第２回
アジア分析科学シンポジウム

Sep.9～10, 2015
福岡

公益社団法人　日本分析化学会
The Japan Society for Analytical Chemistry

2nd Asian Symposium on Analytical Sciences
Program

Room Z: 9th (9:20~17:30) 10th (9:00~12:00)
Room E: 9th afternoon (16:00~17:00)
Room H: 9th afternoon (13:30~15:20)
Room L: 9th morning (9:30~10:55) 10th (9:50~10:10)
Room P: 9th afternoon (15:00~16:00))

Room Z

September 9th

Opening Remark : Norio Teramae (Former president of JSAC)

Chair: Sigeori Takenaka

Z1001A* 9:30 ~ 10:10 **Plenary Lecture**

Nucleic Acid Based Bioengineering: Opportunities in Diagnostics and Therapeutics

○I-Ming Hsing (The Hong Kong University of Science and Technology)

Chair: Kenji Sueyoshi

Z1002A* 10:10 ~ 10:30

Microfluidic immunoassay devices for clinical diagnostics

○Manabu Tokeshi (Hokkaido University)

Z1003A* 10:30 ~ 10:50

Cell-free microfluidic vascular models for nanoDDS

○Naoki SASAKI (Toyo University)

Z1004A* 10:50~11:10

Generation of mono-disperse droplets in liquids by inkjet and its applications

○Hulie Zeng (Tokyo Metropolitan University)

Chair: Shin-ichi Zaitsu

Z1005A* 13:30 ~13:50

Sensing by Sensitization: The role of Auger ionization on the stability of

semiconductor quantum dots

○BIJU, Vasudevan (Health Research Institute, AIST)

Z1006A* 13:50~14:10

Light scattering microspectroscopy of single nanoparticles

○Tuyosi ASAHI (Ehime University)

Z1007A* 14:10~14:30

Bio-Raman Research on Live Cells and Small Molecules

○Morita, Shin-ichi (Tohoku University)

Chair: Shin-ichi Morita

Z1008A 14:30~14:45

Determination of Hexachlorocyclohexane Isomers by Femtosecond Laser Using Gas Chromatography / Multiphoton Ionization / Mass Spectrometry

○ Xixiang Yang, Tomoko Imasaka, Totaro Imasaka (Kyushu Univ., Engineering, Kyushu Univ., Design, Kyushu Univ., CFC)

Z1009A* 14:45~15:05

Faraday rotation microscope imaging of weak magnetic samples under pulsed magnetic field

○ Suwa, Masayori, Tsukahara, Satoshi, Watarai, Hitoshi (Osaka Univ., Science, Osaka Univ., INSD)

Chair: Ryoichi Ishimatsu

Z1010A* 16:00~16:20

Development of Fluorochromic Sensors Utilizing Suzuki-Miyaura Cross-coupling Strategy

○YAMADA, Kouji (EES, Hokkaido Univ.)

Z1011A 16:20~16:35

Flow Injection Chemiluminescence Determination of Ascorbic Acid Using Rhodamine B

○ Tamer Hasanin, Yasuaki Okamoto, Terufumi Fujiwara (Hiroshima University)

Z1012A* 16:35~16:55

Potential-Dependent Encapsulation of Ionic Species in Charged Dendrimers

at Liquid|Liquid Interfaces

○Hirohisa Nagatani (Kanazawa University)

Chair: Hirohisa Nagatani

Z1013A* 16:55~17:15

The thin layer electrolysis cell with the aqueous and the organic phases and its application to coulometric determination and separation of ions

○Yumi Yoshida, Junya Uchida, Shotaro Nakamura, Kohji Maeda (Kyoto Institute of Technology)

Z1014A 17:15~17:30

Formation of Organic Ion Associate Phase from Aqueous Solution for Enrichment and Determination of Trace Heavy Metals in Environmental Water Samples By GF-AAS

○Syeda Mushahida-Al- Noor, Ryo Murashima, Takuya Okazaki, Noriko Hata, Shigeru Taguchi, Hideki Kuramitz (Univ. Toyama)

Room Z

September 10th

Chair: Masaaki Kai

Z2001A* 9:00~9:40 **Plenary Lecture**

Platforms of Luminescence in Analytical Chemistry for Nucleic Acids

○Jianzhong Lu, Masaaki Kai, (School of Pharmacy, Fudan University, School of Pharmaceutical Sciences, Nagasaki University)

Chair: Noritada Kaji

Z2002A* 9:40~10:00

Development of selective analytical techniques based on the unique characteristics of quinone

○Naoya Kishikawa (Nagasaki University)

Z2003A 10:00~10:15

A Single Probe to Sense Al(III) Colorimetrically and Cd(II) by Turn-On Fluorescence in Physiological Conditions and in Live Cells.

○Chirantan Kar, Soham Samanta, Aiyagari Ramesh, Gopal Das (Keio Univ.,

Indian Inst. of Technology Guwahati)

Z2004A 10:15~10:30

Creation of artificial luciferases and their applications for molecular imaging technologies

○Sung-Bae Kim (Environmental Management Research Institute, AIST)

Z2005A* 10:30 ~ 10:50

Planarization of cell membrane on supported lipid bilayers

○Takashi Kaneta, Tomomi Maki (Okayama University)

Chair: Naoya Kishikawa

Z2006A* 11:00 ~ 11:20

Nanowires for analyzing biomolecules

○Takao Yasui, Takeshi Yanagida, Sakon Rahong, Noritada Kaji, Tomoji Kawai, Yoshinobu Baba (Nagoya Univ., Engineering, Nagoya Univ., Nanobio., Kyushu Univ., IMCE, Osaka Univ., ISIR, AIST Health Research Institute)

Z2007A* 11:20 ~ 11:40

Stabilities of DNA secondary structures inside confined nanospace

○Akira Yamaguchi, Kazuyoshi Nasu, Ryoko Yoshida, Shigeki Wakaume (Ibaraki University)

Z2008A* 11:40 ~ 12:00

Speciation of sulfur in thin films and solutions: X-ray absorption fine structure study at Hiroshima synchrotron radiation center

○Shinjiro Hayakawa, (Hiroshima University)

Room L

September 9th

Chair: Katsumi Uchiyama

L1001A* 9:30 ~9:50

Microwave-assisted rapid fabrication of monolithic stationary phases for capillary liquid chromatography

○Lee Wah Lim, Toyohide Takeuchi (Gifu University)

L1002A 9:50 ~ 10:05

Evaluation of chiral recognition ability of quinoline-based oligoamide

foldamers with one-handed helical structure

○Hiroki Noguchi, Victor Maurizot, Ivan Huc, Makoto Takafuji, Hirotaka Ihara (Kumamoto Univ., Univ. of Bordeaux. CNRS, Kumamoto Inst. for Photo-electro Organics (PHOENICS))

L1003A* 10:15 ~ 10:55 **Plenary Lecture**

Applications of Functional Metabolomics in Investigating the Biological Functions of Small Molecules

○Guowang Xu, Peng Gao, Peiyuan Yin, Xin Lu (Dalian Institute of Chemical Physics, CAS)

Room L

September 10th

Chair: Yoshihiro Saito

L2001A* 9:50 ~10:10

Specific Liquid Chromatographic Separations by a C60-fullerene Bonded Silica-monolithic Capillary Prepared via Perfluorophenyl azide,

○Takuya Kubo, Madoka Tsuzuki, Toyohiro Naito, Mingdi Yan, Koji Otsuka, (Kyoto Univ., UMASS, Lowell)

L2002A 9:50 ~10:05 cancelled

Room E

September 9th

Chair: Takeshi Mori

E1012A* 16:00～16:20

Multi-functional Nanopipette Probe for Single-cell Analysis of Tissue Models

○Hitoshi Shiku, Yuji Nashimoto, Hidenori Ito, Yuanshu Zhou, Yasufumi Takahashi, Kosuke Ino, Tomokazu Matsue (Tohoku Univ. WPI-AIMR)

E1013A* 16:20~16:40

A Sputtered Nanocarbon Film Electrode as a Platform for Detecting Biomolecules

○Dai Kato (Biomedical Research Institute, AIST).

