08:25-08:30, Yoshiki Chujo (a vice president of the CSJ)

Session 1

Chair: Yanyi Huang (Peking University)

1) 08:30-08:50, Kenjiro Hanaoka (The University of Tokyo)

Title: Development of Far-red to Near-Infrared Small-molecule Fluorophores and their Applications to Multicolor Imaging

2) 08:50-09:10, Chunhai Fan (Shanghai Institute of Applied Physics, Chinese Academy of Sciences)

Title: Creating dynamic organization with DNA nano structures

3) 09:10-09:30, Yousuke Takaoka (Tohoku University)

Title: Self-assembling turn-on nanoprobe for endogenous protein sensing

4) 09:30-09:50, Xing Chen (Peking University)

Title: Live-Cell bioorthogonal Raman imaging

5) 09:50-10:10, Hiroshi Abe (Hokkaido University)

Title: DNA templated chemical reaction for detection of nucleic acids

10:10-10:30, Coffee break

(10:28-10:30, Introduction of the CSJ journals by Shinichi Suzuki)

Session 2

Chair: Katsunori Tanaka (RIKEN)

6) 10:30-10:50, Xingyu Jiang (National Center for NanoScience and Technology of China)

Title: Live imaging of mitocondria in cells

7) 10:50-11:10, Tomoya Hirano (Tokyo Medical and Dental University (TMDU))

Title: Development of Various Fluorescent Sensors Based on the Construction of Fluorescent Compound Library

8) 11:10-11:30, Zhi Zhu (Xiamen University)

Title: Nucleic Acid Aptamers for Bioanalysis and Biomedicine

9) 11:30-11:50, Shin Mizukami (Osaka University)

Title: Development of smart ^{19}F MRI probes for in vivo imaging

12:00-13:30, Lunch

(group photograph)

Session 3

Chair: Zhi Zhu (Xiamen University)

10) 13:40-14:00, Jing Zhao (Nanjing University)

Title: Inorganic Bioimaging: Cell and Synthetic Approaches

11) 14:00-14:20, Katsunori Tanaka (RIKEN)

Title: Therapeutic In Vivo Synthetic Chemistry: Total Synthesis of Bioactive Compounds in Live Animals

12) 14:20-14:40, Peng Chen (Peking University)

Title: Protein chemistry in living cells

13) 14:40-15:00, Mitsuru Hattori (The University of Tokyo)

Title: Development of Bioluminescent Probes for Analysis of Intracellular Environment and Signaling

15:00-15:20, Coffee break

Session 4

Chair: Kenjiro Hanaoka (The University of Tokyo)

14) 15:20-15:40, Qing Huang (Shanghai Institute of Applied Physics, Chinese Academy of Sciences)

Title: Synchrotron radiation based X-ray imaging for cells

15) 15:40-16:00, Toshitada Yoshihara (Gunma University)

Title: Phosphorescent molecular probes for imaging oxygen levels in living cells and hypoxic tissues

16) 16:00-16:20, Fuyou Li (Fudan University)

Title: Upconversion luminescent materials for bioimaging

17) 16:20-16:40, Hiromu Kashida (Nagoya University)

Title: Highly-sensitive RNA detection by using In Stem Molecular Beacon

18) 16:40-17:00, Yan He (Hunan University)

Title: Single molecule spectroscopy of plasmonic metal nanoparticles

18:00-20:00, Dinner (Quanjude Peking Duck)

Development of Far-red to Near-Infrared Small-molecule Fluorophores and their Applications to Multicolor Imaging

Kenjiro HANAOKA
Graduate School of Pharmaceutical Sciences, The University of Tokyo
Tokyo 113-0033, Japan

Fluorescence imaging is one of the most powerful techniques for visualization of the temporal and spatial biological events in living cells, and fluorescence probes are very useful for this technique. For the development of fluorescence probes, the fluorophores are important, for example, fluorescein has been widely utilized as the fluorescent core for a large number of fluorescence probes. On the other hand, fluorescence probes in green region have been sufficiently developed, however, those in far-red to near-infrared (NIR) color region are still needed. Therefore, we set out to develop fluorescence probes in far-red to NIR color region, since we expected that they would be useful for multi-color imaging with various kinds of indicators having green fluorescence such as GFP. So far, we have developed a novel fluorescein analogue, TokyoMagenta (TM), in which the O atom at the 10 position of the xanthene chromophore of fluorescein is replaced with a Si atom. The absorption and emission wavelengths of TM were about 90 nm longer than those of fluorescein. We further developed a red fluorescence probe for β-galactosidase and Ca^{2+}. We have also developed a novel red fluorescent dye, 2Me SiR600, in which the O atom of Rhodamine Green at the 10 position of the xanthene moiety is replaced with a Si atom, as a scaffold for probes to detect protease activity. Furthermore, the NIR fluorescent dyes based on Si-rhodamines have been developed, and could be utilized for the development of fluorescence probes targeted to calcium ions and antibody labeling reagents.

In the talk, I will talk about hypoxia-sensitive fluorescence probes. We focused on the fluorescence quenching property of azo compounds which are utilized for dark quenchers and developed green and red fluorescence probes, MAR and MASR, for detecting hypoxia on the basis of rhodamine green and SiR600 scaffolds. This kind of azo compounds were also selectively reduced by reductases under hypoxic conditions.

