4th RSC-CSJ Joint Symposium 2013

Chemical Biology Research by Young Investigators

March 24, 2013, the 93rd CSJ Annual Meeting
Ritsumeikan University
Biwako-Kusatsu Campus, Shiga, JAPAN

RSC | Advancing the Chemical Sciences

Royal Society of Chemistry & Chemical Society of Japan

4th RSC – CSJ Joint Symposium 2013

—Chemical Biology Research by Young Investigators—

- Date: March 24th (SUN) 9:00-17:40
- Venue: Room S8 (CO-LEARNING HOUSE I C306), Ritsumeikan University, Biwako-kusatsu Campus(Shiga, Japan)
- Hosted by The Chemical Society of Japan
- Co-hosted by Royal Society of Chemistry

☐Chair: Kazuya Kikuchi

09:10-09:20 Opening Remarks from CSJ

09:20-09:30 Opening Remarks from UK

☐Chair: Hiroshi Murakami

L-1 09:30-10:10 **Gregory L. CHALLIS** (University of Warwick) page 1

"*Elucidating and Exploiting Novel Biosynthetic C–H Functionalization Reactions*"

☐Chair: Gregory L. Challis

L-2 10:10-10:50 **Hiroshi MURAKAMI** (The University of Tokyo) page 3

"*Development of Non-standard Peptide Inhibitors Using TRAP Display*"

10:50-11:20 Intermission

☐Chair: Kazushi Kinbara

L3 11:20-12:00 **Dominic J. CAMPOPIANO** (University of Edinburgh) page 5

"*Sphingolipid Biosynthesis in Man and Microbes*"

☐Chair: Dominic Campopiano

L-4 12:00-12:40 **Kazushi KINBARA** (Tohoku University) page 7

"*Development of Supramolecular Tools for Manipulation of Biological Molecules*"

12:40-13:40 Lunch Time

☐Chair: Stuart J. Conway

L-5 13:40-14:20 **Motonari UESUGI** (Kyoto University) page 9

"*Small Molecule Tools for Cell Biology and Cell Therapy*"

☐Chair: Motonari Uesugi

L-6 14:20-15:00 **Stuart J. CONWAY** (University of Oxford) page 11

"*Cracking the Histone Code: Inhibitors of the Bromodomain-Acetyl–Lysine Interaction*"

15:00-15:30 Intermission

☐Chair: Rebecca GOSS

L-7 15:30-16:10 **Masayuki INOUE** (The University of Tokyo) page 13

"*Total Synthesis and Biological Evaluation of Polytheonamide B*"

☐Chair: Masayuki Inoue

L-8 16:10-16:50 **Rebecca GOSS** (University of St Andrews) page 15

"*Elucidating and Exploiting Biosynthesis*"

☐Chair: Rebecca Goss

L-9 16:50-17:30 **Kazuya KIKUCHI** (Osaka University) page 17

"*Design, Synthesis and Biological Application of in Vivo Imaging Probes with Tunable Chemical Switches*"

17:30-17:40 Closing Remarks

Welcome Address

Dear Colleagues,

Welcome to the 4th RSC-CSJ Joint Symposium 2013 −Chemical Biology Research by Young Investigators−, to be held on March 24, 2013 at the same venue as the CSJ 93rd Annual Meeting; Ritsumeikan University, Biwako-Kusatsu Campus, Shiga, located by the shore of Lake Biwa, under the co-sponsorship of the Chemical Society of Japan (CSJ) and the Royal Society of Chemistry (RSC). This series of bilateral symposium originates from the first one in 2007 held in Osaka University, followed by the second in 2008 at Queen's University and the third in 2010 at the RSC Burlington House where we signed an International Cooperation Agreement between RSC and CSJ.

There are two features to be mentioned for the present symposium.

Firstly, this 4th Joint Symposium on Chemical Biology Research was originally planned to be held at the CSJ 91st Annual Meeting late March 2011 at Kanagawa University, but unfortunately it was forced to be cancelled by the March 11 Disasters, earthquakes and nuclear power plant accidents. The original plan has now revived to be held at this 93rd Annual Meeting after one-year blank. I would like to thank the symposium chair Prof. Kazuya Kikuchi, and RSC Chief Executive Officer Dr. Robert Parker as well as CSJ Executive Director Mr. Nobuyuki Kawashima, and also Dr. Hirofumi Seike, Representative at the RSC Tokyo Office, for their great efforts to make this symposium possible.

Secondly, this year 2013 marks The 150 Year Anniversary of UK-Japan Academic Interaction, since five young nobles from Choshu clan, so-called Chosu-Five, including Ito Hirobumi and Inoue Kaoru, visited UK in 1863 to study at University College London (UCL). Furthermore, 19 students from Satsuma clan visited UCL in 1865 and Joji Sakurai, now known as the Father of Chemistry in Japan, became a student at UCL in 1876. They were all nurtured mainly by the famous chemist Prof. Alexander Williamson, President of the London Chemical Society (a predecessor of RSC). So, it is a great pleasure for us to hold the UK/Japan Joint Symposium in this special year, by thanking all the young talents from Japan and many scientists from UK for their pioneering contribution to establish the strong bridge between two countries UK and Japan, and between RSC and CSJ. I really hope that this joint symposium will further strengthen the bridge for our scientific cooperation.

For this symposium nine distinguished chemists in the area of chemical biology, four from UK and five from Japan, will present their research results and exchange ideas for further development. Chemical biology is a basic science for both "life innovation" and "green innovation", which have been claimed as the science and technology policy for creation of sustainable society. I really hope that this bilateral symposium will contribute greatly not only to the rapidly developing chemical biology field but also a lot of related fields. It is also my great expectation that all of the attendants strengthen your friendship and find new friends throughout the symposium for further collaborations.

Kohei Tamao

President
The Chemical Society of Japan

Welcome Address

Dear colleagues,

The Royal Society of Chemistry (RSC) is delighted to be co-organising with the Chemical Society of Japan (CSJ) this RSC-CSJ Joint Symposium on Chemical Biology. We would like to thank Professors Kazuya Kikuchi and Nobuyuki Kawashima for all their help and support to make this event possible. This symposium is one of the activities jointly organised following the signing in 2010 of an International Cooperation Agreement between the RSC and the CSJ. We hope it will be another successful and memorable CSJ-RSC activity that will facilitate and foster future collaborations between the societies and scientists in both countries.

The RSC is a learned society, concerned with advancing the chemical sciences, developing its applications, and disseminating chemical knowledge. It is also a professional body that maintains professional qualifications and sets high standards of competence and conduct for professional chemists. We have 48,000 members worldwide drawn from all areas of the chemical sciences, including chemical biology. Our Chemical Biology Interface Division aims to improve interdisciplinary research at the chemistry-biology interface, and promote the importance of this area to the government, industry, academia and education. In addition to developing young talents and advancing the chemical sciences both in the UK and internationally, the RSC is a major publisher of research journals, magazines, databases and books that cover all areas of the chemical sciences.

The RSC is also pleased to have the opportunity to work more closely with the Japanese chemical science community through Dr Hirofumi Seike, our Representative for Japan, who is based full-time in our office in Tokyo. This office is located in the CSJ building in Tokyo and we are very grateful to the CSJ for all their help and advice in establishing this office. Dr. Seike is happy to answer your queries about any aspects of the RSC including its activities in Japan, its membership services and publishing activities.

Each of the speakers in this symposium is an international expert in the area of chemical biology, which underpins the development of new medicine and the understanding of biological processes. We hope that the presentations will stimulate the exchange of ideas and experiences between all participants. We thank each of the speakers and all the participants for their contributions to this symposium.

I offer a very warm welcome to what promises to be an exciting scientific event. We hope that the symposium will provide a springboard for future activities and that it will foster new research collaborations. We look forward to continuing our close partnership with CSJ, with the possibility of more joint CSJ-RSC activities in the future.

