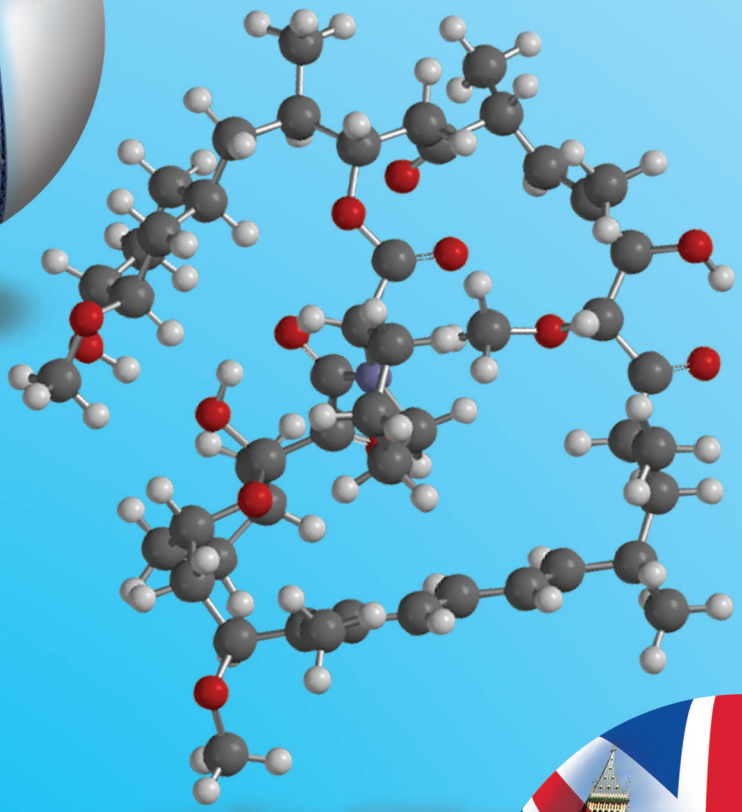
RSC-CSJ Joint Symposium

Chemical Biology



The 91st CSJ Annual Meeting

Date March 28, 2011

Venue Kanagawa University

UK-Japan Chemical Biology Symposium 2011

Date : **March 28, 2011 9:10-17:40**
Venue : **SD (Bldg 23, Rm 310), Yokohama Campus, Kanagawa University, Japan**

		～ Program ～	Page
		Chair: *Kazuya Kikuchi*	
	09:10-09:20	**Yasuhiro Iwasawa** (President, CSJ)	
	09:20-09:30	**Robert Parker** (CEO, RSC)	
		Chair: *Motonari Uesugi*	
L 1	09:30-10:10	**Gregory L. Challis** (Warwick University)	1
		"Novel Catalytic Chemistry in Bioactive Natural Product Biosynthesis"	
		Chair: *Gregory L. Challis*	
L 2	10:10-10:50	**Motonari Uesugi** (Kyoto University)	3
		"Small Molecule Tools for Cell Biology and Cell Therapy"	
	10:50-11:20	～ Intermission ～	
		Chair: *Kazushi Kinbara*	
L 3	11:20-12:00	**Dominic Campopiano** (University of Edinburgh)	5
		"Sphingolipid Biosynthesis in Man and Microbes"	
		Chair: *Dominic Campopiano*	
L 4	12:00-12:40	**Kazushi Kinbara** (Tohoku University)	7
		"Development of Supramolecular Tools for Regulation of Biological Events"	
	12:40-13:40	～ Lunch Time ～	
		Chair: *Nicholas J Westwood*	
L 5	13:40-14:20	**Hiroshi Murakami** (The University of Tokyo)	9
		"Development of Non-standard Peptide Inhibitors Using flexible in Vitro Translation System"	
		Chair: *Hiroshi Murakami*	
L 6	14:20-15:00	**Nicholas J Westwood** (University of St Andrews)	11
		"Recent Advances in Chemical Genetics"	
	15:00-15:30	～ Intermission ～	
		Chair: *Rebecca Goss*	
L 7	15:30-16:10	**Masayuki Inoue** (The University of Tokyo)	13
		"Total Synthesis and Biological Evaluation of Polytheonamide B"	
		Chair: *Masayuki Inoue*	
L 8	16:10-16:50	**Rebecca Goss** (University of East Anglia)	15
		"Elucidating and Exploiting Biosynthesis"	
		Chair: *Rebecca Goss*	
L 9	16:50-17:30	**Kazuya Kikuchi** (Osaka University)	17
		"Molecular Imaging Probes with Tunable Switches for in Vivo Applications"	
		Closing Remarks	
	17:30-17:40	**Daisuke Uemura** (Keio University)	

Welcome Address

Dear Colleagues

At the 91st Annual Meeting of The Chemical Society of Japan CSJ and the Royal Society of Chemistry will hold a joint UK/Japan Chemical Biology Symposium, inviting first-rate chemical biologists from both countries to present their latest research.