E1014A* 16:40~17:00

Ferrocenylnaphthalenediimide-based Electrochemical telomerase assay

(ECTA) as oral cancer screening

○Shinobu Satou, Mana Hayakawa, Kazuhiro Tominaga, Tatsuji Nishihara, Shigeori Takenaka (Kyutech Kyushu Dental Univ.)

Room H

September 9th

Chair: Tohru Saitoh

H1012A* 13:30~13:50

Development and Application of ICP-MS Techniques for Elemental Analysis in Chemical Metrology

○Yanbei Zhu (National Metrology Institute of Japan, AIST)

H1013A* 13:50~14:10

Determination method for measurement of methylmercury in soils and sediments

○Hitosi Kodamatani, Takashi Tomiyasu (Kagoshima University).

H1014A 14:10~14:25

Development of dendrimer modified magnetic chitosan for magnetic separation of Cu(II) ion and humic acid in water,

○Satya Candra Wibawa Sakti, Yasuyuki Narita, Shunitz Tanaka (Grad. Sch. Env. Sci. Hokkaido. Univ.)

Chair: Ryo Kanzaki

H1015A* 14:25~14:45

Removal of Polar Organic Pollutants from Wastewaters by Surfactant-Assisted Coagulation,

○Tohru, Saitoh (Kitami Institute of Technology)

H1016A* 14:45 ~15:05

Triple-Quadrupole Inductively Coupled Plasma-Mass Spectrometry Combined with Selective Cs Adsorption and Ion Exchange Chromatographic Separation for 135Cs and 137Cs analysis

○Jian Zheng, Wenting Bu, Keiko Tagami, Yasuyuki Shikamori, Kazumi Nakano, Shigeo Uchida, Nobuyoshi Ishii (NIRS, Japan, Agilent, Japan).

H1017A　15:05~ 15:20

Measurement of translational and rotational energies for photodesorbed CO from CO-H2O ice

○Shohei Matsuda, Yusuke Kurotani, Motoki Yamazaki, Akihiro Yabushita (Kyushu Univ., Kyoto Univ.)

*: invited lecture

Poster Session

September 9th (15:00~16:00)

P1101A

Spectroscopic study of gold nanoparticles- protein mixture solutions

○Xin Qiao, Nobuyoshi Miyamoto, Xing-Zheng Wu (Fukuoka Institute of Technology)

P1102A

Combination of fluorescence and beam deflection method for monitoring materials movements across a plant surface

○Xiaoyan Wu, Hironari Kubo, Tomomi Inoue, Xing-Zheng Wu (Fukuoka Inst. of Technology, National Inst. for Environmental Studies)

P1103A

Development of palm-sized ELISA system for the rapid and on-site diagnosis of infection disease,

○Kazuhiro MORIOKA, Harpal Singh, Hizuru Nakajima, Akihide Hemmi, Masami Sugamata, Le Van An, Sazaly AbuBakar, Hulie Zeng, Shungo Kato, Ming Yang, Katsumi Uchiyama (Tokyo Metropolitan Univ. NIID, Mebius Advanced Technology, Hue Univ. of Medicine and Pharmacy, Univ. of Malaya)

P1104A

Ionic polymer-grafted porous silica particles for HPLC stationary phase

○ Mohammad Shahruzzaman, Yutaka Kuwahara, Makoto Takafuji, Hirotaka Ihara (Kumamoto Univ., Kumamoto Inst. of Photo-Electro Organics (PHOENICS))

P1105A

Development of hybrid certified reference material of 1H and 19F for quantitative NMR

○Taichi Yamazaki, Sachiko Taniguchi, Nobuyasu Hanari, Toshiyuki Asakai, Ryoko Iwasawa, Masahiko Numata (National Metrology Institute of Japan, AIST)

P1106A

Potential-dependent adsorption of water-soluble porphyrins at liquid|liquid interfaces studied by polarization-modulation total internal reflection fluorescence spectroscopy

○ Sho Yamamoto, Hirohisa Nagatani, Kotaro Morita, Hisanori Imura (Kanazawa University)

P1107A

Gel-filtration separation of protein-coated gold nanoparticles

○Shuushi Tamura, Kazuhiko Fujiwara, Hirotoshi Matsumura, Masafumi Odaka, Nobuaki Ogawa (Akita University)

P1108A

Effect of a Chemical Functionalization of Gold Nanoparticles on their Cytotoxicity

○Yuki Nagano, Kazuhiko Fujiwara, Hirotoshi Matsumura, Masafumi Odaka, Nobuaki Ogawa (Akita University)

P1109A

DNA quantification via nucleobase measurement by high performance liquid chromatography tandem mass spectrometry

○Sachie Shibayama, Shin-ichiro Fujii, Taichi Yamazaki, Akiko Takatsu (National Metrology Institute of Japan, AIST)

P1110A

Resonance Raman spectroscopic studies for elucidating N2O formation in nitric oxide reductase mechanism

○Hirotoshi Matsumura, Pierre Moenne-Loccoz, Nobuaki Ogawa (Akita Univ., Inst. of Environ. Health, OHSU)

Z1001A*
[ASAS講演]

Nucleic Acid Based Bioengineering : Opportunities in Diagnostics and Therapeutics

(The Hong Kong University of Science and Technology) ○I-Ming Hsing

Nucleic acid sequences and biomolecules (e.g., proteins, ATP) are important bio-analytes for pathogen detection and disease diagnostics. Because of technology advances in DNA synthesis, nowadays the cost and the delivery time for short synthetic DNA sequences are much reduced. Using DNA self-assembly principle, many interesting DNA nanostructures have been demonstrated for applications in diagnostics and therapeutics. A very interesting one was the concept of hybridization chain reaction (HCR) where a cascade of hybridization could be initiated only in presence of a specific DNA sequence [1]. This HCR strategy converts a short sequence of DNA hairpins, through repetitive of enzyme-free "polymerization" steps, to a linear DNA polymer with a molecular weight inversely correlated to the concentration of the specific initiating DNA strand. Although this HCR strategy could only achieve a linear version of amplification event, this concept of triggering assembly has attracted a lot of academic and company's interests for highly sensitive and enzyme-free biosensing applications.

Our team has recently demonstrated a non-linear version of HCR where a 2D DNA dendrimer could be grown after a DNA analyte triggers the reaction [2]. This new approach requires no enzyme and does not need an additional PCR-type amplicon enrichment step. The working principle of this new approach involves the use of several specifically-designed reactant sequences according to the sequence of the analyte target. In the presence of an analyte single-stranded DNA sequence, the target DNA would hybridize to a complementary toehold region of a designed double-stranded DNA. This initiating step would trigger a cascade of autonomous and catalytic hybridization reactions, leading to the formation of a two-dimensional branch of DNA self-assembly dendrimer-like network. This branched-growth algorithm mimics PCR-like exponential amplification and it can be easily developed in to ultrasensitive fluorescent, electrochemical [3] and colorimetric sensing platforms, respectively.

References

[1] R.M. Dirks and N.A. Pierce, "Triggered amplification by hybridization", *PNAS*, **101**, 15275, (2004).

[2] F. Xuan, T. Fan, I-M Hsing, "Electrochemical Interrogation of Kinetically-Controlled Dendritic DNA/PNA Assembly for Immobilization-Free and Enzyme-Free Nucleic Acid Sensing", *ACS Nano*, **9**, 5027–5033 (2015).

[3] F. Xuan, XT Luo, I-M Hsing, "Triggering Hairpin-Free Chain-Branching Growth of Fluorescent DNA Dendrimers for Nonlinear Hybridization Chain Reaction", *Journal of the American Chemical Society*, **136**, 9810–9813 (2014).

Acknowledgement

This funding support from the Research Grants Council of the Hong Kong SAR Government (GRF# 601212) is acknowledged.

Z1002A*
[ASAS講演]

Microfluidic immunoassay devices for clinical diagnostics

(Hokkaido University) ○Manabu Tokeshi

Immunoassays are widely used for medical diagnostics, food analysis, and biological studies. They represent some of the most vigorous activities in the field of microfluidics. The reduced consumption of sample and reagents and provision of rapid analyses are inherently possible by miniaturizing immunoassay systems.

