never cease to grow. Advance your quantitative workflows to meet these demands with the next-level performance and productivity of the Thermo Scientific™ TSQ™ Plus portfolio of triple quadrupole mass spectrometers. Address current and future challenges with enhanced acquisition speed, sensitivity, and operational simplicity—all combined with proven robustness and reliability. Easy calibration, intuitive software, and predefined method templates ensure users of all skill levels obtain high-confidence results.

Thermo Scientific™ Vanquish™ Horizon UHPLC system and Thermo Scientific™ TSQ Altis™ Plus triple quadrupole mass spectrometer

Thermo Scientific™ Vanquish™ Flex UHPLC system and Thermo Scientific™ TSQ Quantis™ Plus triple quadrupole mass spectrometer

Thermo Scientific™ Vanquish™ Core HPLC system and Thermo Scientific™ TSQ Fortis™ Plus triple quadrupole mass spectrometer

Find performance at

thermofisher.com/AdvancedQuantitation

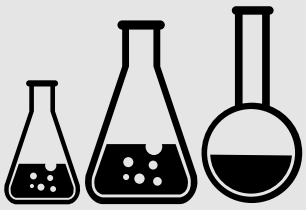
Scan for more information

ThermoFisher SCIENTIFIC

BIO BASIC ASIA PACIFIC

SERVICES

- DNA Sanger Sequencing Service
- Next Generation Sequencing
- Gene Synthesis
- Peptide Synthesis
- Antibody Production

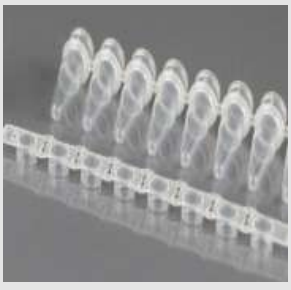


PRODUCTS

- Molecular Biology Reagents & Kits
- Culture Media
- Buffers
- Biochemicals
- Labware (Pipette tips, PCR consumables, Filtration etc.)





Bio Basic Asia Pacific Pte Ltd was established in Singapore in 2016, committed to offer a variety of directly-manufactured life science products and lab services in the areas of Life Sciences and Molecular Biology. Bio Basic Asia Pacific is greatly recognized for providing DNA Sanger Sequencing Service with a rapid turn-around time at a competitive price and local laboratories facility in Singapore. We are a one-stop solution with a broad portfolio of molecular biology products and consumables with the focus on dependability and affordability.

http://biobasic-asia.com/

The organisation of Frontiers in Peptide Science and Drug Discovery Symposium 2022 is brought to you generously by the following sponsors:

Medicilon

恒瑞医药 HENGRUI MEDICINE

GenScript Make Research Easy

ThermoFisher SCIENTIFIC

BIO BASIC ASIA PACIFIC

and supported by:

P²S² Peptides and Proteins Society (Singapore)

The Japanese Peptide Society