Dr Robert Parker
Chief Executive Officer
Royal Society of Chemistry

RSC | Advancing the Chemical Sciences

Gregory CHALLIS

Department of Chemistry,
University of Warwick

➢ Educational Background

- B. Sc. 1994, Imperial College London
- Ph. D. 1998, the University of Oxford
  (advisor: Sir Jack Baldwin FRS)
- Postdoctoral Fellow, 1998-2000, Johns Hopkins University
  (advisor: Craig Townsend)
- Postdoctoral Fellow, 2000-2001, John Innes Centre
  (advisor: Keith Chater FRS)
- Lecturer (Assistant Professor), 2001-2003, University of Warwick
- Senior Lecturer (Associate Professor), 2003-2006, University of Warwick
- Professor (2006)-present, University of Warwick

➢ Scientific Interests

Natural products chemistry and biology, including isolation and structure determination of new bioactive natural products, genomics and genetics of natural product biosynthesis, enzymology of natural product biosynthesis, chemical synthesis of bioactive natural products and intermediates in their biosynthesis, genetic manipulation of bioactive natural product biosynthesis pathways to produce new analogues, molecular mechanism of action of bioactive natural products and their biological function. A highly multidisciplinary approach to these problems is taken encompassing high field NMR spectoscopy, mass spectrometry, HPLC, organic synthesis, bioinformatics, microbiological methods, molecular genetic manipulation, recombinant protein overproduction and purification, and enzyme assay development.

➢ Recent Papers

1. "Cytochrome P450–Catalyzed L-Tryptophan Nitration in Thaxtomin Phytotoxin Biosynthesis" S. M. Barry, J. A. Kers, E. G. Johnson, L. Song, P. R. Aston, B. Patel, S. B. Krasnoff, B. R. Crane, D. M. Gibson, R. Loria, G. L. Challis, *Nat. Chem. Biol*. **2012**, *8*, 814-816.

2. "Regio- and Stereodivergent Antibiotic Oxidative Carbocyclizations Catalyzed by Rieske Oxygenase-Like Enzymes" P. K. Sydor, S. M. Barry, O. M. Odulate, F. Barona-Gomez, S. W. Haynes, C. Corre, L. Song, G. L. Challis, *Nat. Chem*. **2011**, *3*, 388-392.

3. "Identification of a Bioactive 51-Membered Macrolide Complex by Activation of a Silent Polyketide Synthase in *Streptomyces ambofaciens*" L. Laureti, L. Song, S. Huang, C. Corre, P. Leblond, G. L. Challis, B. Aigle, *Proc. Natl. Acad. Sci. USA*, **2011**, *108*, 6258-6263.

4. "AcsD Catalyzes Enantioselective Citrate Desymmetrization in Siderophore Biosynthesis" S. Schmelz, N. Kadi, S. A. McMahon, L. Song, D. Oves-Costales, M. Oke, H. Liu, K. A. Johnson, L. G. Carter, C. H. Botting, M. F. White, G. L. Challis, J. H. Naismith, *Nat. Chem. Biol*. **2009**, *5*, 174-182.

5. "2-Alkyl-4-hydroxymethylfuran-3-carboxylic Acids, Antibiotic Production Inducers Discovered by *Streptomyces coelicolor* Genome Mining" C. Corre, L. Song, S. O'Rourke, K. F. Chater, G. L. Challis, *Proc. Natl. Acad. Sci. USA*. **2008**, *105*, 17510-17515.

6. "A New Family of ATP-dependent Oligomerization–Macrocyclization Biocatalysts" N. Kadi, D. Oves-Costales, F. Barona-Gomez, G. L. Challis, *Nat. Chem. Biol.* **2007**, *3*, 652-656.

7. "Discovery of a New Peptide Natural Product by *Streptomyces coelicolor* Genome Mining" S. Lautru, R. J. Deeth, L. M. Bailey, G. L. Challis. *Nat. Chem. Biol*. **2005**, *1*, 265-269.

L-1

Elucidating and Exploiting Novel Biosynthetic C–H Functionalization Reactions

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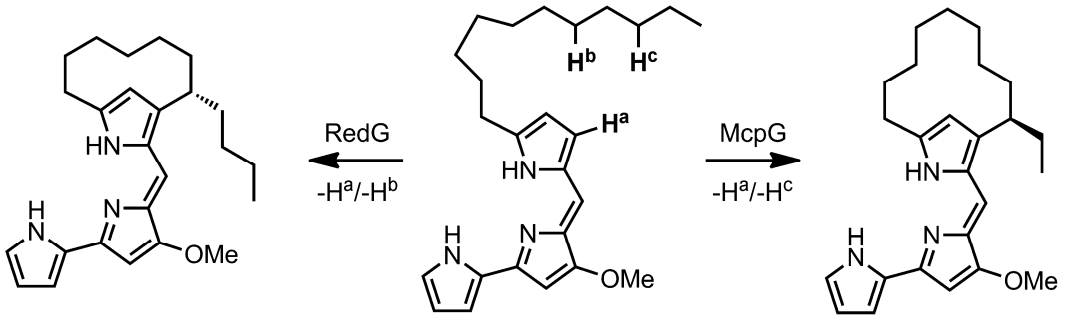
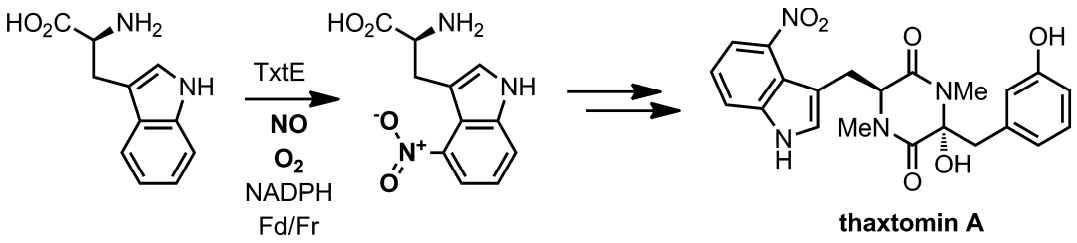
C–H functionalization reactions are key steps in the biosynthesis of numerous bioactive natural products. Such reactions include specific hydroxylation, chlorination and desaturation of unactivated carbon atoms, as well as a variety of oxidative cyclization reactions, exemplified by the conversion of the tripeptide ACV to the bicyclic penicillin nucleus.

Here we report two unprecedented types of enzyme-catalyzed C–H functionalization reaction in natural product biosynthesis. RedG and McpG, novel Rieske non-heme iron dependent oxygenase-like enzymes, catalyse the regio- and stereodivergent oxidative carbocyclization of undecylprodigiosin to streptorubin B and metacycloprodigiosin, respectively, and TxtE, a unique cytochrome P450, catalyzes regiospecific nitration of L-tryptophan, the first committed step in thaxtomin phytotoxin biosynthesis. Efforts to elucidate the catalytic mechanisms of these enzymes and to exploit them for the production of novel streptorubin B and nitrotryptophan derivatives will be described.



streptorubin B **undecylprodigiosin** **metacycloprodigiosin**



thaxtomin A

Hiroshi MURAKAMI

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Graduate School of Arts and Sciences,
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➢ Educational Background

- B.S. 1995, Okayama University
- Ph.D. 2000, Okayama University (Prof. Masahiko Sisido)
- Postdoctoral Fellow, 2000-2001, Okayama University
- Postdoctoral Fellow, 2001-2003, State University of New York at Buffalo (Prof. Hiroaki Suga)
- Assistant Professor, 2003-2009 The University of Tokyo (Prof. Hiroaki Suga)
- Associate Professor, 2009-, The University of Tokyo (Principal Investigator)

➢ Scientific Interests

Chemical Biology and Biomolecular Engineering: Directed evolution of new functional biomolecules that are useful for biological and therapeutic applications.

➢ Recent Papers

1. "Reevaluation of the D-Amino Acid Compatibility with the Elongation Event in Translation" Fujino, T.; Goto, Y.; Suga, H.; Murakami, H.*, *J. Am. Chem. Soc.* **2013**, *135*, (5), 1830-7

2. "Genetically Encoded Libraries of Nonstandard Peptides" Kawakami, T.; Murakami, H.*, *J. Nucl. Acids* **2012**, 713510.

3. "Bases in the Anticodon Loop of tRNA(Ala)(GGC) Prevent Misreading" Murakami, H.*; Ohta, A.; Suga, H.*, ***Nat. Struct. Mol. Biol.*** **2009**, *16*, (4), 353-8.

4. "Diverse Backbone-cyclized Peptides via Codon Reprogramming" Kawakami, T.; Ohta, A.; Ohuchi, M.; Ashigai, H.; Murakami, H.; Suga, H.*, *Nat. Chem. Biol.* **2009**, *5*, (12), 888-90.

5. "Structural Basis of Specific tRNA Aminoacylation by a Small In Vitro Selected Ribozyme" Xiao, H.; Murakami, H.; Suga, H.; Ferre-D'Amare, A. R.*, *Nature* **2008**, *454*, (7202), 358-61.