The advancement of “green innovation” with the aim of creating an environmental/energy advanced nation and “life innovation” to create a healthy society is a highly regarded science and technology policy. Furthermore, radically strengthening support of these policies, strongly supporting basic research, and reinforcing those who support science and technology is also well regarded. Strengthening education and nurturing young talent are extremely important for developing a sustainable society. Chemical biology is a basic science of both green and life innovation, in particular a nucleus for life innovation development, as well as relevant in biology, physics, engineering, and medicine related disciplines and is an important forefront discipline.

In 2007, the Japan/UK Green Sustainable Chemistry Symposium at Osaka University, and in 2008 the Joint Meeting on Green Chemistry Sustainability at Queen’s University, Belfast were held by the CSJ and RSC. Built on the success of these joint symposia, in 2010 a further effort, the RSC-CSJ Joint Symposium on Catalysis for Sustainable Society, focusing on young Japanese and British scientists was held at the newly renovated Chemistry Hall in the RSC Burlington House, London, where there was lively discussion among the participants. During this symposium, a signing ceremony was held for a scientific exchange agreement between the RSC and CSJ. CSJ President Iwasawa and Executive Director Kawashima, and RSC former President Garner and Chief Executive Pike were present to sign the agreement. Execution of a scientific and business cooperation agreement between RSC, the second largest, and CSJ, the third largest chemical societies in the world, is of significant importance. We are very grateful to Dr. Sarah Thomas for organizing the event.

The UK/Japan Chemical Biology Symposium to be held on 28 March 2011 at Kanagawa University will be based on this scientific exchange agreement. Invited speakers will include four from the UK and five from Japan giving a total of nine. Dr. Sarah Thomas will be responsible for the UK delegation and Professor Kazuya Kikuchi will be responsible for the Japan delegation. We are grateful to these two people for planning this fascinating and socially relevant symposium. I expect that through the great scientists in attendance, this international conference will contribute greatly to this field of science and technology, and more than ever advance chemical biology. Furthermore, I expect more than ever advancement through scientific collaboration.

Prof. Yasuhiro Iwasawa
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. This symposium is the first activity organised following the signing last year of an International Cooperation Agreement between the RSC and the CSJ and we hope it will be the first of many CSJ-RSC activities that will facilitate and foster future collaborations between the societies and scientists in each country.

The RSC is a learned society, concerned with advancing chemistry as a science, developing its applications, and disseminating chemical knowledge, and a professional body that maintains professional qualifications and sets high standards of competence and conduct for professional chemists. We have 47,500 members worldwide drawn from all areas of the chemical sciences. We are active in industry, academia and education and play an important role in shaping science policy both in the UK and internationally. In addition the RSC is a major publisher of research journals, magazines, databases and books that cover all areas of the chemical sciences.

The RSC is very pleased to announce that it is opening its first office in Japan. This 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. A full time member of staff will work from the office and will be 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 that they will present. 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.

Once again a very warm welcome to what promises to be an exciting symposium. We hope that this event will be the first of many joint CSJ RSC activities in the future.

Dr Robert Parker
Chief Executive Officer
Royal Society of Chemistry

Gregory CHALLIS

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Education:

B.Sc. 1994, Imperial College London
Ph.D. 1998, 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 elucidation of bioactive natural products; molecular mode of action of bioactive natural products; molecular genetics and biochemistry of natural product biosynthesis and its regulation; engineered biosynthesis of novel natural product analogues; and synthesis of natural products, biosynthetic intermediates and probes of biosynthetic mechanism.

Recent papers:

1. 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, and G.L. Challis. ***Nat. Chem.*, 3**, in press (2011).

2. Stereochemical elucidation of streptorubin B. S.W. Haynes, P.K. Sydor, C. Corre, L. Song and G.L. Challis. ***J. Am. Chem. Soc.***, **133**, 1793-1798 (2011).

3. A butenolide intermediate in methylenomycin furan biosynthesis is implied by incorporation of stereospecifically ^{13}C-labelled glycerols. C. Corre, S.W. Haynes, N. Malet, L. Song and G.L. Challis. ***Chem. Commun.***, **46**, 4079-4081 (2010).

4. P. Patel, L. Song and G.L. Challis. Distinct extracytoplasmic siderophore binding proteins recognize ferrioxamines and ferri-coelichelin in *Streptomyces coelicolor* A3(2). ***Biochemistry***, 49, 8033-8042 (2010).

5. S. Schmelz, N. Kadi, S.A. McMahon, L. Song, D. Oves-Costales, M. Oke, H. Liu, K.A. Johnson , L. Carter, M.F. White, G.L. Challis and J.H. Naismith. AcsD catalyzes enantioselective desymmetrization of citric acid in siderophore biosynthesis. ***Nat. Chem. Biol.*, 5**, 174-182 (2009).