Recently, we have developed a new immunoassay platform called an "immuno-pillar chip", which has the desired features for easy-to-use detection of biomarkers and enterotoxins [1–3]. It has hydrogel microstructures, fabricated inside a microchannel, with many antibody molecules immobilized onto 1 μm diameter polystyrene beads or hydrogel. For detection of disease markers, we confirmed the chip provides rapid analysis with high sensitivity, it is easy-to-use, and it uses small volumes of the sample and reagent. Moreover, multiplex assay was also possible.

We have realized fluorescence polarization immunoassay (FPIA) on a microchip in about 1 minute [4]. FPIA is a homogeneous competitive immunoassay which is based on measuring fluorescence polarization after competitive binding of an analyte and a tracer to an antibody. We successfully carried out a quantitative analysis of theophylline in sera of patients having been medicated by theophylline by a microchip-based FPIA [5]. Very recently, we developed a unique detection system for FPIA using a liquid crystal and an image sensor [6].

Future challenges and potentials of microfluidic immunoassay devices for clinical diagnostics will be also discussed.

[1] Lab Chip, 10, 3335–3340 (2010).
[2] J. Microbiol. Methods, 92, 323–331 (2013).
[3] Anal. Methods, 7, 5092–5095 (2015).
[4] Lab Chip, 9, 996–971 (2009).
[5] Anal. Bioanal. Chem., 401, 2301–2305 (2011).
[6] Anal. Chem., under revision.

Z1003A*
[ASAS講演]

Cell-free microfluidic vascular models for nanoDDS

(Toyo University) ○Naoki Sasaki

Nanoparticle-based drug delivery system (nanoDDS) has been widely utilized to deliver anticancer drugs and diagnostic agents from blood vessels to target tissues. A crucial issue concerning nanoDDS is to estimate transport of nanoparticles to target tissues. Although nanoparticles can permeate vascular pores and interstitium surrounding the blood vessels, since the size and shape of the vascular pores and the composition of the interstitium are essentially non-uniform, conventional animal testing and recent cell-based microfluidic devices are unable to precisely evaluate the effects of physical parameters (e.g. pore size and nanoparticle size) on permeation.

In this presentation, we present a cell-free microfluidic device to estimate permeation of nanoparticles through vascular walls. Porous membranes possessing uniform pores were integrated into a microfluidic device and utilized as artificial vascular walls. The effects of pore size and pressure difference across the pores on nanoparticle permeation were examined under a fluorescent microscope. The experimentally determined permeability coefficient of 1.0 μm-pore membrane against 100 nm-diameter nanoparticles agreed well with the theoretical value obtained from conventional theory for convective transport of hard spheres through the pores. In addition, the present device was successfully applied to nanoparticles with different materials' nature and size [1]. We also present a microvascular-interstitium model on microfluidic devices. We employed magnetic resonance imaging to observe transport of two types of different molecular-weight contrast agents into the model interstitium. The ratio of the transport rates of agents agreed with the ratio calculated from diffusion coefficients of the agents [2]. Our method can be utilized as an initial nanoparticle screening technique to minimize animal testing and accelerate the development of suitable nanoparticles.

REFERENCES

1) N. Sasaki et al., Proceedings of Micro Total Analysis Systems 2013, 1818–1820 (2013).
2) N. Sasaki et al., Analytical Biochemistry, 458, 72–74 (2014).

Z1004A*
[ASAS講演]

Generation of mono-disperse droplets in liquids by inkjet and its applications

(Tokyo Metropolitan University) ○Hulie Zeng

The great potential of inkjet for the generation of mono-disperse droplets in liquids will be presented. i) As a liquid/liquid droplet generator, inkjet was used to produce mono-disperse water droplets in oil (W/O) and oil droplets in water (O/W). Strategies for regulated droplet generation in various liquids were experimentally explored. The diameter of the generated mono-disperse droplet could be accurately tuned by varying the driving voltage and pulse width exerted on the piezo slide of inkjet. ii) Controllable preparation of mono-disperse particles with various structures will be discussed. Uniform droplets of an aqueous polymer solution were ejected from the inkjet in an organic phase. The subsequent dehydration of the droplets by solvent extraction resulted in the formation of porous polymer microspheres. Particles with a narrow size distribution could be obtained in a single step. The relationship between the final particle diameter and the initial polymer droplet size were examined. The diameters of the particles could be precisely controlled by changing the waveform exerted on the inkjet, thus producing particles with diameters in the range of 15–60 μm with a coefficient of variation (CV) in the range of 2.7–4.8%. It was also possible to produce hollow porous polymer particles by simply decreasing the concentration of the polymer solution. Finally, we also expect great potentials of inkjet in quantitative droplet extraction and droplet interfacial enhanced fluorescence fields.

References
1) (a) H. Zeng, J. Yang, D. Katagiri, S. Xue, H. Nakajima, K. Uchiyama, Sensor. Actuat. B Chem. (2015), (b) J. Yang, D. Katagiri, S. Mao, H. Zeng, H. Nakajima, K. Uchiyama, RSC Adv., 5, 7297–730 (2015).
2) (a) H. Zeng, Y. Weng, S. Ikeda, Y. Nakagawa, H. Nakajima, K. Uchiyama, Anal. Chem., 84, 10537–10542 (2012), (b) F. Chen, S. Mao, H. Zeng, S. Xue, J. Yang, H. Nakajima, J.-M. Lin, K. Uchiyama, Anal. Chem., , 85, 7413–7418 (2013), (c) H. Zeng, N. Seino, K. Uchiyama, T. Nakagama, J. Chromatogr. A, 1216, 3337–3342 (2009).

Z1005A*
[ASAS講演]

Sensing by Sensitization : The role of Auger ionization on the stability of semiconductor quantum dots

(Health Research Institute, AIST) ○Biju, Vasudevan

Photoactivated molecules and nanoparticles often react with molecules in the surroundings and suffer from irreversible photochemical transformations. Oxidative damage, also called photobleaching, caused by reactive oxygen species plays major roles on such transformations, which clips the durability of fluorophores and photosensitizers in a variety of applications—from bioimaging to solar cells.[1,2] For example, despite the superior photoluminescence properties of semiconductor quantum dots when compared with the fluorescence of organic dye molecules, self-sensitized generation of singlet oxygen and the subsequent oxidative degradation of capping ligands, shell materials and the core pose major challenges in the applications of these tiny crystals to optoelectronic devices and single-molecule detection systems. Interestingly, the release of an electron (Auger ionization) from a photoexcited quantum dot places it in the ultrafast carrier recombination cycle and inhibits the generation of singlet oxygen, preventing self-sensitized oxidation. Nonetheless, Auger ionization intermittently places single quantum dots in the dark state and induces undesired photoluminescence blinking, which is considered to be the defense mechanism against the generation of singlet oxygen and oxidation.[2] However, the incomplete information about relations among Auger ionization, non-radiative carrier recombination, blinking, and oxidation is an obstacle in the clarification of the defense mechanism. By looking at singlet quantum dots exposed to high-intensity coherent photons, oxygen, and singlet oxygen scavengers, we disclose how Auger ionized quantum dots preserve their photoluminescence by beating singlet oxygen-mediated oxidation.

References :
[1] E. S. Shibu, S. Sugino, K. Ono, H. Saito, A. Nishioka, S. Yamamura, M. Sawada, Y. Nosaka, V. Biju, Angew. Chem. Int. Ed. 2013, 52, 10559–10563.
[2] S. Yamashita, M. Hamada, S. Nakanishi, H. Saito, Y. Nosaka, S. Wakida, V. Biju, Angew. Chem. Int. Ed. 2015, 54, 3892–3896.

Z1006A*
[ASAS講演]

Light scattering microspectroscopy of single nanoparticles

(Ehime University) ○Tuyosi Asahi

Nanoparticles have received much attention for their interesting optical and electronic properties, which originate from their sizes, shapes, and high surface-to-volume ratio. In the last two decates, size and shape dependent optical absorption and emission have been reported for various kinds of nanoraticles of semiconductors, metals and organic molecular systems, however the conventional spectroscopy gives information on an ensemble average of many particles. Thus, spectroscopic measurements of individual nanoparticles are important and indispensable to elucidate the fundamental properties of nanoparticles. Here, spectroscopic analysis techniques of non-fluorescent single nanoparticles are introduced. Recent progress in optical microscopes and light detection equipment enable us to investigate optical properties of single nanopartiles by measuring light scattering spectra from an isolated particle using a dark-field illumination setup of an optical microscope. We will present several experimental techniques of steady and time-resolved spectroscopy of non-florescent nanoparticles, and will demonstrate their applications to optical imaging and spectroscopic analysis of metal and organic nanoparticles.