6. "Ribosomal Synthesis of Bicyclic Peptides via Two Orthogonal Inter-side-chain Reactions" Sako, Y.; Morimoto, J.; Murakami, H.; Suga, H.*, *J. Am. Chem. Soc.* **2008**, *130*, (23), 7232-4.

7. "Messenger RNA-programmed Incorporation of Multiple *N*-Methyl-amino Acids into Linear and Cyclic Peptides" Kawakami, T.; Murakami, H.; Suga, H.*, *Chem. Biol.* **2008**, *15*, (1), 32-42.

8. "Reprogramming the Translation Initiation for the Synthesis of Physiologically Stable Cyclic Peptides" Goto, Y.; Ohta, A.; Sako, Y.; Yamagishi, Y.; Murakami, H.; Suga, H.*, *ACS Chem. Biol.* **2008**, *3*, (2), 120.

9. "A Highly Flexible tRNA Acylation Method for Non-natural Polypeptide Synthesis "Murakami, H.; Ohta, A.; Ashigai, H.; Suga, H.*, *Nat. Methods* 20 06, **3**, (5), 357-9.

L-2

Development of Non-standard Peptide Inhibitors Using TRAP Display

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In vitro selection using a peptide library linked with its genotype (DNA/RNA) are powerful tools to discover novel functional peptides. Compared with high-throughput screening of chemically synthesized peptide libraries, the selection methods can deal with much larger libraries of up to 10^{13} peptides, which dramatically increases the probability of getting peptides with desired properties.

To apply this methodology for a non-standard peptide that contains non-proteinogenic structure for stabilizing the peptide in *in vivo*, we developed a cell-free translation system whose genetic code was reprogrammed to introduce non-proteinogenic amino acids. Using the translation system, we studied the compatibility of many types of non-proteinogenic amino acids including *N*-methyl amino acids, D-amino acids, *β*-amino acids with the translation. We found that the compatibility of these amino acids was strongly depended on the structure of the side chain. For example, D-amino acids with non-bulky alkyl or aromatic side chains (Ala, Ser, Cys, Met, Thr, His, Phe, and Tyr) showed the incorporation efficiencies comparable with the corresponding L-isomer, and these with uncharged hydrophilic or alkyl side chains (Asn, Gln, Val, and Leu) showed decreased incorporation efficiencies. The D-amino acids with other property (Arg, Lys, Asp, Glu, Ile, Trp, and Pro) were not incorporated into a peptide.

Concurrently with the above screening of non-proteinogenic amino acids, we also developed a new *in vitro* display method, TRAP (Transcription–translation coupled with Association of Puromycin-linker) display method, that enables high-speed *in vitro* selection of functional peptides, peptidomimetics via a simple procedure. Using TRAP display, macrocyclic non-standard peptides that bind to human serum albumin were selected. Six rounds of selection using TRAP display were performed only in approximately 14 h. We have also applied TRAP display for the *in vitro* selection of macrocyclic non-standard peptides against vascular endothelial growth factor receptor 2 (VEGFR2). The eight macrocyclic-peptide libraries consisted of combinations of four variants of amino acid linkers for cyclization and two versions of elongator amino acid compositions, including four backbone-modified non-proteinogenic amino acids. Affinity selection from these libraries, yielded multiple anti-VEGFR2 macrocyclic peptide leads. By further screening of the chemically synthesized lead peptides for antagonizing activity, we identified a potent macrocyclic peptide that inhibited VEGF-induced VEGFR2 autophosphorylation, proliferation and angiogenesis of vascular endothelial cells.

Through the combination of the codon reassignment and the TRAP display enables high-speed selection of functional non-standard peptides, it will facilitate the generation of various new non-standard peptides that are useful for biological and therapeutic applications.

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➢ Educational Background

- B. Sc. 1988, University of Glasgow
- Ph. D. 1994, University of Edinburgh
  (advisor: Robert L. Baxter)
- Postdoctoral Fellow, 1992-1995, University of Leicester, UK
  (advisor: William V. Shaw)
- Postdoctoral Fellow, 1995-1996, University of Edinburgh, UK
  (advisor: Robert Baxter)
- Lecturer 1997-2007, University of Edinburgh
- Senior Lecturer, 2007-present, University of Edinburgh
- Royal Society of Edinburgh/Scottish Executive Research Fellow, 2006.

➢ Scientific Interests

Chemical Biology: Natural product biosynthesis, enzyme mechanism, protein structure, host-pathogen interactions, dynamic covalent chemistry, biocatalysis, directed evolution.

➢ Recent Papers

1. "An Improved Racemase/Acylase Biotransformation for the Preparation of Enantiomerically Pure Amino Acids" Baxter, S., Royer, S., Grogan, G., Brown, F., Holt-Tiffin, K. E., Taylor, I. N., Fotheringham, I. G. , Campopiano, D. J., *J. Am. Chem. Soc.* **2012**, *134*, 19310-19313.

2. "Role of a Conserved Arginine Residue during Catalysis in Serine Palmitoyltransferase" Lowther, J., Charmier, G., Raman, M. C., Ikushiro, H., Hayashi, H., Campopiano, D. J., *FEBS Lett* **2011**, *585*, 1729-1734.

3. "Structural, Mechanistic and Regulatory Studies of Serine Palmitoyltransferase" Lowther, J., Naismith, J. H., Dunn, T. M., Campopiano, D. J., *Biochem. Soc. Trans.* **2012**, *40*, 547-554.

4. "The Serine Palmitoyltranserase *from Sphingomonas wittichii* RW1: An Interesting Link to an Unusual Acyl Carrier Protein" Raman, M. C. C., Johnson, K. A., Clarke, D. J., Naismtih, J. H., Campopiano, D. J. *Biopolymers, Special Issue on Natural Products* **2010**, *93,* 811-822.

5. "Serine Palmitoyltranserase Displays a Novel Mechanism of Cycloserine Inhibition" Lowther, J., McMahon, S., Johnson, K. A., Yard, B. A., Carter, L. G Raman, M. C., Naismith, J. H., Campopiano, D. J., *Molecular Biosystems, Emerging Investigators*, *Special Issue* **2010**, *6*, 1682-1693.

6. "Nucleophilic Catalysis of Acylhydrazone Equilibration for Protein-directed Dynamic Covalent Chemistry" Bhat, V. T., Caniard, A. M., Luksch, T., Brenk, R., Campopiano, D. J.,* Greaney, M. F.*, *Nat. Chem.* **2010**,*2,* 490-497.

7. "Insights into How Nucleotide-binding Domains Power ABC Transport" Newstead, S., Fowler, P. W., Bilton, P., Carpenter, E. P., Sadler, P. J., Campopiano, D. J.*, Sansom, M. S. P.*, Iwata, S.*, *Structure* **2009**, *17*, 1213-1222.

8. "The External-aldimine form Of Serine Palmitoyltranserase; Structural, Kinetic, and Spectroscopic Analysis of athe Wild-Type Enzyme and HSAN1 Mutant Mimics" Raman, M. C., Johnson, K. A., Yard, B. A., Lowther, J., Carter, L. G., Naismith, J. H.*, Campopiano, D. J.*, *J. Biol. Chem.* **2009**, *284,* 17328-17339.

9. "The Structure of Serine Palmitoyltransferase; Gateway to Sphingolipid Biosynthesis" Yard, B. A., Carter, L. G., Johnson, K. A., McMahon, S., Dorward, M., Liu, H., Oke, M., Overton, I., Barton, G. J., Naismith, J. H., Campopiano, D. J., *J. Mol. Biol.* **2007**, *370,* 870-886.

L-3

Sphingolipid Biosynthesis in Man and Microbes

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Sphingolipids (SLs) are essential components of all eukaryotic and many prokaryotic membranes where they play essential structural roles. SLs are long chain bases (lcbs) composed of fatty acids and a polar head group (derived from L-serine). In recent years SLs have also been shown to act as potent signaling molecules that participate in various cellular functions. For example, sphingosine 1-phosphate is a potent signal molecule that it known to have five G-protein coupled receptors (GPCRs) in the nuclear membrane. Errors in sphingolipid metabolism have been implicated in a number of inflammatory diseases and cancer. Recent efforts by a large consortium (Lipidmaps) have sought to inventory the large, diverse and chemically-complex family of SLs using high resolution structural techniques such as mass spectrometry. With this catalogue in hand it is now possible to study the regulation of SL biosynthesis in healthy and diseased cells and provide a systems biology roadmap of SL metabolism. It is hoped that our understanding may lead to the development of therapeutic agents specifically directed at targets from SL pathways.

