分子科学研究所 共同利用研究 分子研研究会

Molecular Imaging for Systems Biology

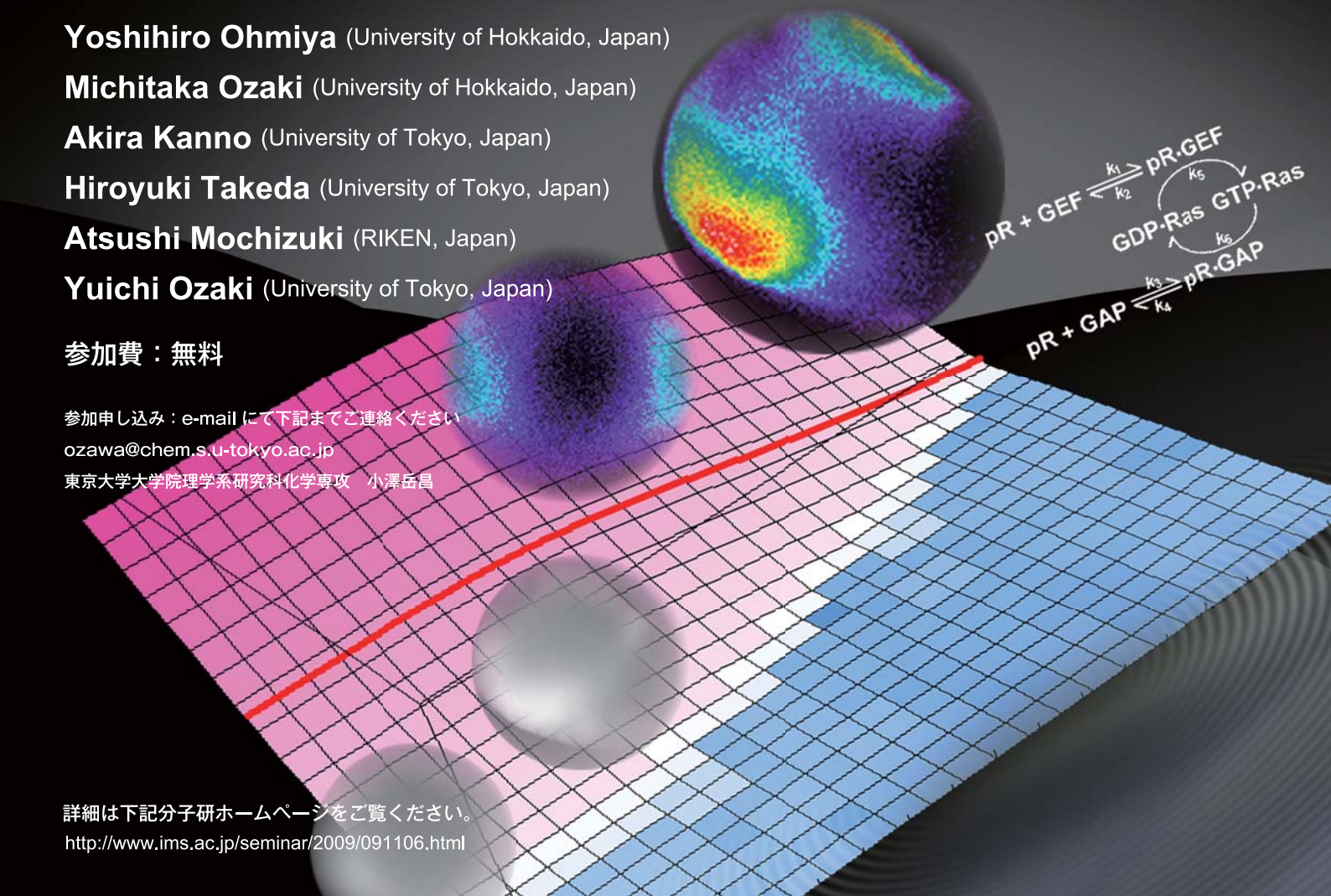
生体分子イメージングの技術開発とシステムズバイオロジー

2009
11/6 FRI
9:00–18:00

岡崎コンファレンスセンター
（愛知県岡崎市明大寺町字伝馬８－１）

■SPEAKER

Michael R. H. White (University of Liverpool, UK)
Robert E. Campbell (University of Alberta, Canada)
Takeharu Nagai (University of Hokkaido, Japan)
Fu-Jen Kao (National Yang-Ming University, Taiwan)
Mingyuan Gao (The Chinese Academy of Sciences, China)
Muhammad Awais (University of Liverpool, UK)
Yoshihiro Ohmiya (University of Hokkaido, Japan)
Michitaka Ozaki (University of Hokkaido, Japan)
Akira Kanno (University of Tokyo, Japan)
Hiroyuki Takeda (University of Tokyo, Japan)
Atsushi Mochizuki (RIKEN, Japan)
Yuichi Ozaki (University of Tokyo, Japan)



参加費：無料

参加申し込み：e-mail にて下記までご連絡ください
ozawa@chem.s.u-tokyo.ac.jp
東京大学大学院理学系研究科化学専攻　小澤岳昌

詳細は下記分子研ホームページをご覧ください。
http://www.ims.ac.jp/seminar/2009/091106.html

Preface

Dear attendees:

We are delighted to invite you to the Seminar of Institute for Molecular Science "Molecular Imaging for Systems Biology", which will be held on November 6, 2009, in Okazaki, Japan. This meeting is a part of the international collaboration program between Japan and UK (JST and BBSRC), which started on April 1, 2009.

This particular meeting will highlight emerging and innovative technologies of biomolecular imaging and their practical application to the field of systems biology. The whole understanding of living systems requires for uncovering spatiotemporal changes of different signaling molecules and their connections in living cells. Biomolecular imaging technologies will provide more helpful information in order for understanding the biological systems. The session of the meeting will include such cutting edge topics as the invention of new techniques of fluorescence and bioluminescence imaging, microscope with new area and their applications, and systems biological analysis in living cells and model organisms.

We are proud to have many distinguished speakers who are active in the front line of these fields.

We sincerely hope that you enjoy this meeting and communication with attendees.

Finally, this seminar could not take place without the hard work and dedication of the Secretary Mrs. Shimizu and group members of Professor Urisu. We would like to acknowledge them for all their efforts and patience in arranging the meeting.

Sincerely,

October 1st, 2009

Takeaki Ozawa (The University of Tokyo, Japan)

Tsuneo Urisu (Institute for Molecular Science)

Program

November 6 Friday

Opning Remarks

10:30-10:40 Takeaki Ozawa Graduate School of Sciences, University of Tokyo, Japan

Keynote Lectures

10:40-11:20 Michael R. H. White School of Biological Sciences, University of Liverpool, UK
"Spatial and temporal information coding by the NF-κB system"

11:20-12:00 Robert E. Campbell Department of Chemistry, University of Alberta, Canada
"Moulding fluorescent proteins into new biotools: engineering of protein surfaces, topology, and chromophores"

Lunch

Session 1: Fluorescence and MRI Imaging

13:00-13:30 Takeharu Nagai Research Institute for Electronic Science, Hokkaido University, Japan
"Toward understanding biological phenomena by genetically-encoded molecular spies"

13:30-14:00 Fu-Jen Kao Institute of Biophotonics Engineering, National Yang-Ming University, Taiwan
"Visualizing Cellular Metabolism with Fluorescence Lifetime of Reduced Nicotinamide Adenine Dinucleotide"

14:00-14:30 Mingyuan Gao Institute of Chemistry, the Chinese Academy of Sciences, China
"Magnetic Nanocrystals: from Preparations to Bioapplications"

14:30-14:50 Muhammad Awais School of Biological Sciences, University of Liverpool, UK
"Measuring dynamic protein interactions in living cells over time"

Coffee Break

Session 2: Systems Biology

15:10-15:40 Hiroyuki Takeda Graduate School of Sciences, University of Tokyo, Japan
"Coupling cellular oscillators in vertebrate segmentation: analysis with high-resolution imaging of gene transcription"

15:40-16:10 Atsushi Mochizuki Theoretical Biology Laboratory, RIKEN Advanced Science Institute, Japan
"Structure of regulatory networks and dynamics of bio-molecules: Predicting unknown from known"