Z1007A*
[ASAS講演]

Bio-Raman Research on Live Cells and Small Molecules

(Tohoku University) ○Morita, Shin-ichi

Recently it became possible to measure Raman spectra of a single live cell using a standard Raman microscope. Using Raman signals, we are capable of, for instance, analyzing the distribution of bio-molecules. Also, many killer applications were opened. It is however difficult to distinguish Raman bands of similar molecules. Raman analysis, therefore, is ambiguous and complicated. Plus, bio-Raman data are extremely large. i) Disentangling of complicated large data is of our interest, in this respect. For instance, Raman spectral data of live cells are complicated and large, especially when we observe time courses to cellular differentiation (typically several days). It is almost impossible to understand those data in an intuitive manner. Spectral analysis is, therefore, essential to extract and visualize intrinsic information. We have developed mathematically analytical methods compatible to bio-Raman research. ii) Use of Raman-tagged small molecules is another promising approach to purify our scientific interest. As markers, let us assume introducing large fluorescent chromophores to small molecules such as lipids and some of nucleic acids. Target small molecules are perturbed by larger labels, far from giving original properties. Dot-like Raman probes such as alkyne-tags, therefore, are expected as effective markers even to small molecules. We are developing Raman tags, for instance, utilizing the resonance Raman effect to enhance the brightness of molecular emissions. iii) We are developing a bio-Raman microscope for monitoring dynamics of cellular differentiation, proliferation, and apoptosis. To do so, living cells have to be incubated on the microscope stage for several days ; plus, the positions of moving cells have to be confirmed continuously. Using the bio-Raman microscope, it was possible to define cellular states in a non-destructive and non-labeling manners.

Z1008A
[ASAS講演]

Determination of Hexachlorocyclohexane Isomers by Femtosecond Laser Using Gas Chromatography/Multiphoton Ionization/Mass Spectrometry

(Kyushu Univ., Engineering[1] • Kyushu Univ., Design[2] • Kyushu Univ., CFC[3])
○Xixiang Yang[1] • Tomoko Imasaka[2] • Totaro Imasaka[3]

Hexachlorocyclohexane (HCH) is the most abundant organochlorine compound pesticide in air and water all over the world. The HCH mixture, heavily used before especially in Asian countries, contains 60–70% α-HCH, 5–12% β-HCH and 10–15% γ-HCH along with minor percentages of other isomers. The effects on killing the pests for each isomers are also different, which has an order as $\gamma > \alpha > \delta \gg \beta$. The γ-HCH, known as lindane, has been used both as an agricultural insecticide and a pharmaceutical treatment for lice. In 2009, the production and agricultural use of lindane was banned under the Stockholm Convention on persistent organic pollutants. However, the HCH still exists in the environment for its stable structure and heavy use before. Therefore, it is important to distinguish the different isomers to evaluate the effects on human beings and the environment.

Gas chromatography combined with time-of-flight mass spectrometry, which was developed in our laboratory, was used to measure the isomers of HCH. Two kinds of GC column, HP-5 column (30 m long, 0.25 mm inner diameter, 0.25 μm film thickness) as well as a γ-DEX 120 column (30 m long, 0.25 mm inner diameter, 0.25 μm film thickness) were employed for isomer separation. Different oven temperature programs were tried and the best one for separation was selected. The third harmonic emission of a Ti : sapphire laser (800 nm, 35 fs, 1 kHz, 4 mJ, Libra, Coherent Co.) was employed for multiphoton ionization.

As a result, the four HCH isomers were successfully separated by both of the GC column. But with γ-DEX 120 column, the mixture HCH sample were separated as well as the (−)γ-HCH and (+)γ-HCH for its chiral nature of the stationary phase. According to the reference[1], the elution order of γ-HCH is first (−)γ-HCH and then (+)γ-HCH. A two-dimensional display of GC/MS along the scales of the flight time and the retention time, which is available by using the present instrument, was useful for identification of these compounds. This research suggests a new approach for identifying different isomers of chiral pesticides in trace analysis and environmental monitoring.

Reference
1. R.L.Falconer *et al. Sci. Total Envrion.* 160/161 (1995) 65–74

Z1009A*
[ASAS講演]

Faraday rotation microscope imaging of weak magnetic samples under pulsed magnetic field

(Osaka Univ., Science[1] • Osaka Univ., INSD[2]) ○Suwa, Masayori[1] • Tsukahara, Satoshi[1] • Watarai, Hitoshi[2]

A lot of effort to invent a novel imaging technique has been devoted, because direct observation of any events provides strong evidence in every field of science. In this study, we have developed Faraday rotation (FR) microscope imaging, which should be able to enhance paramagnetic species and aromatic compounds. FR is an optical gyration phenomenon in every material under a magnetic field. Although FR in a weak magnetic material is unfortunately very small, the rotation angle is proportional to the strength of the magnetic field. A pulse magnet is one of the most effective ways to obtain a high magnetic field using relatively simple and conventional instruments. We have constructed the experimental setup for observing FR microscope image under pulsed magnetic field.

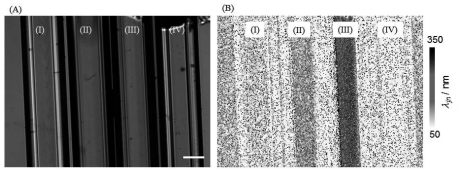


Figure 1. The normal microscopic image (A) and the resonance wavelength image (B) of *n*-dodecane(I), *o*-xylene(II), 1-methylnaphthalene(III) and empty cell(IV). The scale bar in (A) indicates 200 μm

A pulsed magnetic field of 2 T in ～1 ms was generated in a homemade air-core coil of 4 μH by discharge of a capacitor of 4000 μF in an LCR circuit. A Xe flash lamp was used as incident light in order to synchronize with the pulse of the magnetic field. The flash light was collimated with an achromatic lens and passed through a band-pass filter, a calcite polarizer, a sample in the coil, an objective, and an analyzer, which was set to 45 degree clockwise with respect to the polarizer as viewed from the light source. The image of the sample was focused onto a CCD camera. The FR angle was estimated for each pixel by comparing the images acquired in the presence and the absence of the magnetic field. The wavelength dispersion of the FR angle was observed by changing the band pass filter.

The quantitative FR microscope images of organic liquids could be successfully acquired and distinguished based on their aromaticity in FR images. The wavelength dispersion curve of the FR of most organic compounds was able to be theoretically analyzed by Faraday B-term, affording the characteristic resonance wavelength, λjn.[1, 2] The values of λjn obtained for all pixels gave the 2D images as shown in Fig. 1.[3]

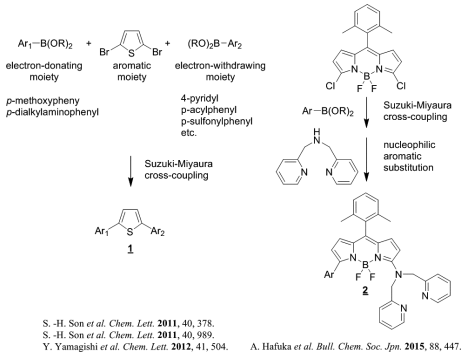
Furthermore, we could improve the sensitivity of the FR imaging by using microsecond magnet, which suppressed Joule heating and mechanical oscillation and increased the repetition rate. The improved microscope enabled us to observe the FR image of polystyrene and polymethyl methacrylate microparticles and clearly distinguish them.[4]

References
1. Barron, L., *Molecular Light Scattering and Optical Activity. 2nd ed.* ; Cambridge University Press : 2004.
2. Suwa, M. et al., *Anal. Sci.* **2013**, *29*, 113–119.
3. Suwa, M. et al., *Anal. Chem.* **2013**, *85*, 5176–5183.
4. Suwa, M. et al., *J. Magn. Magn. Mater.* **2015**, *393*, 562–568.