We have been studying SL biosynthesis with a goal to increase our understanding of how each enzyme in the pathway catalyses a particular reaction and how it is regulated. Later stages of SL biosynthesis are species-specific but all core SLs are synthesized from the condensation of L-serine and a long-chain fatty acid thioester such as palmitoyl-CoA (C16). This is catalysed by the enzyme serine palmitoyl transferase (SPT). SPT is a pyridoxal 5' phosphate (PLP)-dependent enzyme that catalyses the formation of the alpha-oxoamine product, 3-ketodihydrosphingosine (KDS) through a decarboxylative, Claisen-like condensation reaction. Eukaryotic SPTs are membrane-bound, heterodimeric enzymes encoded by two genes (lcb1, lcb2) whereas bacterial enzymes are cytoplasmic homodimers encoded by a single gene. Due to the technical problems associated with studying the mammalian enzymes we have used bacterial SPTs (from Sphingomonas strains) as models to study their structure and mechanism using various spectroscopic methods including x-ray crystallography. We found the PLP-binding site at the dimer interface of the bacterial SPT homodimer. Mutations in human SPT cause hereditary sensory autonomic neuropathy type 1 (HSAN), a rare disease characterized by loss of feeling in extremities and severe pain. The molecular basis of how these mutations perturb SPT activity is subtle and is not simply loss of activity. It is thought that the mutant SPT is promiscuous and generates toxic sphingolipids. We used our bacterial SPT structure to mimic the human HSAN1 mutants and found that mutations on one subunit impact on the enzyme activity and structure of the dimer. We have also explored the nature of SPT inhibition using both enantiomers of the drug cycloserine. We have begun to investigate the complete biosynthetic pathway using various Sphingomonas species as model organisms. We are using knowledge gained from these studies to probe the more complex mammalian systems.

Kazushi KINBARA

Institute of Multidisciplinary Research for Advanced Materials, Tohoku University

➢ Educational Background

- B. S. 1991, University of Tokyo
- Ph. D. 1996, University of Tokyo (advisor: Kazuhiko Saigo)
- Research Associate, 1996-2001, The University of Tokyo
- Lecturer, 2001-2006, The University of Tokyo
- Associate Professor, 2006-2008, The University of Tokyo
- Professor, 2008-Present, Tohoku University

➢ Scientific Interests

- Development of biomimetic molecules
- Supramolecular chemistry of macromolecules
- Protein engineering

➢ Recent Papers

1. "Ion Permeation by a Folded Multiblock Amphiphilic Oligomer Achieved by Hierarchical Construction of Self-assembled Nanopores" T. Muraoka, T. Shima, T. Hamada, M. Morita, M. Takagi, K. V. Tabata, H. Noji, K. Kinbara, *J. Am. Chem. Soc.* **2012**, *134,* 19788–19794.
2. "Application of Photoactive Yellow Protein as a Photoresponsive Module for Controlling Hemolytic Activity of Staphylococcal α-Hemolysin" M. Ui, Y. Tanaka, Y. Araki, T. Wada, T. Takei, K. Tsumoto, K. Kinbara, *Chem. Commun.* **2012**, *48,* 4737–4739.
3. "Mimicking Multipass Transmembrane Proteins: Synthesis, Assembly and Folding of Alternating Amphiphilic Multiblock Molecules in Liposomal Membranes" T. Muraoka, T. Shima, T. Hamada, M. Morita, M. Takagi, K. Kinbara, *Chem. Commun.* **2011**, *47*, 194–196.
4. "Adhesion Effects of a Guanidinium Ion Appended Dendritic 'Molecular Glue' on the ATP-driven Sliding Motion of Actomyosin" K. Okuro, K. Kinbara, K. Takeda, Y. Inoue, A. Ishijima, T. Aida, *Angew. Chem., Int. Ed.* **2010**, *49,* 3030–3033.
5. "Shape-directed Assembly of a 'Macromolecular Barb' into Nanofibers: Stereospecific Cyclopolymerization of Isopropylidene Diallylmalonate" Y. Miyamura, K. Kinbara, Y. Yamamoto, V. K. Praveen, K. Kato, M. Takata, A. Takano, Y. Matsushita, E. Lee, M. Lee, T. Aida, *J. Am. Chem. Soc.* **2010**, *132*, 3292–3294.
6. "A Tubular Biocontainer: Metal Ion-induced 1D Assembly of a Molecularly Engineered Chaperonin" S. Biswas, K. Kinbara, N. Oya, N. Ishii, H. Taguchi, T. Aida, *J. Am. Chem. Soc.* **2009**, *131*, 7556–7557.
7. "Molecular Glues Carrying Multiple Guanidinium Ion Pendants via an Oligoether Spacer: Stabilization of Microtubules against Depolymerization" K. Okuro, K. Kinbara, K. Tsumoto, N. Ishii, T. Aida, *J. Am. Chem. Soc.* **2009**, *131*, 1626–1627.
8. "Toward Long-distance Mechanical Communication: Studies on a Ternary Complex Interconnected by a Bridging Rotary Module" H. Kai, S. Nara, K. Kinbara, T. Aida, *J. Am. Chem. Soc.* **2008**, *130,* 6725–6727.

L-4

Development of Supramolecular Tools for Manipulation of Biological Molecules

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Engineered macromolecules capable of interfacing biological events have become more important in the field of nanobiotechnology. In particular, development of molecular tools that are able to control function of proteins is a hot issue in both materials science and nanomedicine. For example, polymerization/depolymerization event of microtubules, tubular assemblies of α/β−tubulin heterodimers, play essential roles in mitosis. In connection with this, paclitaxel, which stabilizes microtubules against depolymerization, has been applied for anticancer drugs.

We have developed 'molecular glue' which is capable of non-covalent attachment to various wild-type proteins via a multivalent salt-bridge formation between guanidinium (Gu^+) ions and oxyanionic groups that exist ubiquitously in proteins. Gu^+ is known to strongly associate with several oxyanion functionalities such as carboxylate and phosphate groups by electrostatic and hydrogen bonding interactions. Various biomolecules such as proteins, lipids, and nucleic acids have anionic functionalities, and therefore can be hybridized with Gu^+ groups. Actually, we have found that the molecular glues efficiently stabilize protein assemblies such as microtubules (MTs) and actomyosin, and prevent them from dissociation into subunits.

On the other hand, we have focused on polyethylene glycol (PEG), which is a linear polymer widely used for industrial materials to medicines. Nonionic, water-soluble and nontoxic characters of PEG are particularly useful for pharmaceutical applications. Covalent attachment of PEG chains to a target molecule such as a drug or a therapeutic protein, is know as "PEGylation", that inhibits protein aggregation and prolongation of drugs' circulatory time. However, it is also known that linear PEG chains tend to cover the surface of proteins to cause serious deactivation of their functions. Here we propose novel molecules composed of ethylene oxide skeleton, as "structuralized" PEG molecules, that adopt rigid 2D or 3D structures while holding the merits of PEG. As the first target, we designed triangle molecules composed of tetraethylene glycol as the linker unit and pentaerythritol as the vertex unit, and found that they have characteristic properties compared with the linear PEG molecules.

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➢ Educational Background

- B.S. 1990, Kyoto University
- Ph.D. 1995, Kyoto University (advisor: Yukio Sugiura)
- Postdoctoral Fellow, 1995-1998, Harvard University (advisor: Gregory L. Verdine)
- Assistant Professor, 1998-2005, Baylor College of Medicine
- Associate Professor (tenured), 2005-2009, Baylor College of Medicine
- Professor, 2005-, Kyoto University

➢ Scientific Interests

- Chemical Biology: Discovery, design, synthesis, and biological use of small organic molecules with unique biological activity

➢ Recent Papers

1. "A Small Molecule that Promotes Cardiac Differentiation of Human Pluripotent Stem Cells under Defined Cytokine- and Xeno-free Conditions" Minami, I., et al. *Cell Reports* in press, **2012**.

2. "Synthesis and Evaluation of Diarylthiazole Derivatives that Inhibit Activation of Sterol Regulatory Element-binding Proteins" Kamisuki, S., et al. *J. Med. Chem.* **2011**, 54(13), 4923-4927.

3. "A Mitochondrial Surface-specific Fluorescent Probe Activated by Bioconversion" Kawazoe, Y., Shimogawa, H., Sato, A., Uesugi, M. *Angew. Chem. Int. Ed.* **2011**, 50(24), 5478-81.