6. M. Zerikly and G.L. Challis. Strategies for the discovery of new natural products by genome mining. ***ChemBioChem***, **10**, 625-633 (2010).

7. C. Corre, L. Song, S. O'Rourke, K.F. Chater and G.L. Challis. 2-Alkyl-4-hydroxymethylfuran-3-carboxylic acids, antibiotic production inducers discovered by *Streptomyces coelicolor* genome mining. ***Proc. Natl. Acad. Sci. USA***, **105**, 17510-17515 (2008).

8. N. Kadi, S. Arbache, D. Oves-Costales and G.L. Challis. Identification of a gene cluster that directs putrebactin biosynthesis in *Shewanella* species: PubC catalyzes ATP-dependent cyclodimerization of *N*-hydroxy-*N*-succinyl-putrescine. ***J. Am. Chem. Soc.* 130**, 10458-10459 (2008).

L1

Novel catalytic chemistry in bioactive natural product biosynthesis

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Natural products have fascinated chemists for decades, not least because of the remarkable range of potent and useful biological activities they possess. Their unrivalled structural and stereochemical diversity and complexity makes them extremely challenging targets for chemical synthesis, and as such they continue to provide the most rigorous testing ground for new synthetic methods. For the same reasons, natural product biosynthetic pathways arguably involve many of the most remarkable and mechanistically-opaque enzyme-catalysed reactions in Nature.

Genomics has revolutionised the study of natural product biosynthetic pathways and the genes encoding the entire biosynthetic machinery for a microbial natural product can now often be identified in a matter of weeks. One of the greatest unresolved challenges in the field of natural products chemistry and biology is to develop a comprehensive molecular mechanistic understanding of the remarkably selective, yet versatile array of enzyme-catalysed reactions involved in natural product biosynthesis. The necessity to replace traditional fine chemical manufacturing processes, based on dwindling petrochemical resources, with new processes that exploit renewable resources, has provided considerable additional impetus to this important field of interdisciplinary research.

The discovery, mechanistic investigation and exploitation of novel enzyme-catalysed reactions on the biosynthetic pathways to structurally and biologically-interesting natural products will be presented. Examples include: key oligomerisation-macrocyclisation reactions for the assembly of clinically-used iron chelators; highly enantioselective desymmetrisation of citric acid in pathogenicity-conferring siderophore biosynthesis; regiospecific indole nitration at C-4 of L-tryptophan en route to potent phytotoxins; regio and stereodivergent oxidative carbocyclisations in antimalarial antibiotic assembly; and in *trans* hydroxylation during polyketide chain assembly in the biosynthesis of a novel complex of anticancer macrolides.

Motonari UESUGI

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Institute for Chemical Research, Kyoto University

Education:

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 selected papers:

1. Cell-morphology profiling of a natural product library identifies bisebromoamide and miuraenamide A as actin-filament stabilizers. Sumiya, E., Shimogawa, H., Sasaki, H., Tsutsumi, M., Yoshita, K., Ojika, M., Suenaga, K., Uesugi, M.. ***ACS Chem. Biol.*** In press.

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

3. Marine Natural Product Aurilide Activates the OPA1-Mediated Apoptosis by Binding to Prohibitin**.** Sato, S. Murata, A., Orihara, T., Shirakawa, T., Suenaga, K., Kigoshi, H., Uesugi, M. ***Chem. Biol.*** 18 (1), 131-139 (2011).

4. A Small Molecule that Blocks Fat Synthesis by Inhibiting the Activation of SREBP**.** Kamisuki, S., Mao, Q., Abu-Eliheiga, L., Gu, Z., Kugimiya, A., Kwon, Y., Shinohara, T., Kawazoe, Y., Sato, S. Asakura, K., Choo, H., Sakai, J., Wakil, SJ., Uesugi, M. ***Chem. Biol.*** 16 (8), 882-892(2009).

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

6. 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.*** 131(13), 4774-4782 (2009).

7. Polyproline-rod approach to isolating protein targets of bioactive small molecules: isolation of a new target of indomethacin. Sato, S., Kwon, Y., Kamisuki, S., Srivastava, N., Mao, Q., Kawazoe, Y., Uesugi, M. ***J. Am. Chem. Soc.*** 129(4), 873-880 (2007).

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

9. A Wrench-Shaped Synthetic Molecule that Modulates a Transcription Factor-Coactivator Interaction. Shimogawa, H., Kwon, Y., Mao, Q., Kawazoe, Y., Choi, Y., Asada, S., Kigoshi, H., Uesugi, M. ***J. Am. Chem. Soc.*** 126, 3461-3471 (2004).