16:10-16:30 Yuichi Ozaki Graduate School of Sciences, University of Tokyo, Japan
"High throughput quantification of single cellular signaling events by use of immunostaining and image cytometry"

Coffee Break

Session 3: Bioluminescence Imaging

16:50-17:20 Yoshihiro Ohmiya Research Institute of Genome-based Biofactory, National Institute of Advance Industrial Science and Technology, Japan
"Application of luciferases for *in vivo* bioluminescence imaging"

17:20-17:50 Michitaka Ozaki Department of Molecular Surgery, Hokkaido University School of Medicine, Japan
"Bio-imaging of Surgical Stress*, dynamic analysis of liver oxidative stress and damage*"

17:50-18:10 Akira Kanno Graduate School of Sciences, University of Tokyo, Japan
"Bioluminescent Probes to Visualize Biological Functions in Living Cells"

Closing Remarks

18:10-18:20 Tsuneo Urisu Institute for Molecular Science

"Spatial and temporal information coding by the NF-κB system"

Michael R. H. White

School of Biological Sciences, University of Liverpool, Crown Street, Liverpool,

L69 7ZB UK

The Nuclear Factor kappa B (NF-κB) transcription factor regulates cellular stress responses and the immune response to infection. NF-κB activation results in oscillations in nuclear NF-κB abundance (1). To define the function of these oscillations, we treated cells with repeated short pulses of tumor necrosis factor alpha (TNFα) at various intervals to mimic pulsatile inflammatory signals. At all pulse intervals analyzed, we observed synchronous cycles of NF-κB nuclear translocation. Lower frequency stimulations gave repeated full-amplitude translocations, whereas higher frequency pulses, gave reduced translocation, indicating a failure to reset. Deterministic and stochastic mathematical models predicted how negative feedback loops regulate both the resetting of the system and cellular heterogeneity. Altering the stimulation intervals gave different patterns of NF-κB-dependent gene expression, supporting a functional role for oscillation frequency (2).

This system is one of a number of biological cycles that have been discovered. Other examples include calcium signalling, transcription cycles, p53, the segmentation clock, the circadian clock, the cell cycle and seasonal rhythms. Are such cycles are a fundamental theme in the integration of biological systems? This could provide a mechanism to explain the robustness of cellular decision making processes.

(1). D.E. Nelson, A.E.C. Ihekwaba, M. Elliott, J. Johnson, C.A. Gibney, B.E. Foreman, G. Nelson, V. See, C.A. Horton, D.G. Spiller, S.W. Edwards, H.P. McDowell, J.F. Unitt, E. Sullivan, R. Grimley, N. Benson, D. Broomhead, D.B. Kell & M.R.H. White. (2004) Oscillations in NF-kB signaling control the dynamics of gene expression. Science 306: 704-8.

(2). L. Ashall, C.A. Horton, D.E. Nelson, P. Paszek, C.V. Harper, K. Sillitoe, S. Ryan, D.G. Spiller, J.F. Unitt, D.S. Broomhead, D.B. Kell, D.A. Rand, V. Sée, and M.R.H. White. (2009) Pulsatile stimulation determines timing and specificity of NF-kappa B-dependent transcription. Science, 324: 242-246.

Name: Michael R. H. White, Ph.D.

Position:
Professor, School of Biological Sciences, University of Liverpool, Liverpool, UK

Education:
- 1988–1991 External PhD studentship, London University and Amersham International plc
- 1981–1985 Biochemistry degree, Keble College, Oxford University

Major Professional Experiences:
- 2004–present Professor of Cell Imaging, School of Biological Sciences, University of Liverpool
- 2003–2004 Reader in Biological Sciences, School of Biological Sciences, University of Liverpool
- 1999–2002 University Senior Lecturer in Biological Sciences, School of Biological Sciences, University of Liverpool
- 1996–1999 School of Biological Sciences, University of Liverpool
- 1995–1996 University Lecturer in Biochemistry, Department of Biochemistry, University of Liverpool
- 1994–1995 Senior Research Scientist, Amersham International plc
- 1991–1994 Visiting Fellowship (seconded from Amersham International plc), Department of Biochemistry, Oxford University
- 1985–1988 Research and Development Biochemist, New Technologies Department, Amersham International plc

Research Interests:
Mike White applied the firefly luciferase reporter for gene expression imaging in mammalian cells while working at Amersham. Following a move to Liverpool University, he founded the Centre for Cell Imaging for non-invasive multi-parameter imaging of cellular processes. He has recently concentrated on the dynamics and function of NF-kappaB signaling and showed that NF-kappaB signaling involves transcription factor oscillations between the nucleus and cytoplasm. These oscillations maintain NF-kappaB-dependent transcription. Through the combination of cell imaging and mathematical modelling, this has opened up a new understanding of the way in which this important signaling pathway defferentially regulates transcription and cell fate.

Moulding fluorescent proteins into new biotools: engineering of protein surfaces, topology, and chromophores

Robert E. Campbell[1]

[1] University of Alberta, Department of Chemistry, Edmonton, Alberta T6G 2G2, Canada (robert.e.campbell@ualberta.ca)

At the heart of the Campbell group's research program lies the extraordinarily useful family of fluorescent proteins (FPs) which includes the green FP (GFP) from *Aequorea* jellyfish and its numerous homologues of various color from coral. All FPs share the ability to autonomously generate a visible wavelength fluorophore from a sequence of 3 amino acids located in the center of their barrel-like structures. FPs are credited with sparking the explosive and continuing growth in the popularity of live cell fluorescence microscopy; culminating in the 2008 Nobel Prize in Chemistry being awarded to 3 pioneers in this area; Shimomura, Chalfie, and Tsien.

Since its inception in 2003, the Campbell research group has worked to mould (that is, engineer) FPs into improved tools for addressing fundamental questions in life science. For example, we have developed several improved alternatives to some of the traditionally preferred hues of FPs [1,2,3] and exploited their improved properties in new applications. Some of our novel applications include the use of FPs for investigating peptide structure in vivo [4], dual-FRET imaging of caspase activation during apoptosis [5], and a FP-based assay of nucleoside transporter activity (with Joe Casey) [6].

In this seminar I will discuss some of our most recent efforts to create FP variants that have physical or spectral properties that are either improved relative to existing variants or are completely novel and have not been previously reported. As the title of this seminar indicates, this discussion will focus on 3 aspects of FPs; chain topology, surface properties, and chromophore chemistry. Specifically, I will present our most recent progress in the following areas: creation of circularly permuted red FPs through manipulation of topology; engineering of monomeric and heterodimeric FPs through manipulation of surface properties; and engineering of photostable and photconvertable FPs by manipulation of chromophore chemistry and environment.

[1] Ai, H., Henderson, J. N., Remington, S. J. & Campbell, R. E. *Biochem. J.* **400,** 531-540 (2006).

[2] Ai, H., Shaner, N. C., Cheng, Z., Tsien, R. Y. & Campbell, R. E. *Biochemistry* **46,** 5904-5910 (2007).

[3] Ai, H., Olenych, S. G., Wong, P., Davidson, M. W. & Campbell, R. E. *BMC Biology* **6,** 13 (2008).

[4] Cheng, Z., Miskolzie, M. & Campbell, R. E. *ChemBioChem* **8,** 880-883 (2007).

[5] Ai, H., Hazelwood, K. L., Davidson, M. W. & Campbell, R. E. *Nat. Methods* **5,** 401-403 (2008).

[6] Johnson, D. E., Ai, H. W., Wong, P., Young, J. D., *et al. J Biol Chem* **284,** 20499-20511 (2009).

Name: Robert E. Campbell, Ph.D.

Position:
Associate Professor, Department of Chemistry, University of Alberta, and Tier II Canada Research Chair in Bioanalytical Chemistry

Education:
- 2000-2003: Postdoctoral fellow at the University of California, San Diego, Department of Pharmacology
- 1994-2000: Ph.D. from the University of British Columbia, Department of Chemistry
- 1990-1994: B.Sc. from the University of British Columbia, Department of Chemistry.