4. "Cell-morphology Profiling of a Natural Product Library Identifies Bisebromoamide and Miuraenamide A as Actin-filament Stabilizers" Sumiya, E., et al. *ACS Chem. Biol.* **2011**, 6(5), 425-31.

5. "Deactivation of STAT6 through Serine 707 Phosphorylation by JNK" Shirakawa, T. Kawazoe, Y., Tsujikawa, T., Jung, D., Sato, S., Uesugi, M. *J. Biol. Chem.* **2011**, 286, 4003-4010.

6. "Marine Natural Product Aurilide Activates the OPA1-Mediated Apoptosis by Binding to Prohibitin" Sato, S., et al. *Chem. Biol.* **2011**, 18 (1), 131-139.

7. "A Small Molecule that Blocks Fat Synthesis by Inhibiting the Activation of SREBP" Kamisuki, S., et al. *Chem. Biol.* **2009**, 16 (8), 882-892.

8. "A Dumbbell-shaped Small Molecule that Promotes Cell Adhesion and Growth" Yamazoe, S., Shimogawa, H., Sato, S., Esko, J. D., Uesugi, M. *Chem. Biol.* **2009**, 16 (7), 773-782.

9. "Wrenchnolol Derivative Optimized for Gene Activation in Cells" Jung, D., Shimogawa, H., Kwon, Y., Mao, Q., Sato, S., Kamisuki, S., Kigoshi, H., Uesugi, M. *J. Am. Chem. Soc.* **2009**, 131(13), 4774-4782.

10. "Polyproline-rod Approach to Isolating Protein Targets of Bioactive Small Molecules: Isolation of a New Target of Indomethacin" Sato, S., et al. *J. Am. Chem. Soc.* **2007**, 129(4), 873-880.

11. "Small Molecule Transcription Factor Mimic" Kwon, Y., Arndt, H., Mao, Q., Choi, Y., Kawazoe, Y., Dervan, P. B., Uesugi, M. *J. Am. Chem. Soc.* **2004**, 126, 15940-15941.

L-5

Small Molecule Tools for Cell Biology and Cell Therapy

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In human history, bioactive small molecules have had three primary uses: as medicines, agrochemicals, and biological tools. Among them, what our laboratory has done in the past was the discovery and use of biological tools. Our laboratory has been discovering and designing small organic molecules with unique activities to them as tools for biological investigation and manipulation.

In addition to tool discovery, our laboratory has recently become interested in exploring another application of small molecules: small molecule tools for cell therapy. Although small molecule drugs will continue to be important, cell therapy will be a powerful approach to curing difficult diseases that small molecule drugs are unable to handle. However, there are a number of potential problems in bringing cell therapy technologies to the clinic, including high cost, potential contamination, low stability, and tumorigenesis. Stable, completely defined small molecule tools, which are usually amenable to cost-effective mass production, may be able to help the clinical use of cell therapy.

Through screening chemical libraries, we have been discovering unique synthetic molecules that modulate or detect fundamental characteristics of human cells useful for cell therapy. Some of such molecules may serve as tools for cell engineering or cell therapy as well as basic cell biological research. This presentation provides a quick overview of our recent research programs with a special emphasis on the discovery and utilization of "adhesamine." This dumbbell-shaped synthetic molecule enhanced attachment and growth of cells by binding to heparan sulfate on cell membrane and thereby clustering syndecan. Using this molecule as a lead, we were able to design small synthetic molecules with fibronectin-like properties, which boost culture, expansion, and transplantation of clinically useful cells.

Other small-molecule tools we newly discovered may be discussed in the presentation as well.

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➢ Educational Background

- B.Sc. 1997, the University of Warwick
- Ph.D. 1997-2000, the University of Bristol
  (advisor: Prof. Jeff Watkins FRS and Dr. David Jane)
- Postdoctoral Fellow, 2001-2003, the University of Cambridge
  (advisor: Prof. Andrew Holmes FRS)
- Lecturer, 2003-2008, the University of St Andrews
- University Lecturer 2008-present, the University of Oxford
- Tutorial Fellow 2008-present, St Hugh's College, Oxford

➢ Scientific Interests

- Chemical Biology: Discovery, design, synthesis, and biological use of small organic molecules with unique biological activity

➢ Recent Papers

1. "Wavelength-orthogonal Photolysis of Neurotransmitters in Vitro" Stanton-Humphreys et al. *Chem. Commun*. **2012**, *48*, 657-659.
2. "3,5-Dimethylisoxazoles Act as Acetyl–Lysine-mimetic Bromodomain Ligands" Hewings et al., *J. Med. Chem.* **2011**, *54*, 6761-6770. (*Highlighted in Faculty 1000*).
3. "Development of Inositol-based Antagonists for the D-*myo*-Inositol 1,4,5-Trisphosphate Receptor" Keddie et al., *Chem. Commun*. **2011**, *47*, 242-244.
4. "Mechanism of Ligand-gated Potassium Efflux in Bacterial Pathogens" Roosild et al., *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 19784-19789.
5. "The Synthesis and Biological Action of Novel 4-Position-modified Derivatives of D-*myo*-Inositol 1,4,5-Trisphosphate" Bello et al., *J. Org. Chem.* **2007**, *72*, 5647-5659.

L-6

Cracking the Histone Code: Inhibitors of the Bromodomain–Acetyl–Lysine Interaction

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Lysine acetylation is a key dynamic protein post-translational modification (PTM). Histones, the core proteins around which DNA is wound, are susceptible to such PTMs (Figure 1A), combinations of which are proposed to form a "histone code" that is involved in regulating gene expression (Figure 1B).[1] Lysine acetylation influences recruitment of transcriptional regulators through interaction with bromodomain-containing proteins (BCPs), which 'read' lysine acetylation state (Figure 1C-E). There are 61 bromodomains, found within 46 proteins; these modules are emerging important therapeutic targets and the protein-protein interactions they mediate are druggable.[2,3] Furthermore, the few BCPs that have been investigated in detail play fundamental cellular roles and show association with specific diseases, including inflammation and cancer.[1]

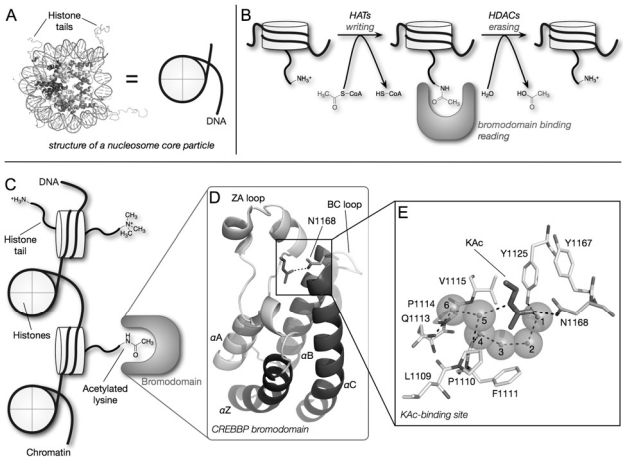

Fig. 1. (A) Histones are the core proteins around which nuclear DNA is packaged. Histone H2A = yellow; Histone H2B = red; Histone H3 = blue Histone H4 = green (PDB ID, 1KX5). (B) Histone acetyl-transferase (HAT) enzymes add an acetyl group to histone lysines and are viewed as "writers"; bromodomains bind to and "read" lysine acetylation; histone deacetylases (HDACs) act as "erasers" and remove histone lysines. (C) Bromodomains are a KAc-recognition domain that act as readers of lysine acetylation state. (D) Bromodomains form a characteristic four-helix bundle comprising helices αZ, αA, αB and αC. (E) Recognition of the KAc residue occurs *via* a direct hydrogen bond between the acetyl carbonyl oxygen atom and N1168 (in CREBBP). A second interaction occurs between the acetyl carbonyl oxygen atom and the phenol of Y1125 (in CREBBP) *via* one of the structured water molecules.

The development of small molecules that selectively prevent the interaction of a specific bromodomain with acetyl-lysine (KAc) is essential to allow dissection of the role that bromodomains play in complex multidomain proteins. These probes will, ultimately, allow us to better understand the cellular roles of BCPs and are essential for the validation of bromodomains as therapeutic targets.