Z1010A*
[ASAS講演]

Development of Fluorochromic Sensors Utilizing Suzuki–Miyaura Cross-coupling Strategy

(EES, Hokkaido Univ.) ○Yamada, Kouji



Fluorochromic sensors are very useful tool, because of their high resolution and sensitivity with accurate quantitative analysis. However, most of the sensors have highly condensed aromatic ring systems, and the design and synthesis of them are difficult using traditional synthetic methods. Suzuki-Miyaura cross-coupling reactions enable C–C bond formation between functional aromatic rings without robust reaction conditions, thus would be available for preparation of the sensors.

We have developed a series of fluorescent solvatochromic dyes **1** excited by visible light and boron-dipyrromethene-based fluoroionophores **2** whose emission wavelength was shifted by the type of captured metal ions as the examples using the coupling reactions. Dyes 1, which were synthesized by the coupling reaction between aromatic dibromide and the electron-withdrawing and electron-donating aromatic borates, showed excellent fluorescent solvatochromism. Unlike conventional synthetic methods, sit is easy to tune the absorption and emission wavelengths and introduce the labeling sites by replacement of three aromatic moieties. The fluoroionophores 2 were synthesized from 3,5-dichloro-boron-dipyrromethene by the coupling and the following nucleophilic aromatic substitution. Compared with the related compounds prepared only by the nucleophilic aromatic substitution, the synthetic and fluorescent quantum yields were improved and it has become possible to adjust the wavelengths.

In this presentation, I will also introduce teaching materials that can visualize the coupling reaction.

Z1011A
[ASAS講演]

Flow Injection Chemiluminescence Determination of Ascorbic Acid Using Rhodamine B

(Hiroshima University) ○Tamer Hasanin・Yasuaki Okamoto・Terufumi Fujiwara

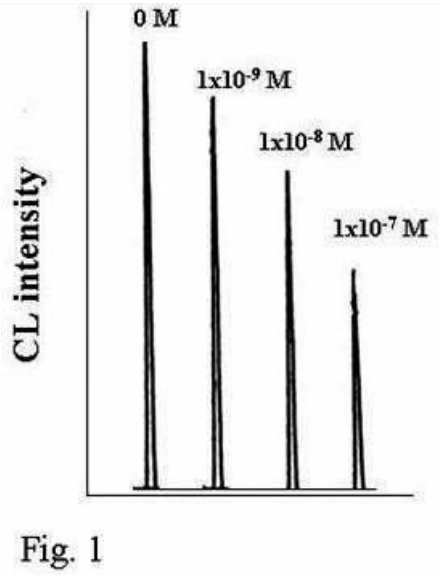


Fig. 1

Ascorbic acid (Vitamin C, AA) has a recommended daily intake of 60 mg. AA found in citrus fruits and vegetable products is mainly a cure for scurvy, drug poisoning, liver disease, allergic reactions and atherosclerosis. Because of its importance, there has been considerable interest in alternative methods of determining the AA content of food products. In this wok, a flow injection chemiluminescence (CL) analysis system for the determination of AA was developed.

The proposed procedure involved : (1) the reduction reaction of tetrachloroaurate (III) with AA ; (2) the on-line extraction of the residual gold (III) from the aqueous hydrochloric acid solution with rhodamine B into toluene, followed by separation of the gold (III)-containing organic phase from the aqueous phase through a microporous Teflon membrane ; (3) measurements of CL produced in a flow cell upon the mixing of the extract stream of gold (III) in toluene with the CL reagent solution of luminol in the reversed micellar medium of cetyltrimethylammonium chloride-water (buffered with sodium carbonate) in 0.38 M 1-hexanol in cyclohexane, which was injected into a reagent stream.

The addition of trace amount of AA to the gold (III) in hydrochloric acid caused a significant lowering of CL intensity obtained in the above procedure. The CL signals at various concentrations of AA are shown in Figure 1 ; the CL intensity decreased with an increase in AA concentration. Under optimized conditions, a detection limit of 1 nM for AA was obtained using the flow injection system.

Z1012A*
[ASAS講演]

Potential-Dependent Encapsulation of Ionic Species in Charged Dendrimers at Liquid|Liquid Interfaces

(Kanazawa University) ○Hirohisa Nagatani

The charge transfer and ion partitioning across an interface between two immiscible electrolyte solutions (ITIES) have been extensively studied for separation sciences, pharmacokinetic analysis, and mass transport in vivo. Dendrimers are unique and nontraditional polymers with a well-defined macromolecular architecture consisting of a core, iterative branch units (interior), and terminal groups. Dendrimers have been demonstrated to be capable of encapsulating various molecules and examined as molecular capsule or container, in which a variety of organic molecules and metal ions can be accommodated in the internal cavity through electrostatic and hydrophobic interactions. Poly (amidoamine) (PAMAM) dendrimer is constructed based on an ethylenediamine core and amidoamine branch units with various terminal groups. The net charge on the PAMAM dendrimer depends on the protonation equilibria of tertiary amines in the interior and terminal groups. The electrostatic interaction between the dendrimer and ionic species, thus, is considerably affected by the pH condition.

This talk will present the characteristic interfacial behavior of amino- and carboxylate-terminated PAMAM dendrimers at the polarized water|1,2-dichloroethane (DCE) interface. The fundamental ion transfer reaction of the dendrimer was successfully analyzed by means of conventional voltammetric techniques and potential modulated fluorescence spectroscopy.[1)] The spectroelectrochemical analysis clearly demonstrated that the interfacial mechanism of the dendrimer involves transfer and adsorption processes depending on the pH condition and the Galvani potential difference.[2)] The molecular encapsulation of anionic fluorescent dye species, i.e. naphthalenesulfonates and porphyrin derivatives, in the PAMAM dendrimer was also investigated at polarized interfaces. The anionic dye species exhibited electrostatic association behavior with the positively charged dendrimer and the stability of the dendrimer-anionic dye associates was varied as a function of pH.[3)] Although the dendrimers stably associated with anionic dye species under acidic conditions, the dye anions were released from the dendrimer at around its transfer potential and then independently transferred across the interface. A negative shift of the transfer potential of dye anion compared to its intrinsic transfer potential was observed for each ion association system. The ion association stability between the dendrimer and dye anions could be estimated from a shift of the transfer potential. The photoreactivity of the ion associates between the PAMAM dendrimer and *meso*-sulfonatophenyl substituted zinc (II) porphyrin was also studied at the polarized water|DCE interface.[4)] The photocurrent response associated with the heterogeneous photoreduction of the water-soluble zinc (II) porphyrin by a lipophilic ferrocene derivative in the organic phase was drastically enhanced by the formation of the dendrimer–zinc (II) porphyrin associates. These findings indicated that the ionic-partitioning property and interfacial reactivity of ionic species can readily be modified through ion association with the charged dendrimer.

References

1) (a) H. Nagatani, "*In Situ* Spectroscopic Characterization of Porphyrins at Liquid Interfaces", in *Handbook of Porphyrin Science*, Vol. 34, K. M. Kadish, K. M. Smith, R. Guilard, eds., World Scientific Publishing, Singapore, pp. 51–96 (2014), (b) H. Nagatani, T. Sagara, *Anal. Sci.*, **23**, 1041–1048 (2007).
2) H. Nagatani, T, Ueno, T, Sagara, *Electrochim. Acta*, **53**, 6428–6433 (2008).
3) (a) H. Nagatani, T. Sakamoto, T. Torikai, T. Sagara, *Langmuir*, **26**, 17686–17694 (2010), (b) H. Sakae, H. Nagatani, K. Morita, H. Imura, *Langmuir*, **30**, 937–945 (2014).
4) H. Nagatani, H. Sakae, T. Torikai, T. Sagara, H. Imura, *Langmuir*, **31**, 6237–6244 (2015).

Z1013A*
[ASAS講演]

The thin layer electrolysis cell with the aqueous and the organic phases and its application to coulometric determination and separation of ions

(Kyoto Institute of Technology) ○Yumi Yoshida・Junya Uchida・Shotaro Nakamura・Kohji Maeda

Electrolytic extraction, in which an ionic species can be extracted or back-extracted between aqueous phase (W) and organic phase (O) by applying the interfacial potential, has unique features comparing with the common solvent extraction. The distribution ratio of the extraction can be changed with the applied potential. Procedures for extraction and back-extraction are attained only by controlling the applied potential and without changing pH or ionic composition in W. Moreover, even if the extracted ionic species is redox-inactive, the amount of the extracted ionic species can be coulometrically determined based on the current caused by the ion transfer.