L2

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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Education:

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, The Scripps Research Institute (advisor: Donald Hilvert)
Lecturer 1997-2007, University of Edinburgh
Senior Lecturer, 2007-, 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.

Recent papers:

1. 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. and Campopiano, D. J. ***Biopolymers,*** **93**, 811-822, *Special issue on natural products* (2010)

2. 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. and Campopiano, D. J., ***Molecular Biosystems,*** **6**, 1682-1693, *Emerging Investigators*, *special issue* (2010)

3. Nucleophilic catalysis of acylhydrazone equilibration for protein-directed dynamic covalent chemistry, Bhat, V. T., Caniard, A. M., Luksch, T., Brenk, R., Campopiano, D. J.,* and Greaney, M. F.*, ***Nature Chemistry***, **2**, 490-497 (2010)

4. Interaction of Human β-Defensin 2 (HBD2) with Glycosaminoglycans, Seo, E. S., Blaum, B. S., Vargues, T., De Cecco, M., Deakin, J. A., Lyon, M., Barran, P. E., Campopiano, D. J. and Uhrín, D., ***Biochemistry***, **49**, 10486-10495 (2010)

5. 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.* and Iwata, S.*, ***Structure***, **17**, 1213-1222 (2009)

6. The external-aldimine form of serine palmitoyltranserase; structural, kinetic and spectroscopic analysis of the wild-type enzyme and HSAN1 mutant mimics, Raman, M. C., Johnson, K. A., Yard, B. A., Lowther, J., Carter, L. G., Naismith, J. H.* and Campopiano, D. J.*, ***J. Biol. Chem.***, **284**, 17328-17339 (2009)

7. 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. and Campopiano, D. J. ***J. Mol. Biol.***, **370**, 870-886 (2007)

L3

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 of 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

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Education:

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. 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, and K. Kinbara, ***Chem. Commun.***, **47**, 194–196 (2011).

2. 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, and T. Aida, ***Angew. Chem., Int. Ed.*, 49**, 3030–3033 (2010).

3. 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, and T. Aida, ***J. Am. Chem. Soc.*, 132**, 3292–3294 (2010).

4. A Tubular Biocontainer: Metal Ion–Induced 1D Assembly of a Molecularly Engineered Chaperonin, S. Biswas, K. Kinbara, N. Oya, N. Ishii, H. Taguchi, and T. Aida, ***J. Am. Chem. Soc.***, **131**, 7556–7557 (2009).

5. Molecular Glues Carrying Multiple Guanidinium Ion Pendants via an Oligoether Spacer: Stabilization of Microtubules against Depolymerization, K. Okuro, K. Kinbara, K. Tsumoto, N. Ishii, and T. Aida, *J. Am. Chem. Soc.*, **131**, 1626–1627 (2009).

6. Toward Long-Distance Mechanical Communication: Studies on a Ternary Complex Interconnected by a Bridging Rotary Module, H. Kai, S. Nara, K. Kinbara, and T. Aida, ***J. Am. Chem. Soc.*, 130**, 6725–6727 (2008).

7. Toward Autonomously Operating Molecular Machines Driven by Transition-Metal Catalyst, K. Tanaka and K. Kinbara, ***Mol. BioSyst.***, **4**, 512–514 (2008).

L4

Development of Supramolecular Tools for Regulation of Biological Events

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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. Furthermore, some polyguanidinium species have been revealed to have the ability to bring proteins or other macromolecules into cells through membranes, and they are expected as carriers in drug, protein, and gene delivery systems.

We designed polyguanidine dendrimers as 'molecular glues' for biomolecules under physiological conditions. The dendritic structure allows for densely packed guanidine functionalities on the surface, which are beneficial for binding with anionic functionalities by a multivalent interaction. In our molecular design, triethyleneglycol was used as the branch unit of the dendrimers, taking into account the fact that it is water-soluble, hardly toxic, and adaptive to physiological conditions. The molecular design also allows incorporation of a variety of functional groups at the core of the dendrimers.

As a demonstration of the interaction between the molecular glues and biomolecules, bovine serum albumin (BSA) was titrated with a luminophore-appended polyguanidine dendrimer in Tris-HCl buffer, where a FRET occurred from the tryptophan unit in BSA to the luminophore. Together with the CD spectral change of BSA upon titration, the molecular glue likely interacts with BSA on its surface. In addition, the molecular glues also efficiently stabilize protein assemblies such as microtubules (MTs) and actomyosin.

Hiroshi MURAKAMI

Graduate School of Arts and Life Sciences, The University of Tokyo

Education:

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

Scientific Interests:

Chemical Biology and Biomolecular Engineering: Directed evolution of biomolecules that regulate cellular function.

Recent papers:

1. Murakami, H.; Ohta, A.; Suga, H., Bases in the anticodon loop of tRNA(Ala)(GGC) prevent misreading. ***Nature Structural & Molecular Biology*** 2009, **16**, (4), 353-8.