Kenjiro HANAOKA received his B.S. in 2000 from Faculty of Pharmaceutical Sciences, The University of Tokyo and then his Ph.D. in 2005 from Graduate School of Pharmaceutical Sciences, The University of Tokyo (Prof. Tetsuo Nagano). He was a JSPS research fellow (2005–2007), and also worked with Prof. Thomas Kodadek at University of Texas Southwestern Medical Center. In 2007, he was appointed as an Assistant Professor at Graduate School of Pharmaceutical Sciences, The University of Tokyo. He is Associate Professor in Graduate School of Pharmaceutical Sciences, The University of Tokyo. His research interests are in the development of chemical biology tools which can visualize or regulate biological phenomena, such as fluorescence probes and inhibitors.

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Creating Dynamic Organization with DNA Nanostructures

Chunhai Fan
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Living bodies are characteristic of dynamic organization. How to reconstruct dynamic organization in vitro remains a grand challenge. DNA nanotechnology provides a promising route to this long-term goal. Here I will present several examples of DNA nanotechnology-enabled dynamic organization, ranging from molecular, nanometer and macroscopic scale.

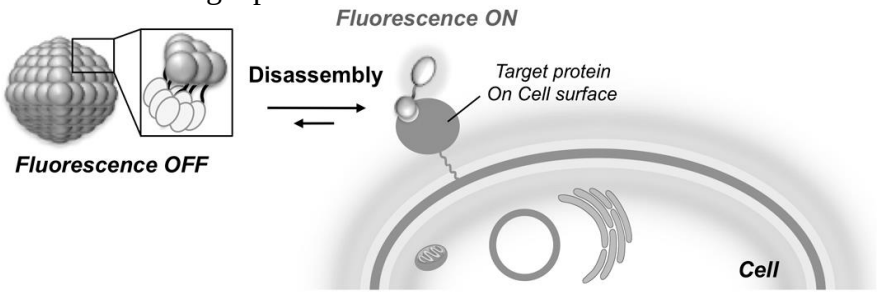
I will first demonstrate how we site specifically organize small molecules and biomacromolecules with self-assembled DNA nanostructures. These nanoassemblies have various applications for lasing, nanoreactors and nanoagents for intracellular imaging and delivery. We can also anchor metal nanoparticles to DNA nanostructures, endowing these soft materials with unique optoelectronic properties. And last, I will show an example that we employ DNA nanostrcutres to engineering macroscopic the biosensing interface. This strategy provides significantly enhanced spatial positioning range and accessibility of the probes on surface over previously reported linear or stem-loop probe structures. We demonstrate the use of this versatile DNA nanostructure-based platform for highly specific and sensitive electrochemical sensing of a wide range of biomolecules.

Self-assembling turn-on nanoprobe for endogenous protein sensing

Yousuke TAKAOKA, Tatsuyuki YOSHII, Keigo MIZUSAWA, and Itaru HAMACHI
Graduate School of Science, Faculty of Chemistry
Tohoku University, Aramakiaza aoba, Sendai, JAPAN

We developed a self-assembling turn-on fluorescent nanoprobes that can be used for fluorescent imaging of protein biomarkers under live cell contexts, as well as in test tube settings.

Visualization of tumor-specific protein biomarkers on cell membranes has the potential to contribute greatly to basic biological research and therapeutic applications. We recently reported a unique supramolecular strategy for specific protein detection using self-assembling ^{19}F-NMR or fluorescent nanoprobes consisting a hydrophilic protein ligand and a hydrophobic detection modality. This method is based on recognition-driven disassembly of the nanoprobes, which induces a clear turn-on fluorescent signal. In the present study, we have successfully extended the range of applicable fluorophores, by newly introducing a hydrophobic module to finely tune the aggregation properties of the probe. This greatly enhanced the flexibility of the probe design. These probes allowed selective imaging of membrane-bound endogenously expressed proteins under live cell conditions. Moreover, an extended nanoprobe was successfully used in a cell-based inhibitor assay for enzyme on the surface of live cells. This highlights the supramolecular approach as reversible sensing method for the target protein.



Yousuke TAKAOKA received his B.S. from Kyushu University and Ph.D (chemical biology) from Kyoto University (supervisor: Prof. Hamachi) in 2005 and 2010, respectively. He worked at the University of Tokyo with Prof. Kenzo Hirose (Postdoc in Neurobiology) and then moved to Kyoto University with Prof. Itaru Hamachi (Assistant Professor in Chemical biology) from 2011 – 2014. He joined Tohoku University with Prof. Minoru Ueda (Chemical biology for Natural Products) in July, 2014. He is lector in Graduate school of Science. His current research interests are protein imaging, protein labeling and protein engineering in living cells.

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Live-Cell Bioorthogonal Raman Imaging

Senlian Hong[1], Tao Chen[2], Xiangdong Tian[3], Liang Lin[1], Ming Xiao[1], Zhong-Qun Tian[3], Yanyi Huang[2], Xing Chen[1]

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Live-cell imaging of biomolecules with high specificity and sensitivity as well as minimal perturbation is essential for studying cellular processes. Here we report the development of a bioorthognal Raman imaging approach that exploits small Raman reporters for visualizing various kinds of biomolecules including nucleic acids, proteins, glycans, and lipids. The Raman reproters including the azide, alkyne, nitrile, and carbon-deuterium bond are small in size and spectroscopically bioorthogonal (background-free). We have demonstrated this strategy by using two Raman imaging techniques, surface-enhanced Raman scattering (SERS) microscopy and stimulated Raman scattering (SRS) microscopy. Our live-cell bioorthognal Raman imaging strategy expands the capabilities of live-cell microscopy beyond the modalities of fluorescence and label-free imaging.