We have undertaken studies to develop inhibitors of the BET family of BCPs. An initial screen for bromodomain-binding fragments led to the discovery that the solvent NMP, dihydroquinazolinone and 3,5-dimethylisoxazoles (3,5-DMIs) can act as KAc mimics.[4] Using a structure-based design approach, we employed the 3,5-DMI moiety as our lead KAc mimic. Optimisation of the lead compound identified **4** as a BRD4(1) ligand with an IC_{50} = 380 nM (AlphaScreen), a K_D = 373 nM (SPR), a good selectivity profile over other bromodomains and a ligand efficiency of 0.41 (Figure 2). Compound **4** has been evaluated in a number of cancer cell lines and has an IC_{50} = 794 nM in MV4;11 acute myeloid leukaemia cells. This molecule will be a useful tool for the study of the BET bromodomains. The scaffold reported represents an excellent prospect for the development of further bromodomains inhibitors.

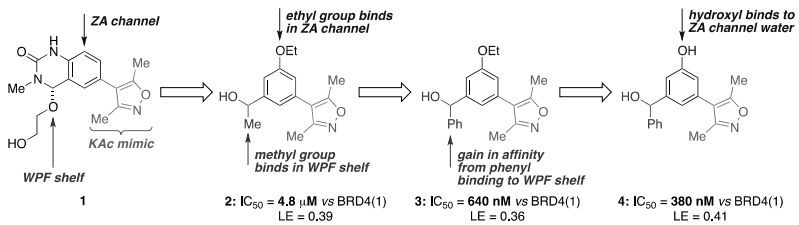

Fig. 2 The optimisation of the lead 3,5-dimethylisoxazole to give a BET-selective probe with IC_{50} = 380 nM *vs* BRD4(1).

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2. Hewings, D. S.; Rooney, T. P. C.; Jennings, L. E.; Hay, D. A.; Schofield, C. J.; Brennan, P. E.; Knapp, S.; Conway, S. J. *J. Med. Chem.* **2012**, *55*, 9393–9413.
3. Conway, S. J. *ACS Med. Chem. Lett.* **2012**, *3*, 691–694.
4. Hewings, D. S.; Wang, M.; Philpott, M.; Fedorov, O.; Uttarkar, S.; Filippakopoulos, P.; Picaud, S.; Vuppusetty, C.; Marsden, B.; Knapp, S.; Conway, S. J.; Heightman, T. D. *J. Med. Chem.* **2011**, *54*, 6761–6770.

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The University of Tokyo

➢ Educational Background

- B.S. 1993, The University of Tokyo
- Ph.D. 1998, The University of Tokyo (advisor: Kazuo Tachibana)
- Postdoctoral Fellow, 1998-2000, Sloan-Kettering Institute for Cancer Research (advisor: Samuel J. Danishefsky)
- Assistant Professor, 2000-2003, Tohoku University (advisor: Masahiro Hirama)
- Lecturer, 2003-2004, Tohoku University
- Associate Professor, 2004-2007, Tohoku University
- Professor, 2007-, The University of Tokyo

➢ Scientific Interests

- Natural Product Synthesis and Bioorganic Chemistry: Synthesis, design and study of biologically important molecules, with particular emphasis on the total synthesis of structurally complex natural product

➢ Recent Papers

1. "Design, Synthesis and Functional Analysis of Dansylated Polytheonamide Mimic: An Artificial Peptide Ion Channel" H. Itoh, S. Matsuoka, M. Kreir, M. Inoue, *J. Am. Chem. Soc.* **2012**, *134*, 14011-14018.

2. "Radical Amination of C(sp^3)–H Bonds Using *N*-Hydroxyphthalimide and Dialkyl Azodicarboxylate" Y. Amaoka, S. Kamijo, T. Hoshikawa, M. Inoue, *J. Org. Chem.* **2012**, *77*, 9959-9969.

3. "Selective Modification of the *N*-Terminal Structure of Polytheonamide B Significantly Changes its Cytotoxicity and Activity as an Ion Channel" N. Shinohara, H. Itoh, S. Matsuoka, M. Inoue, *ChemMedChem*, **2012**, *7*, 1770-1773.

4. "Photochemically-induced Radical Transformation of C(sp^3)–H Bonds to C(sp^3)–CN Bonds" S. Kamijo, T. Hoshikawa, M. Inoue, *Org. Lett.* **2011**, *13*, 5928-5931.

5. "Application of α-Alkoxy Bridgehead Radical for Coupling of Oxygenated Carbocycles" D. Urabe, H. Yamaguchi, M. Inoue, *Org. Lett.* **2011**, *13*, 4778-4781.

6. "Total Synthesis and Bioactivities of Two Proposed Structures of Maresin" K. Sasaki, D. Urabe, H. Arai, M. Arita, M. Inoue, *Chem. Asian J.* **2011**, *6*, 534-543.

7. "CCl_3CN: A Crucial Promoter of mCPBA-mediated Direct Ether Oxidation" S. Kamijo, S. Matsumura, M. Inoue, *Org. Lett.* **2010**, *12*, 4195-4197.

8. "Total Synthesis of Polytheonamide B, the Largest Non-ribosomal Peptide" M. Inoue, N. Shinohara, S. Tanabe, T. Takahashi, K. Okura, H. Ito, Y. Mizoguchi, M. Iida, N. Lee, S. Matsuoka, *Nat. Chem.* **2010**, *2*, 280-285.

9. "Importance of Twisted Side-chain on Potent Toxicity of Antillatoxin: Total Synthesis and Biological Evaluation of Antillatoxin and Analogs" K. Okura, S. Matsuoka, R. Goto, M. Inoue, *Angew. Chem. Int. Ed*. **2010,** *49*, 329-332.

L-7

Total Synthesis and Biological Evaluation of Polytheonamide B

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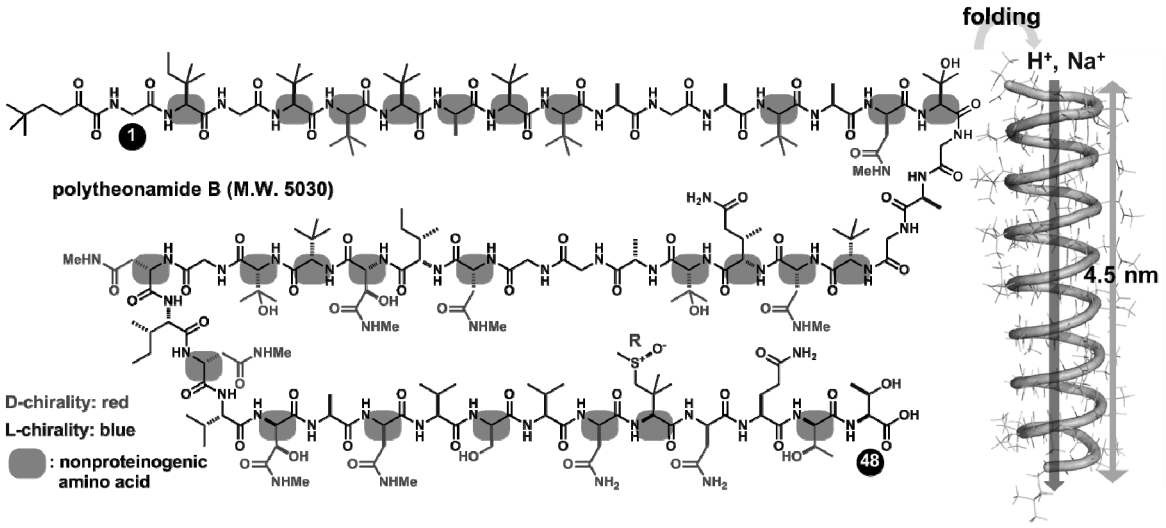
Polytheonamide B, isolated from the marine sponge *Theonella swinhoei*, is a posttranslationally modified ribosomal peptide of molecular weight 5030 Da, and displays extraordinary cytotoxicity. Its 48 amino acid residues include a variety of non-proteinogenic D- and L-amino acids, and the chiralities of these amino acids alternate in sequence. These structural features induce the formation of a stable $\beta^{6.3}$-helix, giving rise to a tubular structure of over 4 nm in length. In the biological setting, this fold is believed to transport cations across the lipid bilayer through a pore, thereby acting as a transmembrane ion channel.