2. Kawakami, T.; Ohta, A.; Ohuchi, M.; Ashigai, H.; Murakami, H.; Suga, H., Diverse backbone-cyclized peptides via codon reprogramming. ***Nature Chemical Biology*** 2009, **5**, (12), 888-90.

3. Xiao, H.; Murakami, H.; Suga, H.; Ferre-D'Amare, A. R., Structural basis of specific tRNA aminoacylation by a small in vitro selected ribozyme. ***Nature*** 2008, **454**, (7202), 358-61.

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L5

Development of Non-standard Peptide Inhibitors Using Flexible *in Vitro* Translation System

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Non-standard peptides isolated as natural products often contain various modifications, such as backbone cyclization, epimerization and *N*-methylation. These modified structures improve the bioactive properties of peptides, making them ideal drug candidates. Therefore, there is great interest in developing new methods for preparing libraries of non-standard peptides to be used for identifying inhibitors against various drug targets.

Here, we will present a novel *in vitro* translation system for the development of non-standard peptide inhibitors. Flexizyme, an artificial acyl-tRNA synthesizing ribozyme, provides us a facile tool for the preparation of a wide array of tRNAs charged with various amino acids. Flexizyme was used for the preparation of acyl-tRNAs carrying non-proteinogenic amino acids, which were subsequently mixed with certain purified *Escherichia coli* translation factors to create a new *in vitro* translation system (the flexible *in vitro* translation system). This system allows for the production of non-standard peptides in a template directed manner. We applied this flexible translation system to prepare a highly diverse library ($>10^{13}$) of non-standard cyclic peptides, and selected inhibitors against human vascular endothelial growth factor receptor 2 (VEGFR2), which is known to play an important role in tumor angiogenesis. We identified several peptides following 5 rounds of selection against VEGFR2, one of which exhibited high affinity binding to VEGFR2 and significantly inhibited the interaction between VEGFR2 and its natural ligand $hVEGF_{165}$. These results serve to validate the usefulness of the non-standard peptide library prepared by the flexible translation system for the development of peptide inhibitors. Moreover, this technology is amenable to producing a variety of non-standard peptides, such as *N*-methyl peptides, D-amino acid containing peptides, thus it could be a powerful tool to discover novel peptide drug candidates against various therapeutic targets.

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NATO Postdoctoral Fellow, 1995-1998, University of Texas, Austin (advisor: Philip D Magnus FRS)
Postdoctoral Fellow, 1999-2001, Institute of Chemistry and Cell Biology, Harvard Medical School (advisor: Matthew Shair and Timothy Mitchison FRS)
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Scientific Interests:

Chemical Biology: Discovery and characterisation of novel molecular probes, protein target identification methods, diversity-oriented synthesis, natural product isolation and synthesis.

Recent papers:

1. Asymmetric catalytic oxidative cleavage of polycyclic systems: The synthesis of atropisomeric diazonanes and diazecanes. Jones, A.M., Liu, G., Lorion M.M., Paterson, S., Lebl, T., Slawin, A.M.Z., Westwood, N.J., ***Chemistry - A European Journal***, in press.

2. Application of the copper catalysed *N*-arylation of amidines in the synthesis of the chemical tool, blebbistatin. Lawson, C.P.A.T., Slawin, A.M.Z., Westwood, N.J., ***Chemical Communications***, 47(3), 1057-1059, (2010).

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4. A small-molecule inhibitor of T.gondii motility induces the post-translational modification of myosin light chain-1 and inhibits myosin motor activity, Heaslip A.T., Leung, M., Westwood, N.J. Ward, G.E., ***PLoS Pathogens***, 6(1), (2010).

5. *Iso-seco*-tanapartholides: Isolation, Synthesis and Biological Evaluation. Makiyi, E.F., Frade, R.F.M., Lebl, T., Jaffray, E.G., Cobb, S.E., Harvey, A.L., Slawin, A.M.Z., Hay, R.T., Westwood, N.J., ***EJOC***, 33, 5711-5715, (2009).

6. Novel Cambinol Analogs as Sirtuin Inhibitors: Synthesis, Biological Evaluation, and Rationalization of Activity. Medda, F., Russell, R.J.M., Higgins, M., McCarthy, A.R., Campbell, J., Slawin, A.M.Z., Lane, D.P., Lain, S., Westwood, N.J., ***Journal of Medicinal Chemistry,*** 52(9), 2673-2682, (2009).

7. Discovery, *in vivo* activity and mechanism of action of a small-molecule p53 activator. Lain S., Hollick J.J., Campbell J., Staples O.D., Higgins M., Aoubala M., McCarthy A., Appleyard V., Murray K.E., Baker L., Thompson A., Mathers J., Holland S.J., Stark M.J.R., Pass G., Woods J., Lane D.P. and Westwood N.J., ***Cancer Cell***, 13(5), 454-463 (2008).