Reference:

1. Lin, L.; Tian, X.; Hong, S.; Dai, P.; You, Q.; Wang, R.; Feng, L.; Xie, C.; Tian, Z.; Chen, X. "A Bioorthogonal Raman Reporter Strategy for SERS Detection of Glycans on Live Cells" *Angew. Chem. Int. Ed.* **2013**, 52, 7266-7271.

2. Hong, S.; Chen, T.; Zhu, Y.; Li, A.; Huang, Y.; Chen, X. "Live-Cell Stimulated Raman Scattering Imaging of Alkyne-Tagged Biomolecules" *Angew. Chem. Int. Ed.* **2014,** 53, 5827-5831.

3. Xiao, M.; Lin, L.; Li, Z.; Liu, J.; Hong, S.; Li, Y.; Zheng, M.; Duan, X.; Chen, X. "SERS Imaging of Cell-Surface Biomolecules Metabolically Labeled With Bioorthogonal Raman Reporters" *Chem. Asian J.* **2014**, DOI: 10.1002/asia.201402151.

Xing CHEN completed his undergraduate degree in Chemistry from Tsinghua University in 2002. Dr. Chen then obtained his Ph.D. in Chemistry from University of California, Berkeley in 2007, where his research focused on chemical biology and bionanotechnology. After completing postdoctoral work at Harvard Medical School in the field of structural biology and immunology, he joined the Peking University faculty in September, 2010. The current research interest of Dr. Chen is focused on chemical glycobiology.

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DNA templated chemical reaction for detection of nucleic acids

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Oligonucleotide-templated reactions are powerful tools for the detection of nucleic acid sequences. One of the major scientific challenges associated with this technique is the rational design of non-enzyme-mediated catalytic templated reactions capable of multiple turnovers that provide high levels of signal amplification.

Herein, we report the development of a nucleophilic aromatic substitution reaction-triggered fluorescent probe. The probe underwent a rapid tem-plated reaction without any of the undesired background reactions. The fluorogenic reaction conducted in the presence of a template provided a 223-fold increase in fluorescence after 30 s compared with the non-templated reaction. The probe provided an efficient level of signal amplification that ultimately enabled particularly sensitive levels of detection. Assuming a simple model for the templated reactions, it was possible to estimate the rate constants of the chemical reaction in the presence and in the absence of the template. From these kinetic analyses, it was possible to confirm that an efficient turnover cycle had been achieved, based on the dramatic enhancement in the rate of the chemical reaction considered to be the rate-determining step. With maximized turnover efficiency, it was demonstrated that the probe could offer a high turnover number of 1500 times to enable sensitive levels of detection with a detection limit of 0.5 pM in the catalytic templated reactions.

Hiroshi Abe received his B.S. (Pharmacy) and Ph. D. (Medicinal Chemistry) from Hokkaido University in 1991 and 2001, respectively. He worked at MIT with JoAnne Stubbe (Postdoc in Biochemistry) and then moved to Stanford with Eric Kool (Postdoc in Bioorganic Chemistry). He joined RIKEN as research scientist in 2005 and became senior research scientist in 2009. He is Associate Professor in Faculty of Pharmaceutical Sciences, Hokkaido University. His current research interests are bio-imaging and drug medicinal chemistry.

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Imaging and Manipulation of Mitoncondria in Live Cells using Molecular Aggregates

Wenwen Liu, Zhuo Wang and Xingyu Jiang

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We show here that an organic molecule that can both image mitochondria and control its activity. This work shows that aggregates of organic molecules in the nanoscale can both image the mitochondria, and selectively inactivate mitochondria to kill tumor cells. This molecule outperforms existing dyes for mitochondrial imaging not only in stability, but also in that it can monitor the mitochondrial membrane potential ($\Delta\Psi m$); it may also serve as highly localized source of singlet oxygen in selective eradication of cells (such as in cancer treatment) when irradiated with light.

Xingyu JIANG obtained his B.S. at the University of Chicago (1999), followed by a PhD. (2004) from Harvard University (Chemistry), working with Professor George Whitesides on microfluidics and cell patterning. After a short postdoctoral fellowship with Professor George Whitesides, he joined the National Center for Nanoscience and Technology in 2005 as a professor where he has remained since and is now also serving as the deputy director of the Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety (Chinese Academy of Sciences). He is a "Hundred Talents Plan" Professor and Top Young Talent of National High-level Talents Special Support Plan. He was awarded the National Distinguished Young Scholars granted by NSFC. His current research interests are surface chemistry, microfluidics, micro/nano-fabrication, cell biology, immunoassays, and nanomedicine.