Major Professional Experiences:
- 02009-present: Associate Professor, Department of Chemistry, University of Alberta
- 2009-present: Tier II Canada Research Chair in Bioanalytical Chemistry (renewal)
- 2003-2009: Assistant Professor, Department of Chemistry, University of Alberta
- 2004-2009: Tier II Canada Research Chair in Bioanalytical Chemistry (renewed)
- 2008: Petro-Canada Young Innovator Award
- 2004-2009: Tier II Canada Research Chair in Bioanalytical Chemistry.
- 2004: Alberta Ingenuity New Faculty Award

Research Interests:
- Protein engineering
- Fluorescence imaging
- Fluorescent proteins
- Genetically encoded biosensors

Toward understanding biological phenomena by genetically-encoded molecular spies

Takeharu Nagai[1,2]

[1] Research Institute for Electronic Science, Hokkaido University,, Sapporo, Japan
[2] Precursory Research for Embryonic Science, Japan Science and Technology Agency, Tokyo, Japan

Our primary goal is to better understand how biological molecules function in space and time. To this end, we are developing several techniques to visualize physiological events at molecular level in living cells and whole body. One approach is the use of the green fluorescent protein and its derivatives (FPs) which are spontaneously fluorescent. To expand color palette of FPs, we recently invented a pH-insensitive ultramarine fluorescent protein, Sirius, with enhanced photostability and an emission peak at 424 nm, the shortest wavelength among fluorescent proteins reported to date. The pH-insensitivity of Sirius makes possible prolonged visualization of biological events in an acidic environment. Combination of FPs with fluorescence resonance energy transfer (FRET) technique allows us to develop functional indicator, thereby we can visualize localized molecular events in their natural environment in vivo. For example, we have developed an ultra-sensitive Ca^{2+} indicator by introducing some modification into Ca^{2+} sensing domain of YC3.60 (Nagai et al 2004). Its small K_d value (20 nM) allows us to detect Ca^{2+} dynamics even at 10-150 nM ranges without affecting cellular viability. Large dynamic range (1300 %) also enables us to detect the signaling pattern in 100,000 cellular networks at single cell resolution, being the largest scales to be achieved so far. In addition to this, we developed "dual FRET" imaging methods by using two FRET pairs, Sirius-CFP and Sapphire-dsRed, thereby we succeeded in simultaneous spatio-temporal visualization of two intracellular events. Furthermore, we applied the FRET technique to make a photoconvertible fluorescent protein, Phamret, which can be highlighted by UV stimulation inducing a change in fluorescence emission from cyan to green color. Phamret can be monitored by single-excitation-dual-emission mode allowing mobility analyses over a broad range of kinetics. In this symposium, I will introduce not only several kinds of FP-based indicators mentioned above but also a technique for high-throughput gene construction that is useful for the high performance indicators.

[1] Imamura et al. *Proc. Natl. Acad. Sci. USA* in press
[2] W. Tomosugi et al. *Nature Methods* **6** (2009) 351.
[3] T. Matsuda, A. Miyawaki and T. Nagai T. *Nature Methods* **5** (2008) 339.
[4] K. Saito K et al.. *Cell Struct Funct.* **33** (2008) 133.
[5] I. Kotera and T. Nagai. *J. Biotechnol.* **13** (2008) 1.
[6] K. Takemoto et al. *Proc. Natl. Acad. Sci. USA* **104** (2007) 13367.
[7] K. Okamoto et al.. *Nature Neurosci* **7** (2004) 1104
[8] T. Nagai et al. *Proc. Natl. Acad. Sci. USA* **101** (2004) 10554.
[9] T. Nagai et al. *Nature Biotechnol.* **20** (2002) 87.
[10] T. Nagai et al. *Proc. Natl. Acad. Sci. USA* **98** (2001) 3197

Name: Takeharu Nagai, Ph.D.

Position:
Professor, Research Institute for Electronic Science, Hokkaido University, Japan

Education:
- 1992 BA: Biology, Tsukuba University, Japan
- 1994 M.D.: Agricultural Science, Tsukuba University, Japan
- 1998 Ph.D.: Medical Science, The University of Tokyo, Japan

Major Professional Experiences:

1995-1998 Special research fellow of Japan Society for Promotion of Science
1998-2001 Special Postdoctoral Researcher of RIKEN
2001-2004 Researcher of PRESTO, JST
2003-2004 Researcher of Brain Science Institute, RIKEN
2005- Professor, Research Institute for Electronic Science, Hokkaido University
2008- Researcher of PRESTO, JST

Research Interests:

Development of functional indicators using FRET and BRET
Development of a method for light-inducible inactivation of biomolecules
Development of new microscopy system for visualization of multifunction in living cells
Understanding the mechanism of pattern formation in multi-cellular organisms

Visualizing Cellular Metabolism with Fluorescence Lifetime of Reduced Nicotinamide Adenine Dinucleotide

V. Gukkasyan[1], T. Buriakina[1], J. Hsu[1], and F.-J. Kao[1,2]

[1]Institute of Biophotonics, National Yang-Ming University, Taipei 11221, Taiwan
[2]Department of Photonics, National Sun Yat-sen University, Kaohsiung 80424, Taiwan

Formulation of the oxidative phosphorylation and its' first observation by means of fluorescence spectroscopy in the 60s of the previous century led to the acceptance of bioenergetics as a new field of studies. The new discipline grew fast with the increasing number of papers, related to the energy generation in mitochondria, advancement of the instrumentation and improvement of observation techniques. As such, fluorescence lifetime imaging microscopy (FLIM) has gained popularity as a sensitive technique to monitor the functional/conformational states of nicotinamide adenine dinucleotide reduced (NADH) – one of the main compounds of the oxidative phosphorylation. We hereby review the development and current application of cellular metabolism observation via NADH FLIM, illustrating it with the examples of both physiological (cell density, apoptosis, necrosis) and pathological states (inhibition of the electron transfer chain).

NADH serves as a co-enzyme and a principal electron donor within the cell for both oxidative phosphorylation (aerobic respiration) and glycolysis (anaerobic respiration). The molecule exists in two functional forms: free and bound. The bound forms can be associated with the energy generation in the form ATP, and the relative quantities of free and bound species can give an insight to the metabolical state of a cell. Aside from the biochemical analysis, which requires extraction of pyridine nucleotides, fluorescence lifetime is the only technique currently providing non-invasive way to assess the free/bound ratio of NADH, whereas the short lifetime at the range of (~300-700 ps) is usually attributed to the free, and the long lifetime (~2500-3000 ps) with the bound forms of NADH.

Since the majority of the NADH activity is related to mitochondria, observation of the mitochondrial NADH redox state has a potential for detecting the changes in the cellular metabolic state and thus properly characterize normal and pathological states. Thus, the technique was able to reveal fine changes of the cellular metabolic activity at different cell density. Moreover, drastic changes in NADH lifetime at apoptosis as opposed to necrosis make the technique as a possible tool for the dosimetry adjustments and efficiency of therapy, involving tumor termination. And finally, the technique has a big potential in the diagnosis of a range of diseases – we demonstrate that the alteration of Complex I and IV of the ETC can be detected and possibly differentiated by NADH FLIM.

We believe that the NADH FLIM has further capacity still underexplored. Combination of the fluorescence lifetime and polarization microscopy may further extend the sensitivity of the technique, providing with the information on the changes in viscosity, size, and conformation of the molecules of interest. Thus, this anisotropy capacity may reveal the bound species, which exhibits shorter lifetime and is otherwise indistinguishable. Many enzymes bind to NADH in the metabolic pathway, and as favored metabolic pathways shift with cancer progression, resulting in the Warburg effect. The change in the distribution of NADH binding sites suggests increased significance of the bound NADH observation as a metabolic indicator. Here application of the fluorescence lifetime anisotropy imaging will provide with the increased sensitivity and the power to distinguish NADH bound to different enzymes, along with the changes in viscosity that is often accompanying the hypoxic conditions. Additionally, many newly developed techniques in the category of nanobio-spectroscopy are expected to further elucidate the roles and mechanisms related to NADH.

[1] D. H. Williamson, P. Lund, and H. A. Krebs, *Biochem J.* **103** (1967) 514.
[2] A. Mayevsky, N. Zarchin, H. Kaplan, J. Haveri, J. Haselgroove, and B. Chance**,** *Brain Res.* **276** (1983) 95.
[3] V. Ghukasyan and F.-J. Kao, *J. of Phys. Chem. C*, **113** (2009) 11532.

Name: Fu-Jen Kao, Ph.D.