L6

Recent Advances in Chemical Genetics

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The search for chemical tools to help us dissect complex biological processes continues. In the recent past this search has been reinvigorated by an improvement in the ability of the academic community to screen for compounds of biological relevance. This has resulted in the discovery of a wide range of new tools. This lecture will focus on the results generated in a collaborative research project between our laboratory and the laboratory of Professor Sir David Lane FRS. The use of a high-throughput screen to identify novel activators of the tumor suppressor protein, p53, has led to the discovery of a new class of sirtuin inhibitors known as the tenovins. Recent SAR studies both with the tenovins and our novel analogues of the sirtuin inhibitor cambinol have led to the further optimisation of these sirtuin inhibitors. A discussion on the optimisation of chemical tools of use in studying SIRT2 function will be provided.

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B.S. 1993, The University of Tokyo
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Postdoctoral Fellow, 1998-2000, Sloan-Kettering Institute for Cancer Research (advisor: Samuel J. Danishefsky)
Assistant Professor, 2000-2003, Tohoku University (advisor: Masahiro Hirama)
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Associate Professor, 2004-2007, Tohoku University
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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. CCl_3CN: A Crucial Promoter of mCPBA-Mediated Direct Ether Oxidation, S. Kamijo, S. Matsumura, and M. Inoue, ***Org. Lett.***, **12**, 4195-4197 (2010)

2. 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, and S. Matsuoka, ***Nature Chem.***, **2**, 280-285 (2010)

3. 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, and M. Inoue, ***Angew. Chem. Int. Ed.***, **49**, 329-332 (2010)

4. Direct Construction of 1,3-Diaxial Diol Derivatives by C-H Hydroxylation, S. Kasuya, S. Kamijo, and M. Inoue, ***Org. Lett.***, **11**, 3630-3632 (2009)

5. Total Synthesis and Bioactivity of Resolvin E2, S. Ogawa, D. Urabe, Y. Yokokura, H. Arai, M. Arita, and M. Inoue, ***Org. Lett.***, **11**, 3602-3605 (2009)

6. Critical Importance of the 9-Membered F-Ring of Ciguatoxin for Potent Bioactivity: Total Synthesis and Biological Evaluation of F-Ring-Modified Analogs, M. Inoue, N. Lee, K. Miyazaki, T. Usuki, S. Matsuoka, and M. Hirama, ***Angew. Chem. Int. Ed.***, **27**, 8611-8614 (2008)

7. Total Synthesis of the C-1027 Chromophore Core. Extremely Facile Enediyne Formation via SmI_2-Mediated 1,2-Elimination, M. Inoue, I. Ohashi, T. Kawaguchi, and M. Hirama, ***Angew. Chem. Int. Ed.***, **47**, 1777-1779 (2008).

Total Synthesis and Biological Evaluation of the Large Non-Ribosomal Peptide Polytheonamide B

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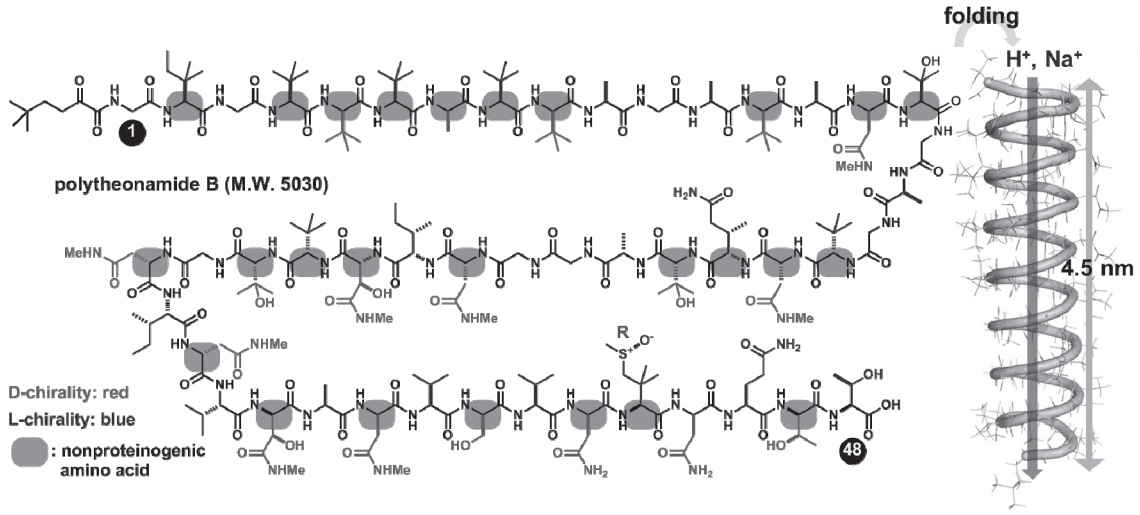
Non-ribosomal peptides (NRPs) are secondary metabolites produced by microorganisms and include number of clinically important natural products such as penicillin, cyclosporine and vancomycin. The diverse and selective biological functions of NRPs originate from their unusual molecular structures. NRPs are biosynthesized by large multifunctional enzyme NRP synthases. They contain not only 20 common amino acids of ribosomal proteins but also various building blocks, and often possess cyclic and branched peptide frameworks. Consequently, NPRs represent attractive targets for total synthesis because of their novel chemical structures and selective biological functions.