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Development of Various Fluorescent Sensors Based on the Construction of Fluorescent Compound Library

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Fluorescent sensors, whose fluorescence properties are changed by each analyte such as ion, small molecule and environment change, have been utilized in various fields of scientific research. For development of such sensors, our group has constructed a library of fluorescent compounds. We chose coumarin as a core structure mainly due to those various fluorescent properties by each substituent and its drug-like structure that would be suitable for development of fluorescent bioactive compounds.
3-azido-and/or-6-bromocoumarins were set as the starting materials, and Suzuki-Miyaura coupling with various aryl boronic acid moieties and Huisgen 1,3-cycloadditon with arylacetylenes were performed to construct the library. From this library, several fluorescent sensors like that for nitric oxide or sodium ion, and multi-analyte sensor, in which the modes of fluorescent changes induced by two analytes were different from each other, that is, one is the change of fluorescent intensity and another is the shift of emission maximum wavelength, could be obtained. In addition, several environment-sensitive fluorescent molecules like viscosity sensor, whose fluorescent maximum wavelength was sifted by the change of solvent viscosity, and pH sensor, which could fluoresce selectively under neutral condition, were also obtained.

As well as those sensors, fluorescent bioactive compounds also could be developed based on this library. For example, fluorescent ligands for progesterone receptor (PR), which is related to female reproductive function and some diseases, could be found from the library. The fluorescence of this ligand was increased in the presence of PR, which suggested that it would be utilized for the study of the physiological function of PR. Thus, our methodology could be utilized as a facile method for development of various functional fluorescent molecules.

Tomoya HIRANO received his B.S. in 1997 from the Faculty of Pharmaceutical Sciences, the University of Tokyo, and M.S. in 1999 and Ph.D. in 2002 from Graduate School of Pharmaceutical Sciences of the University of Tokyo (Supervisor: Professor Tetsuo Nagano). During 2002-2004, he was a JSPS research fellow, and also worked with Professor Kevan M. Shokat at University of California, San Francisco in 2003-2004. He became an Assistant Professor at Graduate School of Biomedical Science, Tokyo Medical and Dental University in 2004, and became an Associate Professor at Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University in 2009. His research interests are in the development and applications of functional molecules for elucidating of physiological functions and medical purpose.

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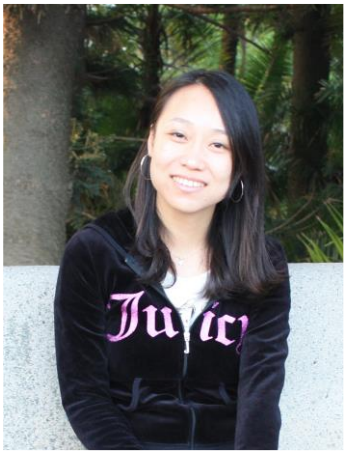
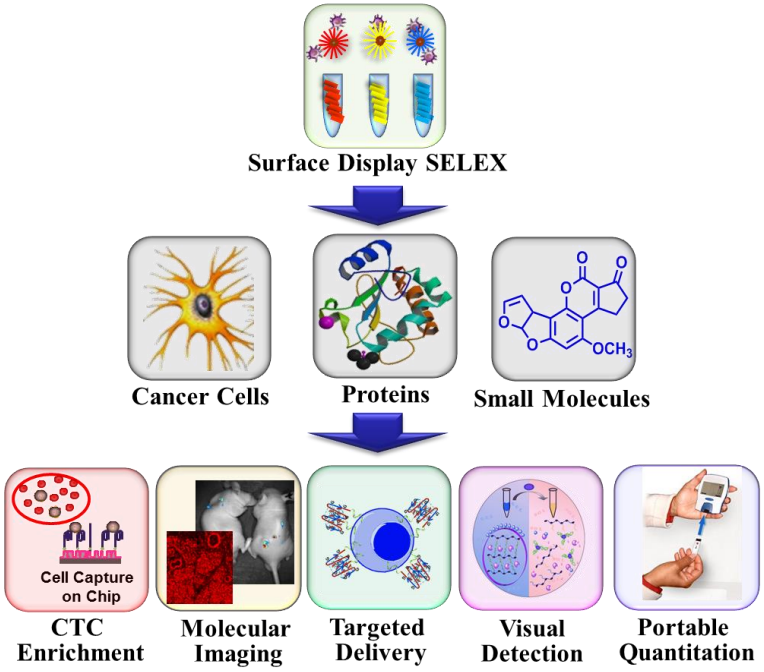
Nucleic Acid Aptamers for Bioanalysis and Biomedicine

Zhi ZHU, Yanling SONG, Xilan LI, Huimin ZHANG, Chaoyong YANG
Department of Chemical Biology, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, 361005, China



Aptamers are single-stranded oligonucleotides that can bind different types of targets, such as small molecules, proteins, cells, and even tissues, with high affinity and specificity. In addition, their robustness, ease of synthesis and modification, high stability, as well as low immunogenesis are some of the defining properties that make aptamers desirable diagnostic and biosensing tools. Therefore, the aptamer probes have found their wide applications in bioanalysis and biomedicine, including biosensing, biomarker discovery, drug screening, targeted delivery, gene regulation and disease diagnosis. In this talk, I will present some recent progress from our group on nucleic acid aptamer selection, optimization, structure modification and their applications in bioanalysis and biomedicine.