We firstly developed the total synthesis route to this unusual peptide. This achievement to gain precise synthetic control of the structure of polytheonamide B provided the first chemical basis for systematically correlating its molecular structure with biological functions. Specifically, structure-activity relationship (SAR) studies of the 21 fully synthetic variants were performed to pinpoint the proteinogenic and non-proteinogenic building blocks within the molecule that were essential to the channel function and the toxicity. These achievements demonstrate the potential benefits of the total synthesis endeavor and the importance of efficient construction of the complex molecule. The knowledge accumulated through these studies will also be useful for rational generation of new tailor-made channel peptides and cytotoxic molecules with desired functions.



Rebecca Jane Miriam GOSS

School of Chemistry, University of St Andrews, UK

➢ Educational Background

- B.Sc. Hons. Dunelm. 1997, University of Durham
- Ph.D. 2001, University of Durham
  (advisor: Professor David O'Hagan, FRSE)
- Postdoctoral Research Associate, 2001-2002, University of Cambridge,
  (advisors: Professor Peter Leadlay, FRS and Professor Jim Staunton, FRS)
- Teaching Fellowship, 2002-2003 Department of Chemistry, University of Nottingham
- Royal Society Dorothy Hodgkin Fellowship 2003-2007
- Lectureship, School of Chemistry, University of Exeter 2003-closed in 2005
- Lectureship, School of Chemistry, University of East Anglia, 2005-2010
- Senior Lectureship, School of Chemistry, University of East Anglia, 2011
- Readership, School of Chemistry, University of East Anglia, 2012
- Reader in Organic/Biomolecular Chemistry, School of Chemistry, University of St Andrews, 2012

➢ Scientific Interests

- The Goss group is interested in the biosynthesis of natural products and in how these biosynthetic pathways may be harnessed to generate natural products of our own design. Many natural products are of medicinal importance. We are also interested in determining the molecular mode of action of drug molecules. It is our aim to couple these two interests, manipulating biosynthetic pathways to expediently access series of otherwise synthetically intractable natural product analogues, which can be utilised in structure activity determination.

➢ Recent Papers

1. "Biogenesis of the Unique Nucleoside of the Uridyl Peptide Antibiotics: Pacidamycin" A. E. Ragab, S. Grüschow, R. J. M. Goss,* *J. Am. Chem. Soc.* **2011**, *133*, 15288-91.
2. "Diversity in Natural Product Families is Governed by More than Enzyme Promiscuity Alone: Establishing Control of the Pacidamycin Portfolio" S. Grüschow, E. Rackham, R. J. M. Goss,* *Chem. Sci*. **2011**, *2*, 2182-2186.
3. *Highlighted in Chemistry World*: http://www.rsc.org/chemistryworld/News/2011/August/11081101.asp (*Highlight in C&EN News **2011**, 89(34), August 22.*)
4. "*RSC Selected Highlight of 'Cutting Edge Chemistry in 2011*" http://www.rsc.org/chemistryworld/News/2011/December/chemistry-articles-most-exciting-events-2011.asp.
5. "Gene Expression Enabling Synthetic Diver sification of Unnatural Products: Chemogenetic Generation Pacidamycin Analogs" A. Deb Roy, S. Grüschow, N. Cairns, R. J. M. Goss*, *J. Am. Chem. Soc.* **2010**, *134*, 1224-12245. (*Highlighted in C.&EN. News, August 23rd, 2010.*)
6. "Pacidamycin Biosynthesis: Identification and Heterologous Expression of the First Uridyl Peptide Antibiotic Gene Cluster" E. J. Rackham, S. Grüschow, A. E. Ragab, S. Dickens, R. J. M. Goss*, *ChemBioChem*. **2010**, *11*, 1700-1709. (*Submitted in February 2010 and published online in July 2010, ahead of a manuscript confirming our results, contributed on August 3rd to PNAS by Professor Christopher Walsh (Harvard)*)
7. "Direct Evidence for the Use of Multiple Antifungals by a Leaf-cutting Ant" J. Barke, R. F. Seipke, S. Grüschow, M. J. Bibb, R. J. M. Goss, D. W. Yu, M. I. Hutchings* *BMC Biol.* **2010**, *8*, 109. (*Labelled "Highly Accessed" http://www.biomedcentral.com/1741-7007/8/109; 5400 downloads in the first month*).

L-8

Elucidating and Exploiting Biosynthesis

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Natural products represent a treasure trove of medicinally relevant compounds: over the past 3 decades over 70% of antimicrobials and over 60% of antitumor agents entering clinical trials have been based on natural products.[i] Generation of natural product analogues is an important area.

A new paradigm in natural product analogue generation, which we have termed Chemogenetics, will be described. A genome scanning approach to determining the biosynthetic genes responsible for the construction of a highly unusual natural product will also be discussed :-

CHEMOGENETICS *a new paradigm in natural product generation*

The generation of analogues of natural products is key to understanding structure activity relationships and improving physicochemical properties. Traditional approaches of analogue generation such as total synthesis and semisynthesis have limitations. We have pioneered a new concept in which a gene is introduced to an organism and coerced to work in concert with an existing biosynthetic pathway. This installs a chemical handle that enables selective derivatisation of the natural product.

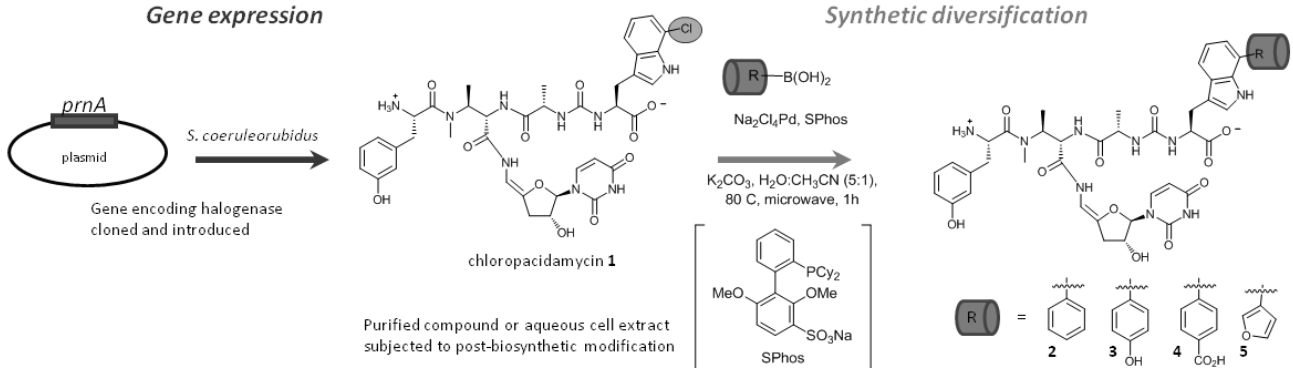

Figure 1. Chemogenetics: gene expression enabling synthetic diversification.

IDENTIFICATION OF THE FIRST URIDYL PEPTIDE ANTIBIOTIC BIOSYNTHETIC CLUSTER: PACIDAMYCIN

The first identification of the pacidamycin biosynthetic cluster and its heterologous expression, using the cutting edge approach of genome scanning will be described.

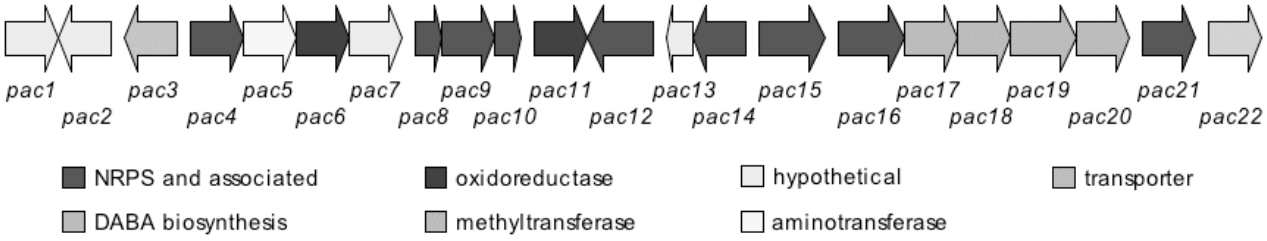

Figure 2. Pacidamycin biosynthetic cluster.

[i] Newman, D. J., Cragg, G. M. *J. Nat. Prod.* **2007,** *70*, 461-477.