Position:
Professor and Chair, Institute of Biophotonics Engineering, National Yang-Ming University, 155, Sec. 2, Li-Nong Street, Beitou District, Taipei 112, Taiwan

Education:
- Doctor of Philosophy (D. Phil) in Physical Sciences at Cornell University (August, 1993)
- Master of Science (MA) in Physical Sciences at Cornell University (August, 1988)
- Bachelor of Science (BA) in Physical Sciences at the National Taiwan University (June, 1983)

Major Professional Experiences:
- Professor and Chair, Institute of Biophotonics, National Yang-Ming University (since August, 2004)
- Chief of Research and Planning, Office of Research Affair, National Sun Yat-sen University (May, 2003-July, 2004)
- Professor, Institute of Electro-Optical Engineering, National Sun Yat-sen University (since August, 2003)
- Professor, Department of Physics, National Sun Yat-sen University (August, 2002-July, 2003)
- Associate Professor, Department of Physics, National Sun Yat-sen University (August, 1993-July, 2002)

List of recent publications and work
- Book: Peter Török and Fu-Jen Kao (Eds.), Optical Imaging and Microscopy-Techniques and Advanced Systems, ISBN 3-540-43493-3, Springer, Berlin, 2003.
- Over 30 SCI Journal Papers
- 14 EI Journal Papers
- 3 Other Referred Journal Papers
- Over 49 International Conference Presentations and Proceedings
- 1 US Patent and 4 domestic patents
- Technical reports and domestic publications

Magnetic Nanocrystals: from Preparations to Bioapplications

Mingyuan Gao

Institute of Chemistry, the Chinese Academy of Sciences, Beijing 100190, China

Superparamagnetic iron oxide nanoparticles have received great attention due to their applications as contrast agents for magnetic resonance imaging (MRI). Until now, the commercial iron oxide-based contrast agents are exclusively produced by hydrolytic synthetic routes, e.g., coprecipitation method. The growth of iron oxide nanoparticles in aqueous media is heavily controlled by a large number of kinetic factors not only because water and hydroxyl ion are involved, but also because complicated surface dynamics are induced by the strong polar aqueous media. Thus the resultant iron oxide particles are generally characterized by wide particle size distributions and relatively low magnetic susceptibility. In contrast, the recently developed thermal decomposition method overcomes the intrinsic drawbacks by adopting a different synthetic principle, i.e., pyrolysis rather than hydrolysis. Consequently the number of kinetic factors for growing iron oxide particles is greatly reduced in the high temperature environment due to the absence of bulk water and the indissolubility of the iron oxides in the non-polar and weak polar solvents. Thus, uniform iron oxide nanocrystals become more facially available through the thermal decomposition method as the surface dynamics are much simplified in comparison with those occurring in aqueous systems.

Nevertheless, acids, alcohols, and amines bearing long hydrophobic chain are required for producing iron oxide nanocrystals to eventually provide them surfaces compatible with non-polar environment. Consequently the direct products of the thermal decomposition syntheses are typically characterized by hydrophobicity. Only through further surface engineering by taking the surface biocompatibility and functionalizability into considerations, they become usable as MRI contrast agents.

We develop an alternative approach to achieve water soluble and biocompatible iron oxide nanocrystals meanwhile inheriting the advantages of the thermal decomposition method by replacing the non-polar or weak polar solvents with strong polar solvents[1,2], which further allows us to effectively combine the particle synthetic chemistry with surface engineering chemistry, consequently different types of biocompatible Fe_3O_4 nanocrystals can be obtained via "one-pot" reactions, which greatly simplifies the procedures for producing high quality MRI contrast agents[3-5]. In addition, we will also report our recent results on in vivo MRI tumor detection by using Fe_3O_4-based molecular probes[4-7].

[1] Z. Li, H. Chen, H. Bao, M. Y. Gao, *Chem. Mater.,* **16** (2004) 1391.
[2] Z. Li, Q. Sun, M. Y. Gao, *Angew. Chem. Int. Ed.,* **44** (2005) 123.
[3] Z. Li, L. Wei, M. Y. Gao, H. Lei, *Adv. Mater.,* **17** (2005) 1001.
[4] F. Hu, L. Wei, Z. Zhou, Y. Ran, Z. Li, M. Y. Gao, *Adv. Mater.,* **18** (2006) 2553.
[5] L. Wei, G. Zhou, Z. Li, L. He, M. Y. Gao, J. Tan, H. Lei, **25** (2007**)** 1442.
[6] S. Liu, B. Jia, R. Qiao, Z. Yang, Z. Yu, Z. Liu, K. Liu, H. Ouyang, F. Wang, and M. Y. Gao, *Molecular Pharmaceutics*, **6** (2009) 1074.
[7] R. Qiao, C. Yang, and M. Y. Gao, *J. Mater. Chem.*, (2009), in press (feature article).

Name: Mingyuan Gao, Ph.D.

Position:
Professor, Institute of Chemistry, the Chinese Academy of Sciences, Beijing, China

Education:
- BA: Polymer Chemistry and Physics, Jilin University, Changchun, China
- M.D.: Polymer Chemistry and Physics, Jilin University, Changchun, China
- Ph.D.: Polymer Chemistry and Physics, Jilin University, Changchun, China

Major Professional Experiences:
- Postdoc (AvH Fellow), Max-Planck-Institute of Colloids and Interfaces, Germany
- Staff scientist (group leader), University of Munich, Germany
- Professor, Institute of Chemistry, the Chinese Academy of Sciences, Beijing, China

Research Interests:
- Synthesis of inorganic nanocrystals with novel properties, unusual shapes and structures
- Biomedical and environmental applications of functional nanomaterials
- Synthesis of organic/inorganic hybrid materials

Measuring dynamic protein interactions in living cells over time

Muhammad Awais, Dave Spiller and Michael R. H. White

School of Biological Sciences, University of Liverpool, Crown Street, Liverpool,

L69 7ZB United Kingdom

Protein-protein interactions critically control for many cellular processes. Fluorescence resonance energy transfer (FRET) microscopy is a useful method to map the extent and location of protein interactions or protein conformational changes within living cells. Previously, we have developed intramolecular FRET probes in which donor and acceptor fluorophores are present on a single host protein which may be a concatenation of interacting domains. By observing changes in the FRET signal it is possible to infer ligand induced conformational changes in nuclear receptors (NRs) and their interactions with other cellular proteins. For signaling processes such as NF-kappaB (NF-κB) where dynamic interactions between separate proteins over time play very important role, the situation is more complex. Intermolecular FRET (when donor and acceptor fluorophores are fused to two different proteins) the stoichiometry of acceptors to donors can vary either between cells or their intracellular compartments. In these cases a FRET signal is far more difficult to observe because of fluorescence spillover. The only reliable ways of determining if interactions are occurring are by acceptor photobleaching or fluorescence lifetime imaging (FLIM). However, the former method only allows one measurement per cell at any given time. To overcome this limitation, we have developed a method in which FRET between a reversible-photoswitchable donor fluorescent protein Dronpa (λex: 503, λem: 518) and a DsRED-express (λex: 557, λem: 579) acceptor is measured repeatedly. The principal of this technique was tested by imaging the interactions between p65, the major component of the NF-κB signaling system and glucocorticoid receptor (GR) which has a vital role in anti-inflammation signaling. A strong FRET signal was observed when Dronpa was repeatedly switched on in defined regions within a microscope field of view using light from a Titanium Saphire laser (810 nm). The Dronpa and FRET signals were observed and modulated by 488nm light from an argon ion laser. This technique allowed the dynamic interactions between p65 and GR to be measured within cells after treatments that selectively activated either the NF-κB or GR signaling pathways. This technique may be generally applicable to any scenario where one wishes to measure and map the location of protein-protein interactions in living cells over time.

Name: Muhammad Awais, Ph.D.

Position:
Postdoctoral Research Fellow, Centre for Cell Imaging, University of Liverpool, Liverpool, UK

Education:
- The University of Tokyo, PhD, March 2005, Japan
- University of Punjab, MSc (First Class), May 1996, Pakistan
- University of Punjab, BSc, December 1992, Pakistan
- Forman Christian College, FSc, July 1990, Pakistan

Major Professional Experiences:
- September 2007-Present, Postdoctoral Research Fellow, Centre for Cell Imaging, School of Biological Science, the University of Liverpool, UK
- April 2006-August 2007, Postdoctoral Fellow, Institute for Molecular Sciences, Okazaki, Japan
- April 2005-March 2006, COE Postdoctoral Fellow, Department of Chemistry, the University of Tokyo, Tokyo, Japan
- May 1997-June 2000, Chemical Process Engineer, Army Welfare Pharmaceuticals, Pakistan

Research Interests:
- Fluorescent and Bioluminescent Cell-Based Assays for monitoring ligand-protein and protein-protein interactions, Cell Imaging, and Molecular Biology.