Zhi ZHU received her B.S. (Chemistry) from Peking University in 2006 and Sc.D. (Analytical Chemistry) from University of Florida in 2011. She joined Xiamen University faculty in 2011. She is Associate Professor in Department of Chemical Biology, College of Chemistry and Chemical Engineering. Her current research is particularly focused on molecular recognition, molecular engineering, point-of-care testing, and microfluidics.
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Development of smart ^{19}F MRI probes for in vivo imaging

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Graduate School of Engineering, Osaka University, Suita 565-0871, Japan
Immunology Frontier Research Center (IFReC), Osaka University, Suita 565-0871, Japan

Magnetic resonance imaging (MRI) is one of the most promising technologies for in vivo studies, because MRI can visualize deep regions in living animals. We have focused on ^{19}F MRI and developed the activatable small-molecule probes to detect protease activities on the basis of a paramagnetic relaxation-based off/on switching principle. However, the in vivo application of these small molecule-based probes was not successful due to the weak signal intensity. Therefore, we improved the sensitivity of ^{19}F MRI probes and succeeded to develop core-shell nanoparticle PFCE@SiO_2, which involves a liquid perfluorocarbon (PFCE: perfluoro crown ether) core and silica shell. The strong ^{19}F MRI signals were successfully detected from deep regions of the living mice bodies. Covering the surface of PFCE@SiO_2 with polyethylene glycol (PEG) elongated the retention time in the blood. When this PEGylated nanoparticle was injected to tumor-bearing mice, the ^{19}F MRI signals were observed from the tumor tissues.

Then, we tried to develop the off/on signal regulation of ^{19}F MRI signals of PFC@SiO_2 nanoparticles. To attenuate ^{19}F MRI signal of PFCE@SiO_2, paramagnetic species were modified on the surface of the nanoparticle. Modification of Gd^{3+} complexes on the nanoparticle surface attenuated the ^{19}F MRI signals in the Gd^{3+}-density-dependent manner. For further application, the paramagnetic species were conjugated to PFC@SiO_2 with an enzyme substrate peptide linker. By using this smart nanoparticle probes, the enzyme reaction was detected by ^{19}F MRI. This result indicates the promising future of ^{19}F MRI for visualization of enzyme activities in deep tissues of living animals.

Shin Mizukami received his B.S. (Pharmaceutical Sciences) and Ph.D. (Pharmaceutical Sciences) from the University of Tokyo in 1997 and 2002, respectively. He worked at National Institute of Advanced Industrial Science and Technology (2002-2004, Postdoc in Materials Science) and then moved to Stanford University (Prof. Eric T. Kool lab) (2004-2005, Postdoc in, Bioorganic Chemistry). He joined Osaka University as Assistant Professor in 2005. He is Associate Professor in Graduate School of Engineering, and Immunology Frontier Research Center (IFReC), Osaka University (Prof. Kazuya Kikuchi lab). His current research interest is development of new methodologies of chemical biology, such as imaging and regulation of biological signals with organic chemistry, materials science, and biochemistry.

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Inorganic Bioimaging: Cellular and Synthetic Approaches

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The inorganic species play important roles in physiological activity and diseases. We have developed several approaches to detect the inorganic species.

Specifically, our work can be divided into three parts:

(1) detecting heavy metal ions based on whole cells. Taking advantage of MerR family metalloproteins, we developed a whole-cell gold biosensor that allows the selective detection of gold ions by naked-eyes (Fig1).

(2) imaging the gasotransmitters by small molecule fluorescent dyes. We designed and synthesized a series dual-function colorimetric and ratiometric fluorescent dyes for H_2S with facile structural manipulation.

(3) developing the first label-free fluorescence method that can selectively detect and image inorganic drug cisplatin. Our method provides a starting point for fast determination of cisplatin.

We hope that these biosensors will be powerful tools to study the roles of inorganic species in physiological activity or diseases.

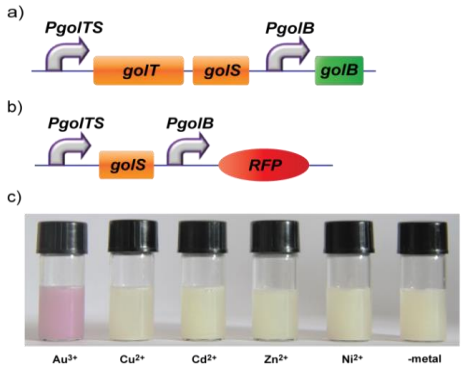

Fig. 1 Whole-cell based gold biosensor

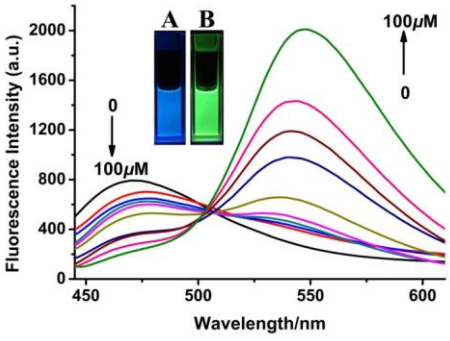

Fig. 2 Ratiometric fluorescent probe for H_2S

Jing ZHAO received his B.S. (Chemistry) from Nanjing University in 1998 and Ph.D. (Inorganic Chemistry) from Yale University in 2005.. He worked at the University of California, Berkeley with Dean Toste (postdoc in organometallic chemistry) and then moved to the University of Chicago with Chuan He (postdoc in chemical biology). He joined Nanjing University faculty in 2008. He is Professor in School of Life Sciences, Nanjing University. His current research interests are imaging inorganic species with fluorescent dyes and cells based on metalloprotein structures.