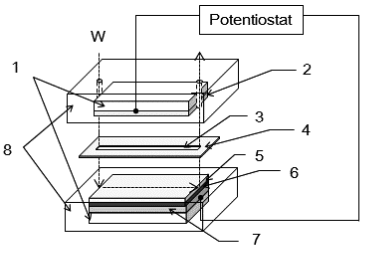


Fig. 1 Construction of thin layer flow cell: (1) polyacrylic block, (2) Ag/AgCl plate, (3) internal flow path (2 mm width, 22 mm length and 50 μm depth), (4) PTFE spacer (50 μm thickness), (5) PTFE porous membrane containing NPOE (30 μm thickness), (6) conducting polymer, (7) Pt plate and (8) poly(dimethylsiloxane).

In electrolytic extraction, thin thickness of the W and O is important for the complete extraction of the objective ion, because extraction kinetics and extraction efficiency are mainly dominated by the diffusion of the objective ion near to the stationary interface. To date, electrolysis cells with only a thin W layer have been reported, and applied to the absolute determination of redox-inactive ions based on ion transfer at the W|O interface [1–4]. In these cells, the absolute determination of a redox-inactive ion using the injection method [1,2], the absolute determination using the continuous flow method [2,4], and the selective absolute determination via the addition of an ionophore into O [2] have been realized. On the other hand, we have developed a thin-layer cell with not only a thin W layer, but also a thin O layer [5,6]. Due to the thin O layer, quantitative back extraction can also be achieved and applied for an absolute determination combined with the stripping technique. Moreover, the thin-layer cell is a two-electrode system, and has a simple laminate structure, contributing to the decrease of the sample volume of redox-inactive ions [5].

The technical breakthrough for preparing the thin O layer is to employ a conducting polymer-coated electrode with functions of both a reference- and a counter electrode in O (reference/counter electrode) [5,6]. We found that the poly (3,4-ethylenedioxythiophene)-coated electrode (PEDOT-E), one of the conducting polymer-coated electrode, was useful for this purpose. In order to use the PEDOT-E as a reference/counter electrode in O, we also evaluated the electrode properties of PEDOT-E in O [6]. Herein, we review the evaluation of the PEDOT-E as a reference/counter electrode for a thin O layer and the performance of the electrolysis cell for the electrochemical extraction of ionic species consisting of thin O and W layers. With the present cell, the absolute determination of a redox-inactive ion using the flow injection method or the stripping method is also described [5].

[1] S. Sawada, et.al., *Anal. Chem*., 2002, **74**, 1177. [2] A. Yoshizumi, et.al., *J. Electroanal. Chem*., 2005, **581**, 275. [3] E. Grygolowicz-Pawlak, et.al., *Anal. Chem*., 2010, **82**, 4537. [4] S. Kihara, et.al., *Anal. Sci*., 2011, **27**, 1. [5] Y. Yoshida, et.al., *J. Electroanal. Chem*., 2013, **707**, 95. [6] Y. Yoshida, et.al., *Anal. Sci*., 2010, **26**, 137.

Z1014A
［ASAS講演］

Formation of Organic Ion Associate Phase from Aqueous Solution for Enrichment and Determination of Trace Heavy Metals in Environmental Water Samples By GF-AAS

（Univ. Toyama）○Syeda Mushahida-Al-Noor・Ryo Murashima・Takuya Okazaki・Noriko Hata・Shigeru Taguchi・Hideki Kuramitz

Introduction Formation liquid organic ion-associate phase（IAP）in aqueous solution was applied to the extraction and GF-AAS determination of trace Cd, Ni and Pb in environmental waters. The optimum conditions were investigated.

Experimental Trace heavy metal in 40 mL sample were converted to complexes with 2-(5-bromo-2-pyridylazo)-5-(*N*-propyl-*N*-sulphopropylamino) phenol (5-Br-PAPS). The pH was adjusted by tetramethylammonium hydroxide（TMAH）. The complexes were extracted into the liquid organic ion-associate of phenolsulphonate anion and benzethonium cation during the phase formation. The viscous organic IAP was dissolved in 0.075 mL of 2M HNO_3 and then 0.025 mL of chemical modifier. Trace metals in water samples were determined by GF-AAS.

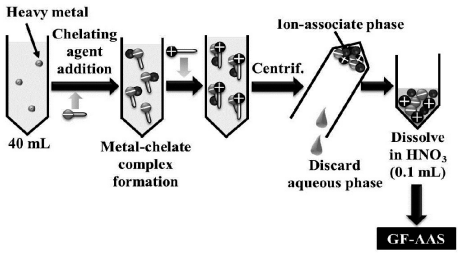


Fig. 1 Procedure of trace heavy metal determination by ion-associate phase extraction

Results and discussion After addition of 5-Br-PAPS, quantitative recovery of trace metals found at pH, ranges from 8 to 10 and the pH was adjusted *ca.* 9 . Trace heavy metals（Cd, Ni or Pb）in different real samples such as river water, spring water and sea water were determined by this method. The recovery of trace metals in environmental water sample was satisfied（100–105%）, which proved the efficacy of this technique. The detection limits were 0.3 ng/L for Cd, 0.2 μg/L for Ni and 0.4 ng/L for Pb and relative standard deviations were found 2–8%.

Conclusion Ion-associate phase extraction technique was successfully applied to attain 400-folds enrichment and GF-AAS determination of trace heavy metals in environmental waters. This technique is simple and rapid, requires single vessel to perform and thus ensure low risk of contamination.

Z2001A*
[ASAS講演]

Platforms of Luminescence in Analytical Chemistry for Nucleic Acids

(School of Pharmacy, Fudan University[1] • School of Pharmaceutical Sciences, Nagasaki University[2])
○Jianzhong Lu[1] • Masaaki Kai[2]

We have reported a series of chemiluminescence (CL) sensors for the detection of different targets, based on special CL reagents as the signaling molecules such as lumnol[1, 2] and 3, 4, 5-trimethoxyl phenylglyoxal (TMPG). TMPG reacts instantaneously with guanine nucleobases (G) of any DNA or RNA sequences to form an unstable CL intermediate for the generation of light. For example, G-free capture DNA sequences were immobilized on the surface of magnetic beads, and then hybridized with one G-rich adenosine-binding aptamer to form our CL sensor for the detection of adenosine.[3] Second, a "sandwich-type" detection strategy was employed for the determination of IgE,[4] where magnetic beads functionalized with capture antibody were reacted with target protein IgE, and then sandwiched with the aptamer-bar-codes which were prepared by assembling polystyrene beads with IgE aptamer. Third, a new amplifying strategy that uses hybridization chain reaction (HCR) was designed to detect specific sequences of DNA, [5] where stable DNA monomers assemble on the magnetic beads only upon exposure to a target DNA. Fourth, a sensitive, specific, simple and rapid CL detection of telomere DNA[6] on the synthesized sephadex beads was performed via a sandwich-type DNA hybridization assay.

In this work, we employed an exponential rolling circle amplification (RCA) and CL instantaneous derivatization technology for the highly specific and sensitive detection of let-7a. Only in the presence of target let-7a, the padlock probe can be ligated and subsequent isothermal RCA process is initiated by DNA polymerase. Following that, long RCA products are digested into many new "target DNA sequences", further initiating numerous new extension processes in a polymerase/nicking amplification mode. Thus, huge numbers of streptavidin aptamers are generated and then accumulated on the magnetic beads for the generation of CL signal. With the employment of instantaneous derivatization reaction between TMPG and G bases on the streptavidin aptamer backbone, a detection limit of 5 amol was obtained with a dynamic range that spanned 5 orders of magnitude under the optimized experimental conditions. Moreover, excellent specificity is achieved by mutating single base on the fully complementary padlock probe and this approach could be easily applied to the detection of let-7a in human lung cells. Upon modification, the approach presented herein could be easily extended to detect other types of miRNA and DNA and even proteins, which enables the developed method to be a universal sensing platform[7] for a variety of target analytes.