Coupling cellular oscillators in vertebrate segmentation: analysis with high-resolution imaging of gene transcription

Hiroyuki Takeda

Department of Biological Sciences, Graduate School of Science, University of Tokyo

A unique feature of vertebrate segmentation (somitogenesis) is its strict periodicity (Fig. 1), which is governed by the numerous cellular oscillators of the segmentation clock [1]. These cellular oscillators are driven by a negative-feedback loop of the Hairy transcription factor (Her1 and Her7 in zebrafish), are linked through Notch-dependent intercellular coupling and display the synchronous expression of clock genes (Fig. 2). Combining our transplantation experiments in zebrafish with mathematical simulations, we recently demonstrated how the cellular oscillators maintain synchrony and form a robust system that is resistant to the effects of developmental noise such as stochastic gene expression and active cell-proliferation. The accumulated evidence indicates that the segmentation clock behaves as 'coupled oscillators', which is a mechanism that also underlies the synchronous flashing seen in fireflies [2, 3].

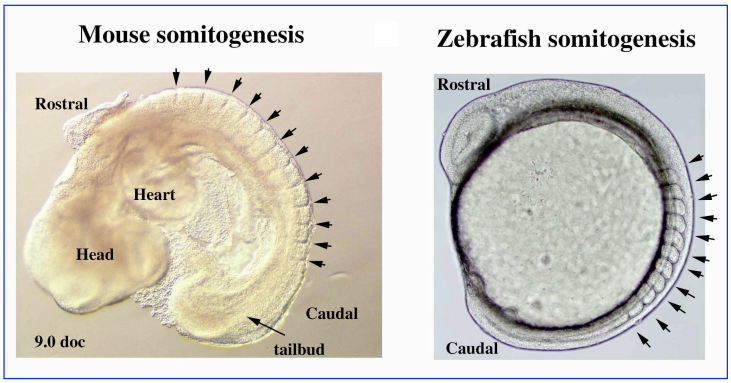


Fig. 1. Vertebrate somitogenesis
Left: mouse embryos (lateral view)
Right: zebrafish embryos (lateral view)
Arrow: somite
A pair of somites on both sides is formed every 2 hours in mouse and 30 minutes in zebrafish in an anterior to posterior direction.

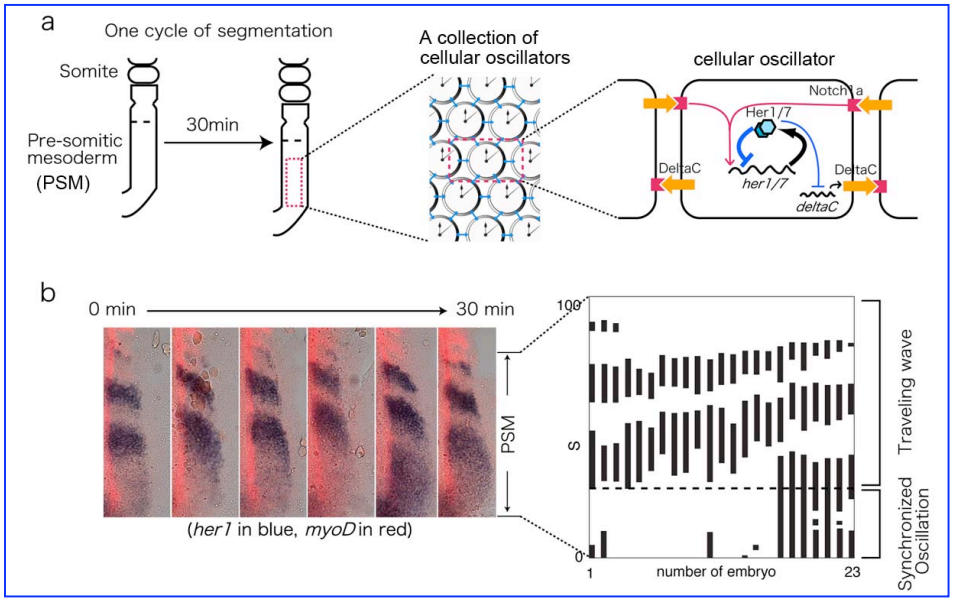


Fig. 2. Segmentation clock in zebrafish
a. Schematic representation of the zebrafish presomitic mesoderm (PSM) and PSM oscillators. b. Representative images of *her1* expression and a graph showing its expression profile. The positions of *her1*-positive cells in 23 embryos are indicated by a solid bar. The *her1* expression domain appears every 30 minutes in the posterior PSM, and travels anteriorly. The mode of *her1* expression changes from a synchronized to traveling mode in the intermediate zone (dotted line).

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Name: Hiroyuki Takeda, Ph.D.

Position:
Professor, Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Japan

Education:
- BA: Zoology, The University of Tokyo, Japan
- M.D.: Zoology, The University of Tokyo, Japan
- Ph.D.: Zoology, The University of Tokyo, Japan

Major Professional Experiences:
- Research Associate, Department of Zoology, School of Science, The University of Tokyo, Japan
- Associate Professor, Department of Biological Sciences, Nagoya University, Japan
- Professor, National Institute of Genetics, Japan
- Professor, Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Japan

Research Interests:
- Vertebrate axis formation
- Vertebrate organogenesis
- Genome Science

Structure of regulatory networks and dynamics of bio-molecules: Predicting unknown from known

A. Mochizuki[1,2,3] and D. Saito[1]

[1] Theoretical Biology Laboratory, RIKEN Advanced Science Institute, Wako 351-0198, Japan
[2] Department of computational intelligence and systems science, Tokyo Institute of Technology, Yokohama, 226-8503, Japan
[3] JST PRESTO

Regulations of biological molecules constitute complex network systems which generate various developmental or physiological functions via dynamics of molecular activities. However, we have very little understanding on the relationship between the structure of network and its dynamical nature. In this study, we introduce a mathematical theory of network dynamics to show the relation between structure of network and dynamics of molecular activities. We show examples of its applications to some experimentally determined regulatory networks. The basic premise is the "functionality": the dynamics of an activity of molecule should be uniquely determined by the activities of controlling entities in the network. We formalize two aspects of this idea, "incompatibility" and "independency". The "incompatibility" determines the upper limit of number of possible steady states of molecular activities realized by a given network. The "independency" determines the possible combinations of states of the system. These constraints (upper limit and possible combinations) on the molecular activities are determined only from the topological structure of a regulatory network without any assumptions of dynamics. Thus, if we found any inconsistencies among the experimental data using the theory, they lead to the prediction of unknown states or unknown regulations. This method was applied to some regulatory networks including the gene network for early development of sea urchin. We found a set of genes whose activity is responsible for the diverse steady states observed during development. Further, the inconsistency with the observed expression pattern indicated the presence of unknown regulation, which has been confirmed by later experiment. We applied this method also to the regulatory network of signal transduction pathway.