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Therapeutic In Vivo Synthetic Chemistry: Total Synthesis of Bioactive Compounds in Live Animals

Katsunori TANAKA

Biofunctional Synthetic Chemistry Laboratory, RIKEN, Saitama 351-0198, Japan
Biofunctional Chemistry Laboratory, A. Butlerov Institute of Chemistry, Kazan Federal University, Kazan 420008, Russia

We are synthetically exploring the overlooked reactivity of the *N*-alkyl unsaturated imines, which are readily derived from the various aldehydes and primary amines in biosystems. The new reactivity of imines could then be used to investigate the biological functions and in vivo dynamics of the natural products, e.g., polyamines and glycans, and even to challenge the multi-step synthesis of the biologically active molecules in live animals. Two recent examples of are discussed.

1. New reactivity of *N*-alkyl unsaturated imines from aminoalcohols and diamines: Synthetic application and their biological functions

We found that α,β-unsaturated imines, obtained from unsaturated aldehyde and aminoalcohols or diamines, could readily participate in the [4+4], [4+2], or [4+2+2] cycloadditions depending on the substrates. These reactions in fact proceed in biosystems and regulate the various biofunctions. For instances, the [4+4] products derived from the polyamines and acrolein contribute new mechanisms underlying acrolein-mediated oxidative stress and diseases.

2. In vivo glycan dynamics studies: application to natural products synthesis in live animals

Efficient and general bioconjugation of various glycan molecules on the proteins and live cells was established based on the rapid 6π-azaelectrocyclization of the *N*-alkyl unsaturated imines. Noninvasive whole body imaging and dissection experiments revealed the organ- and cell-selective distribution of the neoglycoproteins depending on their glycan structures. These glycan-based in vivo delivery systems were efficiently applied to the natural products synthesis in live animal.

Katsunori TANAKA received his received his BS (1996) and Ph.D. (2002) from Kwansei Gakuin University in Japan, under the direction of Professor Shigeo Katsumura. After a post-doc with Professors Koji Nakanishi at Columbia University, New York (2002-2005), he was appointed as an Assistant Professor in Osaka University. He moved to RIKEN (Biofunctional Synthetic Chemistry Laboratory) as an Associate Chief Scientist in 2012. In 2014, he was also appointed as an international Professor, Biofunctional Chemistry Laboratory, Kazan Federal University in Russia. His research interests include exploring new synthetic methods for total synthesis (either in flask, microfluidics, live cells, or animals), biological evaluation, molecular imaging, and molecular recognition of natural products.

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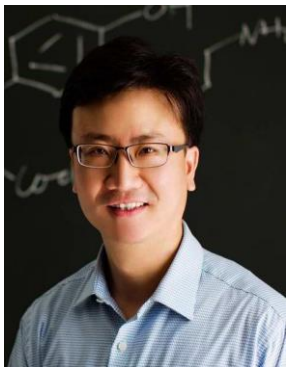
Protein Chemistry in Living Cells

Peng Chen[1,2]

Beijing National Laboratory for Molecular Sciences & Molecular Engineering
Synthetic and Functional Biomolecules Center, Peking University, Beijing 100871, China. [1]
Peking-Tsinghua Center for Life Sciences, Beijing, China.[2]

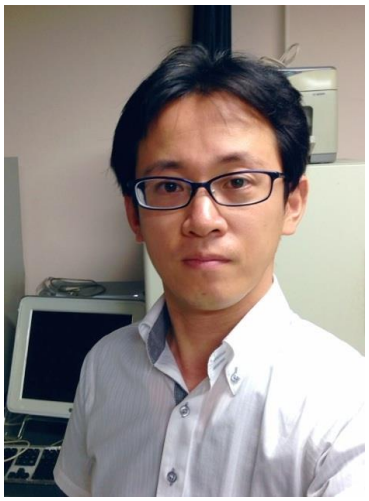
Research in my laboratory has been centered on exploring and applying novel chemistry-based platform technologies to modulate protein-interactions and activities in living cells, particularly with proteins involved in infectious diseases. In recent years, a new technique termed “Genetic-code Expansion” has become a highly powerful method for introducing unnatual amino acids, beyond the canonical twenty, into a protein of interest (POI) in a precisely controlled manner. Employing this strategy, we introduced diverse chemical reactive groups into POIs inside living systems, with the applications ranging from protein conjugation, protein activation to the study and imaging of protein interactions. Together, we aim to develop novel chemical toolbox to study proteins within their native biological context, which is advantageous because these biomolecules can be manipulated with an unprecedented precision.



Peng Chen obtained his BS degree in Chemistry from Peking University in 2002 and Ph.D with Prof. Chuan He in Chemistry at The University of Chicago in 2007. After a postdoctoral training with Prof. Peter Schultz at The Scripps Research Institute between 2007 and 2009, he started his independent career as an Investigator in Chemical Biology at Peking University in July 2009. His Lab is interested in developing and applying novel chemistry tools to investigate protein-based interactions and activities in living cells. He received NSFC Distinguished Young Scholar Award in 2012, China Young Scientists Award in 2013 and Chemical Society Review Emerging Investigator lectureship in 2014.

Development of Bioluminescent Probes for Analysis of Intracellular Environment and Signaling

Mitsuru HATTORI, Hideo TAKAKURA and Takeaki OZAWA
Department of Chemistry, School of Science, The University of Tokyo, Tokyo 1130033, Japan



Bioluminescence technology based luciferase proteins has been widely applied to a quantitative measurement and an imaging for biomolecules. We have developed a new bioluminescence technique based on complementation of split luciferase fragments. It enables to detect the timing of protein-protein interaction by bioluminescence photon counts. Using the technique, we can monitor several protein dynamics, such as the reversible activity, structural changing and modification in living cells.