Polytheonamide B is by far the largest NRP currently known, and displays extraordinary cytotoxicity (EC_{50} = 68 pg/mL, mouse leukemia P388 cells). 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 β-strand-type structure, giving rise to an overall tubular structure over 30 Å in length. In a biological setting, this fold is believed to transport cations across the lipid bilayer through a pore, thereby acting as an ion channel. We recently achieved the first chemical construction of polytheonamide B. Our synthesis relies on the combination of four key stages - syntheses of non-proteinogenic amino acids, a solid phase assembly of four fragments of polytheonamide B, silver mediated connection of the fragments and finally global deprotection. The synthetic material now available will allow studies of the relationships between the conformational properties, the channel functions and the cytotoxicity.



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B.S.C. Hons. Dunelm. 1997, University of Durham
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Postdoctoral Research Associate, 2001-2002, University of Cambridge, (advisors: Professor Peter Leadlay, FRS and Professor Jim Staunton, FRS)
Teaching Fellowship, Department of Chemistry, University of Nottingham , 2002-2003
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

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) Gene expression enabling synthetic diversification 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. Cited already by 2 publications in Nature.*

2) Pacidamycin biosynthesis: Identification and heterologous expression of the first uridyl peptide antibiotic gene cluster, E. J. Rackham, S. Grüschow, A.E. Ragab, S. Dickens, and R. J. M. Goss*, *ChemBioChem.*, **2010,** *11*, 1700-1709.

3) 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, and 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

4) New pacidamycins biosynthetically: probing N and C terminal substrate specificity of an unusual NRPS, A. E. Ragab, S Grüschow, E. J. Rackham, R. J. M. Goss,* *Org. Biomol. Chem.*, **2010**, *8,* 3128-3129

5) Fluororapamycins generated through the exploitation of biosynthesis, R. J. M. Goss,* S. Lanceron, A. D. Roy, S. Spague, Nur-e-Alam, D. L. Hughes, B. Wilkinson, S. J. Moss, *ChemBioChem.,* **2010**, 11, 1439-4227

6) Antimicrobial nucleoside antibiotics targeting cell wall assembly: Recent advances in structure–function studies and nucleoside biosynthesis, M. Winn, R. J. M. Goss, K. Kimura and T. D. H. Bugg,* *Nat. Prod. Rep.*, **2010,** 27, 279-304.

7) A serine carboxypeptidase-like acyltransferase is required for synthesis of antimicrobial compounds and disease resistance in oats, S. T. Mugford, X. Qi, S. Bakht, L. Hill, E. Wegel, R. K. Hughes, K. Papadopoulou, R. Melton, R. J. M. Goss, and A. E. Osbourn* *Plant Cell*, **2009**, 21, 2473-2484

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 .

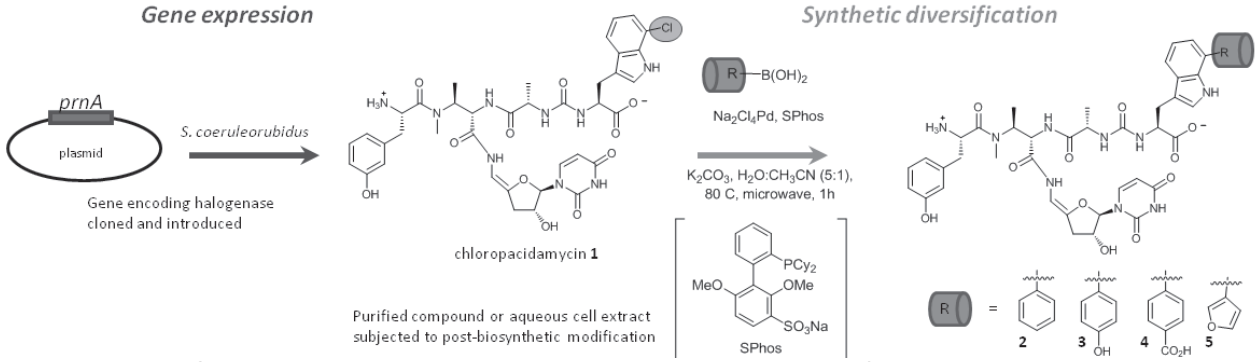

Figure 1. Chemogenetics : gene expression enabling synthetic diversification.