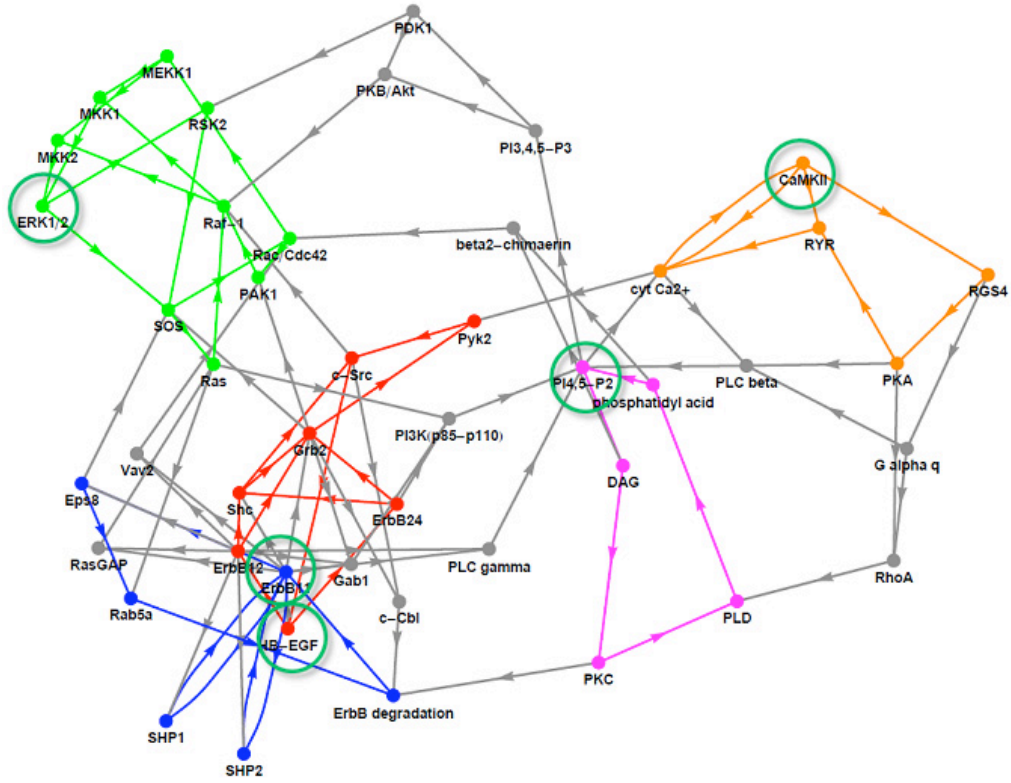

Figure 1: Network of signal transduction and informative nodes.

[1] A. Mochizuki, *J. Theor. Biol.*, **250** (2008) 307-321.

Name: Atsushi Mochizuki, Ph.D.

Position:
Chief Scientist, Theoretical Biology Laboratory, RIKEN Advanced Science Institute, Japan

Education:
- BA: Science, Kyoto University, Japan
- M.D.: Biology, Kyushu University, Japan
- Ph.D.: Biology, Kyushu University, Japan

Major Professional Experiences:
- Assistant Professor, Department of Biology, Kyushu University, Japan
- Associate Professor, Division of Theoretical Biology, National Institute for Basic Biology, Japan
- Chief Scientist, Theoretical Biology Laboratory, RIKEN Advanced Science Institute, Japan

Research Interests:
- Mathematical Biology
- Regulatory networks of biological systems
- Pattern Formation in development
- Evolution of complex systems

High throughput quantification of single cellular signaling events by use of immunostaining and image cytometry

Y. Ozaki[1], S. Uda[1], T. Saito[1], J. Chung[1], H. Kubota[1] and S. Kuroda[1,2]

[1] Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo
[2] CREST, Japan Science and Technology Agency University of Tokyo

Modeling of cellular functions on the basis of experimental observation is increasingly common in the field of cellular signaling. However, such modeling requires a large amount of quantitative data of signaling events, including phosphorylation and the localization and expression of signaling molecules, with high spatio-temporal resolution. There are a variety of techniques for quantitative measurements of signaling activities. Among them, western blotting, flow cytometry, and live cell imaging are the most commonly used, owing to their specificity (Table 1). However, there is no versatile and high throughput technique for quantitative measurements of the signaling activities in adherent cells with single cell resolution. To obtain such data, we developed a fully automatable assay technique, termed quantitative image cytometry (QIC), which integrates a quantitative immunostaining technique and a high precision image-processing algorithm for cell identification. With the aid of an automated sample preparation system, this assay system can quantify protein expression, phosphorylation and localization with subcellular resolution at one-minute intervals. The signaling activities quantified by the assay system showed good correlation with, as well as comparable reproducibility to, western blot analysis. Using the QIC technique, we observed the signaling dynamics of the ERK pathway in PC12 cells, and confirmed that the results of the analyses were consistent with previous reports. In the presentation, we will discuss the utility of this high-throughput and high-content assay system for investigating the systems biology of cellular signaling.

Table 1

	Quantitative image cytometry	Western blot	Flow cytometry of fixed cells	Live cell imaging
Automated sample preparation	Yes	No	No	Not required
Possible signaling interference	No	No	Detaching adherent cells	Introduction of Fluorescent probes
Resolution of quntification	Subcellular structure	Population of cells	Single cell	Subcellular structure
Sample format	Fixed adherent cells	Lysed cells	Fixed cell suspension	Live adherent cells
Phospho-signal	Yes	Yes	Yes	Limited
Probe	Antibody	Antibody	Antibody	Fluorescent proteins
Temporal analysis	Snapshot	Snapshot	Snapshot	Time-lapse
Signal specificity	Antibody specificity and cellular localization	Antibody specificity and molecular weight	Antibody Specificity	Specificity of Fluorescent probe
Multiplex assay	Yes	No	Yes	Yes

Name: Yu-ichi Ozaki, Ph.D.

Position:
Assistant professor, Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, Japan

Education:
- BS: Mathematics, Tsukuba University, Japan
- M.Sc.: Mathematics, Tsukuba University, Japan
- Ph.D.: Biology, The University of Tokyo, Japan

Major Professional Experiences:
- Assistant professor, Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, Japan

Research Interests:
- Systems biology of the ERK signaling network
- Development of high throughput assays for the analysis of cellular signaling

Application of luciferases for *in vivo* bioluminescence imaging

Yoshihiro Ohmiya[1,2], Hidetoshi Akimoto[2], and Chun Wu[3]

[1] Research Institute of Genomic-based Biofactory, National Institute for AIST. Sapporo, Japan
[2] Gradate School of Medicine, Hokkaido University, Sapporo, Japan
[3] Research Institute of Cell Engineering, National Institute for AIST. Sapporo, Japan

In the postgenomic era, molecular imaging has become essential for elucidating whole animal physiology. Genes and proteins in living cells can now be visualized using luciferase-based bioluminescent probes. In this presentation, I do introduce *in vivo* bioluminescence imaging (BLI) using beetle luciferase and Cypridina luciferase probes.

At the first topics, it has been accepted that bone marrow cells infiltrate the brain and play important roles in neuroinflammation. However, there is no good tool for the visualization of these cells in living animals. In this study, we generated mice that were transplanted with GFP- or luciferase-expressing bone marrow cells, and performed *in vivo* fluorescence imaging (FLI) and *in vivo* bioluminescence imaging (BLI) to visualize the infiltrated cells. Brain inflammation was induced by intrahippocampal injection of lipopolysaccharide (LPS). Immunohistochemical investigation demonstrated an increase in the infiltration of bone marrow cells into the hippocampus because of the LPS injection and differentiation of the infiltrated cells into microglia, but not into neurons or astrocytes. BLI, but not FLI, successfully detected an increase in signal intensity with the LPS injection, and the increase of BLI coincided with that of luciferase activity in hippocampus. So, BLI could quantitatively monitor bone marrow-derived cells in brain inflammation *in vivo* [1].

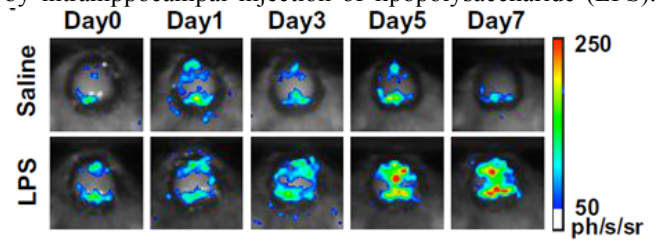

Fig. 1. *In vivo* bioluminescence imaging of bone marrow-derived cells. Representative *in vivo* bioluminescence imaging of bone marrow-derived cells.

At the second topics, we aimed to develop a new far-red luminescence imaging technology for visualization of disease specific antigens on cell surfaces in a living body. First, we conjugated a far-red fluorescent indocyanine derivative to biotinylated *Cypridina* luciferase. This conjugate produced a bimodal spectrum having long-wavelength bioluminescence emission in the far-red region as a result of bioluminescence resonance energy transfer. To generate a new far-red luminescent probe with targeting and imaging capabilities of tumors, we then linked this conjugate to an anti-human Dlk-1 monoclonal antibody via the biotin-avidin interaction. This far-red luminescent probe enabled us to obtain high-resolution microscopic images of Dlk-1-expressing Huh-7 live cells without an external light source, and to monitor the accumulation of this probe in tumor-bearing mice. Thus this far-red luminescent probe is a convenient analytical tool for the evaluations of monoclonal antibody localization in a living body [2].