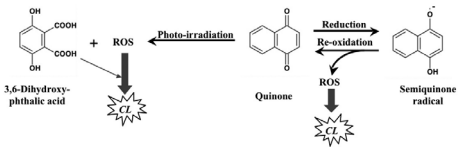
References

1) J. Lu, C. Lau, M. Morizono, K. Ohta, M. Kai, *Anal. Chem.*, **73**, 5979–5983 (2001).
2) H. Zhang, C. Smanmoo, T. Kabashima, J. Lu, and M. Kai, *Angew. Chem. Int. Ed.*, **46**, 8226–8229 (2007).
3) X. Yan, Z. Cao, M. Kai, J. Lu, *Talanta*, **79**, 383–387 (2009).
4) Q, Peng, Z. Cao, C. Lau, M. Kai, J. Lu, JZ, *Analyst*, **136**, 140–147 (2011).
5) X, Wang, C. Lau, M. Kai, J. Lu, JZ, *Analyst*, **138**, 2691–2697 (2013).
6) Ahmed F. M. El-Mahdy, V. Ejupi, T. Shibata, T. Kabashima, J. Lu, M. Kai, *Microchim. Acta*, **182**, 495–503 (2015).
7) A. Fan, Zhijuan Cao, H. Li, M. Kai and J. Lu, *Anal. Sci.*, **25**, 587–597 (2009).

Z2002A*
[ASAS講演]

Development of selective analytical techniques based on the unique characteristics of quinone

(Nagasaki University) ○Naoya Kishikawa



【Introduction】Quinones are interesting compounds which have unique characteristics and several important biological roles. For example, quinones such as ubiquinone have a role in the electron transport chain to maintain biological functions, and also quinone structures are related to some vitamins like vitamin K. While quinones have several beneficial effects, some quinones such as polycyclic aromatic hydrocarbon quinone are regarded as a class of environmental pollutants which can cause a variety of hazardous effects on living cells. Therefore, an effective determination method for quinones is required in many fields. For this purpose, we have developed selective chemiluminescence analytical techniques for quinones based on their unique characteristic, and applied to determine biologically active quinones in environmental, pharmaceutical and biological samples. Firstly, a luminol chemiluminescence method was developed based on the generation of reactive oxygen species (ROS) through the redox cycle reaction of quinone. Secondly, a photo-induced chemiluminescence method was developed based on the formation of ROS and chemiluminescence enhancer by the photo-degradation of quinones.

【Redox reaction based chemiluminescence】In biological systems, quinones are reduced to semiquinone radicals by the enzyme NADPH : quinone reductase. Next, semiquinone radicals react with dissolved oxygen to form superoxide anion, which reacts with biological molecules to cause oxidative stress. On the other hand, chemiluminescence reagents such as luminol can emit chemiluminescence after oxidation by ROS. Therefore, chemiluminescence reagents have been used widely to investigate ROS. From these aspects, we have developed a sensitive and selective assay for quantifying quinones using luminol chemiluminescence. This chemiluminescence method is based on the generation of ROS through the redox reaction between quinone and dithiothreitol (DTT), a reductant, followed by detection of the generated ROS by luminol. This chemiluminescence technique was applied to determine ubiquinone in pharmaceutical and plasma samples.

【Photoinduced chemiluminescence】This method is based on conversion of quinones to ROS including hydrogen peroxide and a fluorescent photoproduct, 3,6-dihydroxyphthalic acid under UV irradiation, and the liberated hydrogen peroxide could be detected by peroxyoxalate chemiluminescence reaction via only mixing with diaryl oxalate. Additionally, it was noticed that 3,6-dihydroxyphthalic acid has a strong enhancing effect on luminol chemiluminescence. The photogeneration of the enhancer in association with the ROS in that photochemical reaction greatly increases the light output of luminol chemiluminescence. This phenomenon allowed the development of a highly sensitive and selective HPLC method for the determination of quinones. These chemiluminescence techniques was successfully applied to determine polycyclic aromatic hydrocarbon quinones in airborne particulates or to determine vitamin K in plasma samples.

Z2003A
[ASAS講演]

A Single Probe to Sense Al(III) Colorimetrically and Cd(II) by Turn-On Fluorescence in Physiological Conditions and in Live Cells.

(Keio Univ.[1] • Indian Inst. of Technology Guwahati[2]) ○Chirantan Kar[1,2] • Soham Samanta[2] • Aiyagari Ramesh[2] • Gopal Das[2]

Highly selective sensing of cation or anion is crucial in many areas of technology, including environmental, biological, clinical, and waste management applications. For heavy- and transition-metal ions, selective sensing are particularly significant due to their high toxicity and essential role in biological systems. Although a number of different concepts in metal-ion sensing have been developed to enhance sensitivity, selectivity, and the dynamic working range, the recognition of congregations of chemical species and of multianalyte mixtures still poses a major challenge. In most of the previous reports of multi-ion detection, the sensor probe is designed with multiple receptor sites for targeting different analyte. Recently a new and alternate strategy has been developed where the probes are designed with a single receptor unit but sensing of multiple analyte is acheived by using an array of different detection method. In a recent work we have developed a pyridine-2-carbohydrazide functionalized conjugated fluorophoric Schiff base ligand $\mathbf{L_1}$ specifically sense Al^{3+} and Cd^{2+} ions through significant changes in their absorption and emission spectral behavior, respectively, in physiological condition. The spectral changes are in the visible region of the spectrum and thus facilitate naked eye detection. Apart from the visible changes, an in-field device application was demonstrated by sensing these ions in paper strips coated with $\mathbf{L_1}$. The crystal structure of the $\mathbf{L_1}$**-Cd** complex provided additional insight of the metal coordination attribute of $\mathbf{L_1}$. Interestingly, fluorescence microscopic studies demonstrated that the ligand $\mathbf{L_1}$ could also be used as an effective probe in imaging experiments for detection of intracellular Cd^{2+} ions in HeLa cells without any toxicity to these model human cells.

Z2004A
[ASAS講演]

Creation of artificial luciferases and their applications for molecular imaging technologies

(Environmental Management Research Institute, AIST) ○Sung-Bae Kim

This presentation demonstrates functional artificial luciferases (ALucs) wholly synthesized for bioassays and molecular imaging. The ALucs bearing epitopes were newly created by amending the sequences of our previously reported ALucs in light of a multi-sequence alignment and hydrophobicity search. The made ALucs are survived in live cells and stable in culture media for 25 days after secretion. The epitopes in ALucs are exposed during the secretion process and indeed valid for column purification and immunological assays. The made ALucs exerted a 9400-times stronger substrate selectivity to a coelenterazine derivative (CTZ i), compared to Renilla reniformis luciferase 8.6–535. A supersecondary structure of ALuc30 was predicted with respect to the X-ray crystallographic information of the coelenterazine-binding protein (CBP). The structure revealed that ALuc30 has a room for accommodating to the iodide of CTZ i. The present study guides on how to create functional artificial luciferases and predicts the structural details with the current bioinformatics technologies.

Reference : Kim et al. Biochem. Biophy. Res. Commun. 448 (2014) 418–423.

Z2005A*
[ASAS講演]

Planarization of cell membrane on supported lipid bilayers

(Okayama University) ○Takashi Kaneta・Tomomi Maki

Membrane proteins play important roles in cellular excretion and uptake of substances, for example, ion channel proteins work as a pump of sodium ion and potassium ion, and transporter proteins excrete organic molecules selectively. Therefore, the analysis of membrane proteins is a key issue in understanding the mechanism of regulations in biological cells. However, extraction of membrane proteins is, in general, difficult without denaturation since they are insoluble in water because of lipophilic property. In this study, we propose a novel method to extract membrane proteins onto supported lipid bilayers (SLBs) as maintaining their functionality.

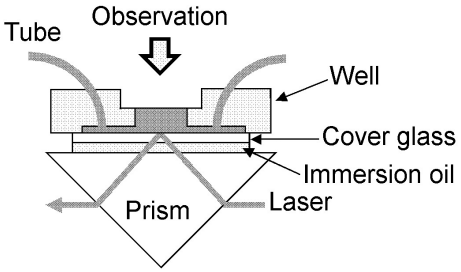


Fig. 1 Experimental Setup

In this study, SLBs were formed on a cover glass treated with a piranha solution by vesicle fusion. Liposomes consisting of 1,2-Dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) and phosphoethanolamine-N-lissamine rhodamin B slfonyl (fluorescent lipid) were prepared according to the following procedure : a mixture of DPPC and the fluorescent lipid dissolved in chloroform was taken into a round-bottomed tube. The solvent was evaporated under a gentle nitrogen flow, after which the tube was put in a vacuum desiccator to remove residual chloroform. The lipid film was rehydrated by adding an aliquot of water to the tube, followed by sonication and extrusion to uniform the size of the liposomes. The formation of SLBs was monitored by a microscope equipped with a CCD camera under total internal reflection conditions where the cover glass was put on a right-angle prism with immersion oil, and a laser emitting at 532 nm was incident into the prism. The experimental setup is shown in Fig. 1.