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Immunology Frontier Research Center, Osaka University

➢ Educational Background

- B.S. 1988, University of Tokyo
- Ph.D. 1994, University of Tokyo (advisor: Masaaki Hirobe)
- Postdoctoral Fellow, 1994-1995, University of California, San Diego (advisor: Roger Y. Tsien)
- Postdoctoral Fellow, 1995-1996, The Scripps Research Institute (advisor: Donald Hilvert)
- Assistant Professor, 1997-2000, University of Tokyo (advisor: Tetsuo Nagano)
- Associate Professor, 2000-2005, University of Tokyo (advisor: Tetsuo Nagano)
- Professor, 2005-, Osaka University, Graduate School of Engineering
- Professor, 2009-, Osaka University, Immunology Frontier Research Center

➢ Scientific Interests

- Chemical Biology: Design, Synthesis and Application of Molecular Imaging Probes A short list of interests and activities
- Fluorescent Probes, Molecular Imaging, MRI Probes, Optical Imaging, in Vivo Imaging

➢ Recent Papers

1. "Development of Fluorogenic Probe with Transesterification Switch for Detection of Histone Deacetylase Activity" R. Baba, Y. Hori, S. Mizukami, K. Kikuchi, *J. Am. Chem. Soc.* **2012**, *134*, 14310-14313.
2. "Development of Protein Labeling Probes with Redesigned Fluorogenic Switch Based on Intramolecular Association and No-wash Live-cell Imaging" Y. Hori, K. Nakaki, M. Sato, S. Mizukami, K. Kikuchi, *Angew. Chem. Int. Ed.* **2012**, *51*, 5611-5614.
3. "No-wash Protein Labeling with Designed Fluorogenic Probes and Application to Real-time Pulse-chase Analysis" S. Mizukami, S. Watanabe, Y. Akimoto, K. Kikuchi, *J. Am. Chem. Soc.* **2012**, *134*, 1623-1629.
4. "In Vivo Fluorescence Imaging of Bone-resorbing Osteoclasts" T. Kowada, J. Kikuta, A. Kubo, M. Ishii, H. Maeda, S. Mizukami, K. Kikuchi, *J. Am. Chem. Soc.* **2011**, *133*, 17772-17776.
5. "Covalent Protein Labeling with a Lanthanide Complex and Its Application to Photoluminescence Lifetime-based Multicolor Bioimaging" S. Mizukami, T. Yamamoto, A. Yoshimura, S. Watanabe, K. Kikuchi, *Angew. Chem. Int. Ed.* **2011**, *50*, 8750-8752.
6. "^{19}F MRI Detection of β-Galactosidase Activity for Imaging of Gene Expression" S. Mizukami, H. Matsushita, R. Takikawa, F. Sugihara, M. Shirakawa, K. Kikuchi, *Chem. Sci.* **2011**, *2*, 1151-1155.
7. "Compound Release System Using Caged Antimicrobial Peptide" S. Mizukami, M. Hosoda, T. Satake, S. Okada, Y. Hori, T. Furuta, K. Kikuchi, *J. Am. Chem. Soc.* **2010**, *132*, 9524-9525.
8. "Photoactive Yellow Protein-based Protein Labeling System with Turn-on Fluorescence Intensity" Y. Hori, H. Ueno, S.Mizukami, K. Kikuchi, *J. Am. Chem. Soc.* **2009**, *131*, 16610-16611.
9. "Design, Synthesis and Biological Application of Chemical Probes for Bio-imaging" K. Kikuchi, *Chem. Soc. Rev.* **2009**, *39*, 2048-2053.

L-9

Design, Synthesis and Biological Application of in Vivo Imaging Probes with Tunable Chemical Switches

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One of the great challenges in the post-genome era is to clarify the biological significance of intracellular molecules directly in living cells. If we can visualize a molecule in action, it is possible to acquire biological information, which is unavailable if we deal with cell homogenates. One possible approach is to design and synthesize chemical probes that can convert biological information to chemical output.

Fluorescence protein labeling by synthetic probes is a powerful approach to investigate protein function and localization inside living cells. This chemistry-based technique utilizes a pair of a protein tag and its specific ligands connected to fluorophores. Its potential advantage is that various fluorescent molecules are available as labeling reagents, and the timing of protein labeling is easily controlled. Because of these characteristics, this method is attracting attention as an alternative of fluorescent proteins. On the other hand, in this labeling system, there is a problem that the fluorescence of free probes inside cells prevents the identification of labeled proteins. Thus, washing procedures are required to remove the free probes from cells. However, if the probes are not completely washed out, the remaining probes cause the reduction of the signal-to-noise ratio. As a solution of this problem, we previously developed a fluorogenic probe for labeling photoactive yellow protein (PYP) tag. PYP is a small protein (14 kDa) derived from purple bacteria, and binds to the thioester derivatives of cinnamic acid/coumarin through transthioesterification with Cys69. Novel fluorogenic probes, which possess 4-hydroxycinnamic acid or 7-dimethylaminocoumarin as a ligand scaffold, were synthesized. The labeling kinetics was significantly improved by these probes. Furthermore, no-wash labeling of intracellular proteins was successfully achieved. The detail of the strategy for probe design, kinetic experiments and live-cell imaging will be reported.

The 150 Years Anniversary of UK-Japan Academic Interaction

2013 marks the 150th anniversary of the arrival at UK the Choshu Five from Japan. The five young nobles from the Choshu clan (Hirobumi Ito, Kaoru Inoue, Yōzō Yamao, Kinsuke Endo, and Masaru Inoue) smuggled to London and studied at University College London (UCL) in 1863 in order to receive a mind-broadening education in readiness for the Meiji restoration. Furthermore, in 1865, 19 students from the Satsuma clan arrived at UCL. They would form the core of the new Japanese government – the first of the modern Japan.

Choshu-Five; the five young nobles fron the Choshu clan

On their return they became the first Prime Minister (Hirobumi Ito), the Minister for Foreign Relations (Kaoru Inoue), Secretary of State in the Ministry of Industries (Yōzō Yamao), founder of the Japanese National Mint (Kinsuke Endō), founder of Japanese railways (Masaru Inoue), the first Minister of Education (Arinori Mori), and the first head of the University of Tokyo (Yoshinari Hatakeyama). These groups were followed by many other Japanese students in successive years, supported by the Edo Shogunate Government and then the new Meiji Government, in a sharing of knowledge and ideas that continues today.

Statue of the young students from the Satsuma Clan (Kagoshima-city, Japan)

In this history, two chemists had a most significant contribution to the UK-Japan interaction in the field of chemistry. Professor Alexander William Williamson was a Professor of UCL (1855-1887) and President of the London Chemical Society, which developed to the Royal Society of Chemistry. Many of the Japanese students including the Choshu-five and Satsuma-19 lived in Professor. Williamson's residence. Professor Williamson looked after all aspects of university life including registration of

Professor Alexander William Williamson

University and arrangement of study subjects. Unfortunately, four Japanese students died during study in UCL. Professor Williamson made their graves in the Brookwood Cemetery, Surrey. Within our 150 Years activities, Prof. and Mrs. Alexander Williamson's Monument Committee has constructed Professor Williamson's monument at the Brookwood Cemetery.

The Logo of the 150 Years Anniversary of UK-Japan Academic Interaction

Professor Joji Sakurai became a student of UCL (1876-1881) and studied chemistry under Professor. Williamson. He was an outstanding student. Thus, he was elected as a fellow of the London Chemical Society (1879). After returning to Japan, he became the first Professor in the Department of Chemistry at the University of Tokyo at the age of 25. And he contributed to the establishment of RIKEN and the Japan Society for the Promotion of Sciences, and served as President of The Chemical Society of Japan (1883-1885). Also, he was selected as Fellow of the Japan Academy (Nippon Gakushi-in) and University of London.

Monument in Memory of Professor WIliamson

UCL, with the support of the Japanese Embassy in London and the British Embassy in Tokyo, is organising a number of events, in the United Kingdom and Japan to celebrate the benefits of international understanding that arose as a result of the arrival in London in 1863 of the first Japanese students in UK. Although we will be celebrating these historic events, we will also be looking forward to the future of the benefits of wider understanding and cooperation between international communities and we are hoping to initiate a fund to encourage wider interactions between Japanese and UK students in the future.

Professor Shin-ichi Ohnuma

University College London,
Organising Committee member,
The 150 Years Anniversary of UK-Japan Academic Interaction

4th RSC – CSJ Joint Symposium 2013

—Chemical Biology Research by Young Investigators—

- Date: March 24th (SUN) 9:00-17:40
- Venue: Room S8 (CO-LEARNING HOUSE I C306), Ritsumeikan University, Biwako-kusatsu Campus (Shiga, Japan)

- Hosted by
  The Chemical Society of Japan
  Royal Society of Chemistry

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