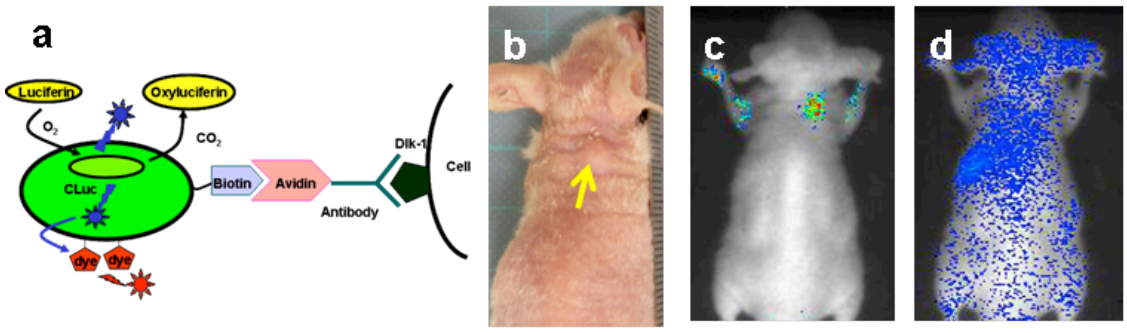

Fig.2-a) Schematic of FBP-IgG showing targeting to tumor cells. b) Photographs of two tumor-bearing mice. c) 4 hours after the administration of FBP-IgG, luciferin was injected and BLI were obtained using a CCD photon imaging system. d) FII of the tumor-bearing mice shown in panel b after administration of FBP-IgG, but before luciferin injection.

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[2] Wu C, Mino K, Akimoto H, Kawabata M, Nakamura K, Ozaki M' Ohmiya Y, *Proc Natl. Ame. Sci,* in press.

Name: Yoshihiro Ohmiya Ph.D.

Position:
Principal Research Scientist, Research Institute of Genome-based Biofactory, National Institute of Advance Industrial Science and Technology

Education:
- BA: Applied Materials Sciences, Gunma University, Japan
- M.D.: Materials Science, Gunma University, Japan
- Ph.D.: Biochemistry, Gunma University, Japan

Major Professional Experiences:

- Associate Professor, Department of Chemistry, Faculty of Education, Shizuoka University, Japan
- Leader of Cell Dynamics Research Group, National Institute of Advance Industrial Science and Technology, Osaka, Japan
- Professor of Department of Photobiology, Graduate School of Medicine, Hokkaido University, Sapporo, Japan

Research Interests:
- Basic and medical application of Bioluminescence system
- Molecular mechanism of bioluminescence system

Bio-imaging of Surgical Stress
dynamic analysis of liver oxidative stress and damage

M. Ozaki[1], S.Haga[1,2], T. Ozawa[3], SJ. Remington[4], N. Morita[5], Y. Ohmiya[6]

[1] *Hokkaido University School of Medicine, Department of Molecular Surgery*
[2]*TheJapanSocietyforthePromotionofScience (JSPS)*
[3]*Department of Chemistry, School of Science, The University of Tokyo*
[4]*Institute of Molecular Biology, Howard Hughes Medical Institute and Department of Physics, University of Oregon*
[5] *Research Institute of Genome-based Biofactory,* [6] *Research Institute of Cell Engineering,*
National Institute of Advanced Industrial Science and Technology (AIST)

Realtime monitoring of cellular conditions lead to better understandings of various physio-pathological phenomena, and will provide options in clinical diagnoses and therapies. We developed redox sensitive GFP (roGFP) and luciferase-based caspase-3 optical probes for in vivo imaging, and tried to visualize the dynamic changes of oxidative stress (OS) and the following damage in mouse liver ischemia/reperfusion (I/R) and partial hepatectomy (PH) models.

[Materials & Methods]

A newly developed roGFP is a mutant GFP replaced with C48S, S65T, S147C, and Q204C which renders the redox sensitive property to GFP. The ratios of fluorescence from excitation at 400 and 480 nm changed in response to chemically induced OS [1]. Also, we developed a novel probe (pcFluc-DEVD) reflecting caspase-3 activity. Split N-/C-terminal ends of luciferase were connected to the substrate sequence (DEVD) for caspase-3 (inactive). Once caspase-3 is activated in cells (DEVD is cleaved), Fluc changes into an active form, restoring luminescence activity [2].

By transfecting adenovirus vectors cording for *roGFP* (Ad*roGFP*) or *pcFluc-DEVD* (Ad*pcFluc*), we investigated whether these probes will monitor redox states and apoptosis in live cells and liver during hypoxia/reoxygenation (H/R) , I/R and PH in mice [3,4].

[Results]

roGFP visualized H/R-induced dynamic changes of cellular redox states. Cellular redox was slightly reduced during hypoxia, but was rapidly but transiently oxidized post-reoxygenation. roGFP well illustrated the anti-oxidative effects of N-acetyl cysteine, catalase, and Ref-1 on H/R-induced cellular OS. Similarly, pcFluc probe reflected cellular caspase-3 activity induced by various pro-apoptotic stimuli dose-dependently (FasL, staurosporine and H/R). These probes also illustrated the redox changes by the repeated stimuli, indicating that these probes functioned reversibly.

In mouse liver I/R experiment, adenovirally transfected roGFP showed two peaks of OS in the post-ischemic liver. The early OS peak, originating from liver cells, was observed within 60 min, and increased its intensity in proportion to the ischemic time and the following liver injury. The second and larger peak of OS, which originates from infiltrating neutrophils, was observed 24 hr post-ischemia or later. pcFluc probe indicated on-going processes of liver damage quantitatively by visualizing the dynamic changes of caspase-3 activities in the post-ischemic liver. These probes together revealed time-/strength-relationships of OS and damages in I/R model.

Regarding PH model of mice, post-PH oxidative stress and apoptotic cell death were not observed at all in young / lean mice. Aged and obesed mice with fatty liver showed marked oxidative stress and injury immediately after PH.

[Summary]

The roGFP redox- and pcFluc caspase-probes successfully illustrated OS and the following damage in vitro and in vivo. By visualizing the organ conditions, these may provide many options for diagnoses and treatments in the future.

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Name:
OZAKI, Michitaka MD, PhD

Position:
Professor, Department of Molecular Surgery, Hokkaido University School of Medicine

Education:
1984 M.D., Okayama University Medical School, Okayama, Japan
1988 Ph.D., Okayama University, Okayama, Japan

Major Professional Experiences:
1988 - 1991: Clinical residency for surgery, Dept of Surgery, Okayama University Medical School, Okayama
1991 - 1997: Department of Surgery, Tokyo Women's Medical College, Tokyo (in clinical liver, pancreas & kidney transplantation program)
1997 - 1998: Research fellow, Surgery, Johns Hopkins University School of Medicine, Baltimore, Maryland
1998 - 1999: Research fellow, Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland
1999 - 2003: Senior Research Scientist, Bioengineering Laboratory, Department of Innovative Surgery, National Research Institute for Child Health and Development, Tokyo, Japan
2003 - 2004: Associate professor, Department of Food and Health Science, Okayama University Graduate School of Medicine and Dentistry, Faculty of Medicine
2004 - 2007: Associate professor, Department of Surgery, Division of Organ Transplantation and Regenerative Medicine, Hokkaido University School of Medicine
2007 - Present: Professor, Department of Molecular Surgery, Hokkaido University School of Medicine

Research Interests:
- Cell/organ stresses including surgical stress and oxidative stress (hypoxia/reoxygenation, ischemia/reperfusion)
- Bio-imaging
- Liver physio-pathology
- Cell/organ transplantation

Bioluminescent Probes to Visualize Biological Functions in Living Cells

Akira Kanno[1], Noki Hida[1], Takeaki Ozawa[1,2]

[1] Department of Chemistry, School of Science, The University of Tokyo
[2] PRESTO, Japan Science and Technology Agency

During the past decades, molecular biology has developed mostly by taking advantage of physical and chemical methods of analysis. In addition, developments of novel methods and advances in biotechniques have provided us with many basic tools to quantify and sense intracellular events in living subjects. Biological tools such as green fluorescent protein (GFP) and luciferase have become indispensable for further understanding of biological systems. Growing demand for simple methods to elucidate such biological processes has promoted developments of general strategies. Among them, dissection and subsequent reconstitution of reporter proteins from its peptide fragments opened a new way for studying the functions of proteins in living cells. We have developed a novel strategy of protein reconstitution system from reporter fragments. Here we present a couple of genetically encoded bioluminescent probes recently developed for monitoring the dynamics of proteins in living subjects.