First, we introduce one of the applications for G-protein coupled receptors (GPCRs) reaction. GPCRs are biological sensors on plasma membrane. To monitoring the activity with specific ligand, two of luciferase fragments were fused individually with GPCR and arrestin, a cytoplasmic protein binder to GPCR. We generated a stable cell line expressing these GPCR and arrestin probes, and showed concentration-dependent bioluminescence responses for a specific ligand. Moreover, the high luminescence intensity of probes enabled meaningful imaging of GPCR signal range from single cell to living subjects.

Second, we focus on an acidification of living tissue. Stress and disorder induce acidification in tissue level. However, spatiotemporal analysis of the pH variation in living tissue remains as an important challenge. We developed a bioluminescence pH indicator based on combination of photo-reactive protein and luciferase-fragment complementation. Bioluminescence of the fusion protein temporary diminished upon light irradiation, and recovered gradually in the dark. We designated the fusion protein as Photo-Inactivatable Luciferase (PI-Luc). The recovery time of bioluminescence (RT) was dependent to pH without subjecting to D-luciferin or ATP concentrations. PI-Luc was induced living mice and tried to construct the RT images with low O_2 condition. Spots showing high RT values were generated in case of ischemic treatment, and the RT values recovered to normal condition with reperfusion. Consequently, PI-Luc can be used to monitor such oxidative stress in living tissues.

Mitsuru HATTORI received his B.S. (Biology) and Sc.D. (Biology) from Nagoya University in 2003 and 2008, respectively. He worked at the University of Tokyo as a Research Fellow of the Japan Society for the Promotion of Science and as a Project Researcher. His current research interest is the development of bioluminescence methods to monitor biomolecules in living organs.

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Synchrotron radiation based X-ray imaging for cells

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Synchrotron-based X-ray microscopy is a non-destructive imaging technique that enables high resolution spatial mapping of metals with elemental level detection methods. Here I will introduce some examples to show the potential usage of this unique microscopy in cell imaging.

As we know, to detect and localize specific biomolecules in cells with high resolution may be the most important goals of cellular imaging. The resolution of synchrotron-based X-ray microscopy can easily extended to the nanoscale, offering great promise for localization of specific intracellular proteins and organelles. On the other hand, Synchrotron-based X-ray microscopy is an advanced imaging technology with excellent elemental specificity, provides a new platform for studying interactions between nanomaterials and living systems. I will show how we use this platform to study the mechanism of cytotoxicity of Qds and Carbon nanodiamonds.

Phosphorescent molecular probes for imaging oxygen levels in living cells and hypoxic tissues

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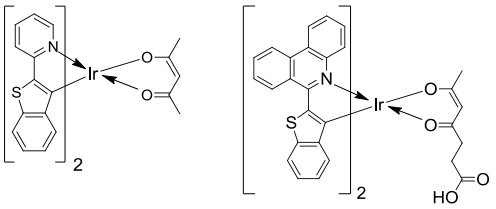
The oxygen levels in tumors are significantly lower than those of normal tissues because of abnormal and/or poorly developed vasculature. Therefore, measurements of oxygen levels of the tissues are of growing importance in detection of tumor and therapeutic treatment of tumor by radiation and photodynamic therapy. Phosphorescence of molecule has been applied to the observation of behavior of oxygen in cells and tissues, because its intensity and lifetime depend remarkably on the concentration of oxygen. Thus, phosphorescent probes such as α-diimine complexes of Ru(II), and Pt(II) and Pd(II) complexes of decorated porphyrins have been developed for tumor imaging.

Iridium(III) complexes exhibit intense phosphorescence with moderately long lifetimes. We used red-emitting iridium complex BTP (Fig. 1), because red emission exhibits deeper tissue penetration [1]. In this study, we investigated the phosphorescent properties of BTP in solutions, and performed the phosphorescence imaging experiment for living cells and tumor-bearing nude mice. The phosphorescence of BTP was remarkably quenched by oxygen molecule in solutions and living cells. The tumor images of nude mice were obtained 1 hour after the injection of BTP. The spectral and photophysical properties of BTP can be easily modified by changing aromatic ligands. We will also present the phosphorescent properties of the improved iridium complexes (BTPHSA, Fig. 1) and the tumor imaging by using these complexes [2, 3].



BTP BTPHSA

Fig. 1 Structures of BTP and BTPHSA

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[2] S. Zhang *et al.*, *Cancer Res.*, *70*, 4490, **2010**.
[3] T. Yoshihara *et al.*, *Proc of SPIE, 8233*, 82330A-1, **2012**.