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.

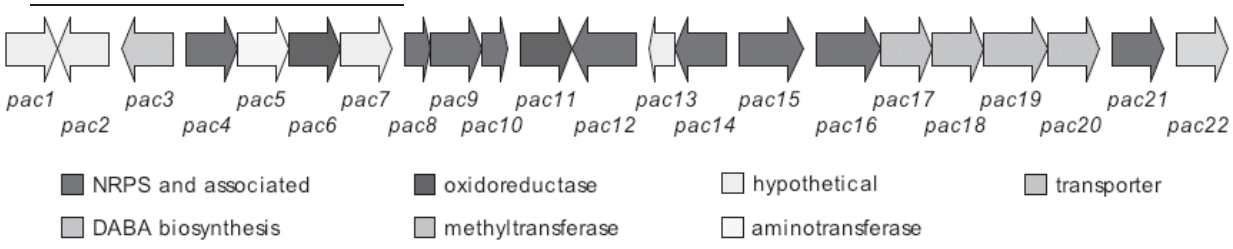

Figure 2. Pacidamycin biosynthetic cluster.

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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)
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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. Compound Release System Using Caged Antimicrobial Peptide, S. Mizukami, M. Hosoda, T. Satake, S. Okada, Y. Hori, T. Furuta, and K. Kikuchi, ***J. Am. Chem. Soc.*, 132,** 9524-9525 (2010)

2. Photoactive Yellow Protein-Based Protein Labeling System with Turn-on Fluorescence Intensity, Y. Hori, H. Ueno, S.Mizukami, and K. Kikuchi, ***J. Am. Chem. Soc.***, **131,** 16610-16611 (2009)

3. Design, Synthesis and Biological Application of Chemical Probes for Bio-imaging, K. Kikuchi, ***Chem. Soc. Rev.*, 39,** 2048-2053 (2009)

4. Covalent Protein Labeling Based on Non-catalytic β-Lactamase and a Designed FRET Substrate, S. Mizukami, S. Watanabe, Y. Hori, and K. Kikuchi, ***J. Am. Chem. Soc.***, **131,** 5016-5017 (2009)

5. Dual Functional Probe to Detect Protease Activity for Fluorescence Measurement and ^{19}F MRI, S. Mizukami, R. Takikawa, F. Sugihara, M. Shirakawa, and K. Kikuchi, ***Angew. Chem. Int. Ed.***, **48,** 3641-3643 (2009)

6. Lanthanide-based Protease Activity Sensors for Time-resolved Fluorescence Measurements, S. Mizukami, K. Tonai, M. Kaneko, and K. Kikuchi, ***J. Am. Chem. Soc.***, **130,** 14376-14377 (2008)

7. Paramagnetic Relaxation-based ^{19}F MRI Probe to Detect Protease Activity, S. Mizukami, R. Takikawa, F. Sugihara, Y. Hori, H. Tochio, M. Wälchli, M. Shirakawa, and K. Kikuchi, ***J. Am. Chem. Soc.***, **130,** 794-795 (2008)

L9

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.

Real-time imaging of enzyme activities *in vivo* offers valuable information in understanding living systems and in the possibility to develop medicine to treat various forms of diseases. Magnetic resonance imaging (MRI) is an imaging modality adequate for *in vivo* studies. Because background signal is hardly detectable, ^{19}F-MRI probes are promising for *in vivo* imaging. A novel design strategy for ^{19}F-MRI probes to detect protease activities is proposed. The design principle is based on the paramagnetic relaxation effect from Gd^{3+} to ^{19}F. A peptide was synthesized, Gd-DOTA-DEVD-Tfb, attached to a Gd^{3+} complex at the N-terminus and a ^{19}F-containing group at the C-terminus. The ^{19}F-NMR transverse relaxation time (T_2) of the compound was largely shortened by the paramagnetic effect of Gd^{3+}. The peptide was designed to have a sequence cleaved by an apoptotic protease, caspase-3. T_2, after cleavage by caspase-3, was extended to cancel the paramagnetic interaction.

Protein tags for labeling proteins of interest (POIs) with small molecule based probes have become important technique as practical alternatives to the fluorescent proteins (FPs) for live cell imaging. We have designed a protein labeling system that allows fluorophores to be linked to POI. The protein tag (BL-tag) is a mutant class A β-lactamase (TEM-1) modified to be covalently bound to the designed specific labeling probes and the labeling probes is consisted with a β-lactam ring (ampicillin, cephalosporin) attached to various fluorophores. A fluorogenetic labeling system can be designed using the unique property of cephalosporin, which release leaving group by subsequent reaction after opening the lactam ring. Highly specific and fast covalent labeling was achieved between the BL-tag and the β-lactam labeling probes under physiological conditions in living cells.

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