Cyclic Luciferase for Real-Time Sensing of Caspase-3 Activities in Living Mammals[1]

Apoptosis is a crucial process involved in pathogenesis and progression of diseases, which is executed by caspases. We developed a genetically encoded bio-luminescent indicator for high- throughput sensing and noninvasive real-time imaging of caspase activities in living cells and animals. Firefly luciferase connected with a substrate sequence of caspase-3 (Asp-Glu-Val-Asp) is cyclized by a DnaE intein. When the cyclic luciferase is expressed in living cells, the luciferase activity greatly decreases due to a steric effect. If caspase-3 is activated in the cells, it cleaves the substrate sequence embedded in the cyclic luciferase and the luciferase activity is restored. We demonstrated quantitative sensing of caspase-3 activities in living cells upon extracellular stimuli. Furthermore, the indicator enabled noninvasive imaging of the time-dependent caspase-3 activities in living mice.

Real-Time Imaging of Dual Protein–Protein Interactions Using Multicolor Luciferases[2]

An effective methodology to assess protein–protein interactions in living cells of interest is protein-fragment complement assay (PCA). Using multicolor luciferase with different spectral characteristics for PCAs, we developed an imaging method for real-time analysis of protein–protein interactions. We demonstrate its utility in spatiotemporal characterization of Smad1–Smad4 and Smad2–Smad4 interactions in early developing stages of a single living *Xenopus laevis* embryo. We also describe the value of this method by application of specific protein–protein interactions in cell cultures and living mice.

These techniques provide general means for quantitative real-time sensing of protein dynamics, and for screening novel pharmacological chemicals among candidates in living subjects.

[1] A. Kanno, Y. Yamanaka, H. Hirano, Y. Umezawa and T. Ozawa, *Angew. Chem. Int. Ed.*, **46** (2007) 7595.
[2] N. Hida, M. Awais, M. Takeuchi, N. Ueno, M. Tashiro, C. Takagi, T. Singh, M. Hayashi, Y. Ohmiya and T. Ozawa, *PLoS ONE*, **4** (2009) e5868.

Name: Akira Kanno, Ph.D.

Position:
- Assistant Professor, Department of Chemistry, School of Science, The University of Tokyo, Japan

Education:
- BA: Chemistry, The University of Tokyo, Japan
- M.D.: Chemistry, The University of Tokyo, Japan
- Ph.D.: Chemistry, The University of Tokyo, Japan

Major Professional Experiences:
- Postdoctoral Research Associate at Department of Chemistry, Graduate School of Science, The University of Tokyo
- Postdoctoral Research Associate at Department of General Systems Studies, Graduate School / College of Arts and Sciences, The University of Tokyo
- Professor, Department of Chemistry, School of Science, The University of Tokyo, Japan

Research Interests:
- Analytical Chemistry
- Protein Chemistry

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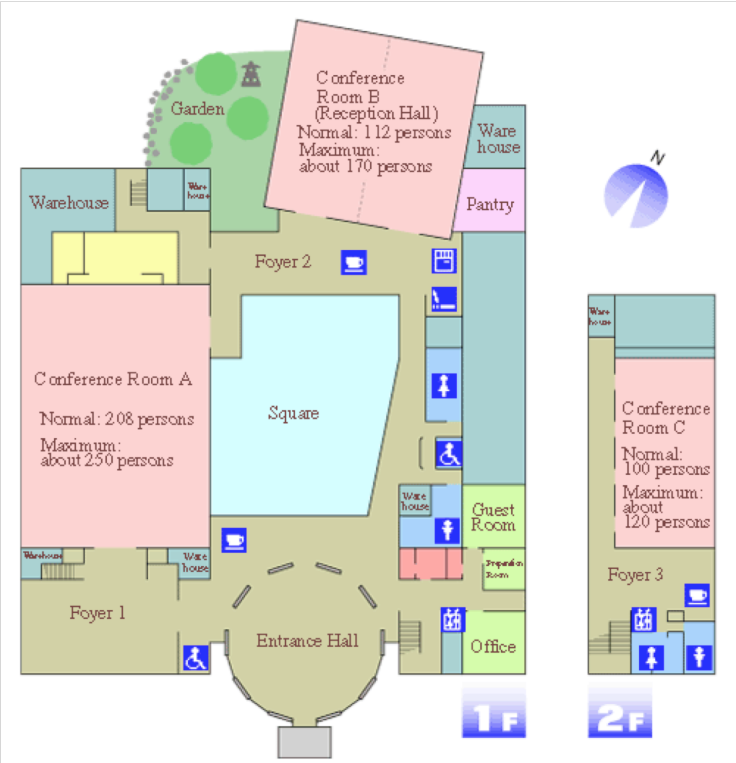

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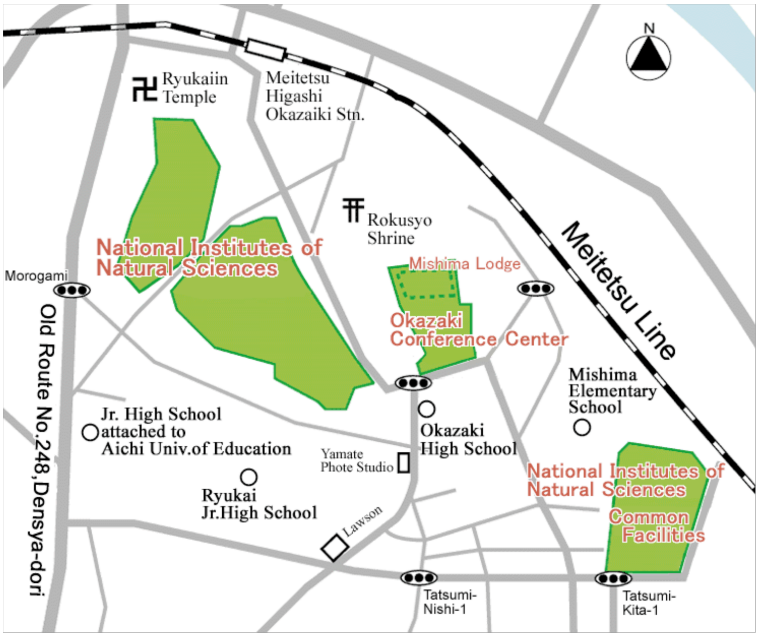
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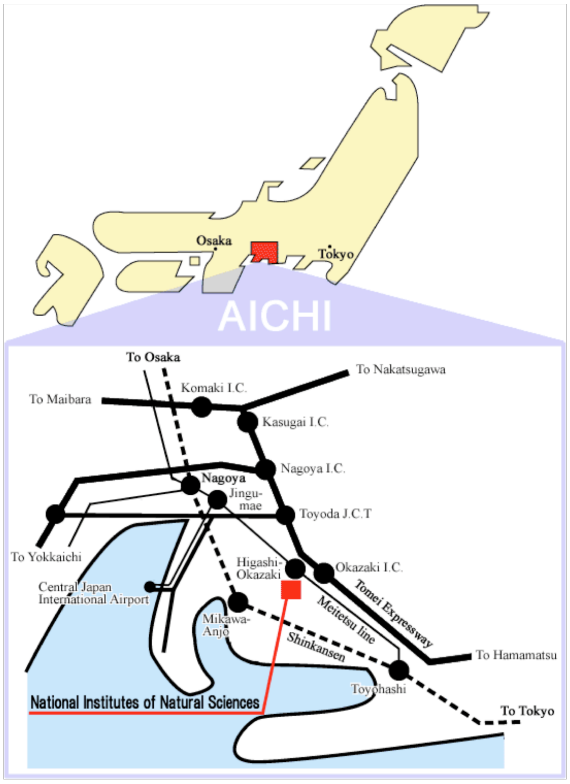
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