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Upconversion luminescent materials for bioimaging

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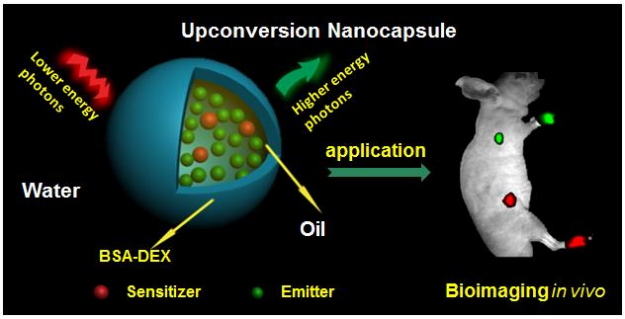
Upconversion luminescence (UCL) is a unique process whereby continuous-wave low-energy light is converted into higher-energy one through sequential multiple photon absorption or energy transfer.[1] Nowadays, the UCL emission can be achieved by ***f-f*** transition of the lanthanide ion and triplet-triplet annihilation (TTA) process (Scheme 1). Lanthanide nanophosphors codoped with Yb^{3+} (sensitizer) and Er^{3+} (activator) become popular to show visible and near-infrared UCL emission under 980 nm excitation.[2-5] Such Yb^{3+}-sensitized upconversion nanoparticles have been applied in bioimaging and sensing. Furthermore, by loading both sensitizer and annihilator into a nanosystem, we have constructed water-soluble, high-effective upconversion nanosystems based on TTA process.[6-8] These upconversion nanosystems have been applied for high-contrast UCL bioimaging *in vivo* under low power excitation.



Scheme 1. Schematic illustration of the upconversion nanocapsules for bioimaging *in vivo*

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Highly-sensitive RNA detection by using In Stem Molecular Beacon

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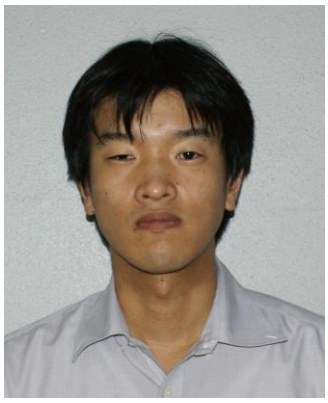

We develop In-Stem Molecular Beacons (ISMBs) detecting target DNA or RNA with high sensitivity. These probes can be used for visualization of mRNA in cells.

Recently, localization of mRNA has been paid much attention due to its relevance to spatial and temporal protein expression. To clarify roles of mRNA localization in living cells, monitoring tools with high sensitivity are strongly required. We have developed In-Stem Molecular Beacons (ISMB) by incorporating fluorophores (perylene) and quenchers (anthraquinone) into stem portion of molecular beacon. ISMB could detect target DNA and RNA with higher sensitivity than conventional molecular beacons. Its detection sensitivity was further improved by the addition of cationic polymer. We also synthesized ISMB tethering multiple Cy3 fluorophores and quenchers (Nitro Methyl Red), which showed high detection ability both with DNA and RNA. ISMB could detect endogenous mRNA in fixed cells.

We developed Serinol Nucleic Acid (SNA), which can hybridize natural DNA and RNA with high affinity. Molecular beacon composed of SNA (SNA-MB) showed extremely high sensitivity for RNA detection; its emission increased by up to 1000 times after the addition of target RNA. In addition, SNA-MB exhibited high resistance to nucleases. We believe ISMB and SNA-MB can be versatile tools for the visualization of various kinds of RNA in living cells.

Hiromu Kashida is an associate professor at the Graduate School of Engineering, Nagoya University. He received his Ph.D. (Engineering) in 2006 from the University of Tokyo under the supervision of Prof. Makoto Komiyama. He became an Assistant Professor at Nagoya University in 2007. He was promoted to Associate Professor in 2011. He received the PCCP prize from the Royal Society of Chemistry in 2011 and The Chemical Society of Japan Award for Young Chemists in 2014. His research interests include the design of artificial nucleic acids and their applications as probes and nano-materials.

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Single molecule spectroscopy of plasmonic metal nanoparticles

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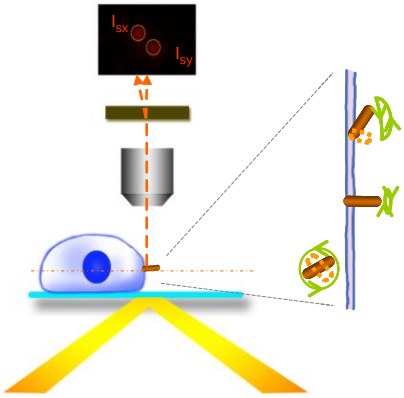

Optical microscopy is a convenient yet powerful strategy for noninvasive studying in complex surrounding with single event resolution. Observing molecules one by one can effectively remove ensemble averaging. Statistical distributions, time-dependent fluctuations and rare events could be revealed from the undisturbed raw data. So far, the optical imaging method for single molecule detection is dominated by fluorescence microscopy techniques. But the inherent photo-instability of dye molecules prevents long-time, dynamic tracking of individual fluorescent probes. A promising alternate for this issue is to use the noble metal plasmon resonance nanoparticles (PRPs) as the optical contrast agent due to their unique size, shape and composition dependent optical features. One attractive optical property of PRPs is their resonance frequency could be finely manipulated via adjusting their size, shape and composition. Another one is their absorption and scattering cross-sections are several orders of magnitude larger than dyes. Moreover, compared with fluorescent molecules, PRPs exhibit no photobleaching, photoblinking, and optical saturation. Here, based on our previous studies on single molecule PRP imaging, we present improved apparatus and techniques that enable us to detect small PRPs and track their translational and rotational motions inside noisy living cells. Our methods could be widely applied to study vital biological events with PRPs as efficient optical contrast agents, and assist the investigation and regulation of the kinetics of nanomachines to achieve highly efficient gene and drug delivery.

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