In order to extract lipid membrane proteins of biological cells onto the SLBs, cell blebs were prepared using HeLa cells stained with octadecyl rhodamne B. HeLa cells were washed with a buffer solution containing 10 mM HEPES, 2 mM CaCl2, and 150 mM NaCl twice, and then were shaken gently in the buffer solution containing 25 mM formaldehyde and 2 mM dithiothreitol for 1 h, resulting in suspension of cell blebs. The upper portion of the buffer solution in the dish was used to fuse the cell blebs onto SLBs.

When liposomes containing the fluorescent lipid were added to a well with the bottom of the hydrophilic cover glass, the fluorescence intensity increased with time, indicating the SLBs formation. However, no fusion was observed by adding the cell blebs onto SLBs although sedimentation of the blebs can be seen. The reason of unsuccessful fusion would be attributed to insufficient affinity between SLBs and membranes of HeLa cells. Thus, it was expected that the composition of the lipids must be optimized in the future work.

Z2006A*
[ASAS講演]

Nanowires for analyzing biomolecules

(Nagoya Univ., Engineering[1]・Nagoya Univ., Nanobio.[2]・Kyushu Univ., IMCE[3]・Osaka Univ., ISIR[4]・AIST Health Research Institute[5]) ○Takao Yasui[1,2]・Takeshi Yanagida[3,4]・Sakon Rahong[1,2]・Noritada Kaji[1,2]・Tomoji Kawai[4]・Yoshinobu Baba[1,2,5]

Nanostructures based on advanced nanotechnology open up a novel research field for biomolecule analysis with the ultrahigh resolution, including a single biomolecule analysis. An electrophoretic operation of biomolecules by utilizing artificial nanostructures embedded in microchannels has emerged as a promising technique since it was first proposed. A number of unique artificial nanostructures have been examined to analyze biomolecules. These highly ordered nanostructures have provided less time-intensive and required fewer manual operations when compared with other conventional methods. Although recent progresses in micro- and nano-fabrication technologies have allowed us to fabricate smaller and more precise artificial nanostructures for analyzing biomolecules, there is still an inherent size limitation of lithographic technology.

Self-assembled one-dimensional nanowires down to several nano-meters have attracted much attention due to not only the fundamental interests in nanoscale-confined properties but also novel nano-device applications, where existing nano-materials have not been applicable. Among various nanowire materials, "oxide nanowire" is most promising candidate for the artificial nanostructures, due to their robust surface properties and fascinating redox surface events especially contacting with water, which are hardly attainable to other conventional semiconductor nanowires. In this talk, we demonstrate the feasibility of bottom-up nanowire array embedded in microchannels using vapor-liquid-solid (VLS) methodology, and the application example for biomolecule analysis.

References

[1] T. Yasui, N. Kaji, R. Ogawa, S. Hashioka, M. Tokeshi, Y. Horiike and Y. Baba, *Nano Lett.*, **15**, 3445–3451 (2015) ; T. Yasui, N. Kaji, M. R. Mohamadi, Y. Okamoto, M. Tokeshi, Y. Horiike and Y. Baba, *ACS Nano*, **5**, 7775–7780 (2011) ; T. Yasui, N. Kaji, R. Ogawa, S. Hashioka, M. Tokeshi, Y. Horiike and Y. Baba, *Anal. Chem.*, **83**, 6635–6640 (2011)

[2] T. Yasui, S. Rahong, K. Motoyama, T. Yanagida, Q. Wu, N. Kaji, M. Kanai, K. Doi, K. Nagashima, M. Tokeshi, M. Taniguchi, S. Kawano, T. Kawai and Y. Baba, *ACS Nano*, **7**, 3029–3035 (2013) ; S. Rahong, T. Yasui, T. Yanagida, K. Nagashima, M. Kanai, A. Klamchuen, G. Meng, Y. He, F. Zhuge, N. Kaji, T. Kawai and Y. Baba, *Scientific reports*, **4**, 5252–5259 (2014).

Z2007A*
[ASAS講演]

Stabilities of DNA secondary structures inside confined nanospace

(Ibaraki University) ○Akira Yamaguchi・Kazuyoshi Nasu・Ryoko Yoshida・Shigeki Wakaume

Self-assembly of nucleotides of fewer than three base pairs is often found in protein-nucleotide conjugations, despite their energetic instability, and is regarded as the potential starting point for the creation of artificial hydrogen-bonded supramolecular complexes. In the present study, we examined duplex formation of 3-mer DNA fragments confined within silica mesopores modified with a positively-charged trimethylaminopropyl monolayer (effective pore diameter = 2.4 nm), and their further stabilization under supercooled conditions ($T < 273$ K) [1]. We loaded 3-mer DNA fragments with donor- or acceptor-dye (d-TTT and a-AAA) into modified silica mesopores and examine their hybridization behaviors using fluorescence energy transfer (FRET) measurements. The FRET results clearly revealed that efficient duplex formation thorough at least two A-T base pairs can be achieved at 233 K, indicating that confined meso-scale cavities to be a novel low-temperature reaction space for hydrogen-bonded supramolecular complexes. In contrast to the linear-duplex of short-DNA fragments, hairpin structure of (CCG) 4 trinucleotide repeat was destabilized by confinement inside the amine-functionalized silica mesopore. The structural stability of DNA inside meso-scale cavity depends on secondary structure of DNA.

[1] H. Arafune et al., Nat. Commun., 5, 5151 (2014).

Z2008A*
[ASAS講演]

Speciation of sulfur in thin films and solutions : X-ray absorption fine structure study at Hiroshima synchrotron radiation center

(Hiroshima University) ○Shinjiro Hayakawa

X-ray absorption edge is specific energy corresponding to the ionization of a core electron, and the fine structures in X-ray absorption spectrum around the absorption edge is widely utilized as a strong tool for investigation of chemical state and local structure of the element of interest. Measurements of X-ray absorption fine structure (XAFS) are usually carried out in the facility equipped with the synchrotron light source, and the relatively low frequency of the beam time is disadvantageous.

Hiroshima University has its own compact synchrotron light source. The range of X-ray energy is limited up to 5 keV, but the soft X-rays from the storage ring is fully utilized. One of the beamlines (BL11) was dedicated for XAFS measurements, and a specially designed He filled chamber is utilized for XAFS measurements of solid and liquid samples. The beamline is equipped with a Si(111) double crystal monochromator, and the X-rays from 2.1 keV to 5 keV are utilized for K edges from P to Ti, and L edges from Sr to Ce.

In this presentation speciation of sulfur in the sulfite solutions and the sulfide thin films will be presented.

L1001A*
[ASAS講演]

Microwave-assisted rapid fabrication of monolithic stationary phases for capillary liquid chromatography

(Gifu University) ○Lee Wah Lim・Toyohide Takeuchi

With the increasing concern of environmentally friendly analysis, miniaturization of chromatographic systems has attracted much attention and the development of capillary-based separation systems has grown tremendously especially after the invention of fused-silica capillaries. Capillary systems do not only reduce the amount of solvents, reagents and packing materials used, but ultra-high sensitive and selective analysis of the most complex samples, particularly when it is directly connected to a mass spectrometer (MS) or a tandem MS/MS detection unit, could also be expected. Capillary columns also make the development of novel stationary phases so much easier and more importantly, affordable.

In this study, we focused our attention on the rapid fabrication of monolithic capillary columns using a microwave device and the experimental conditions were optimized. Monolithic material has attracted much attention since its introduction in the early 1990s and has been studied intensively in recent years due to its high separation efficiency with ultra low flow resistance, which makes it suitable for high speed separation required in the modern analysis nowadays. Generally, monolithic columns can be divided into two types, i.e. silica- and organic polymer-based, and both consist of μm-sized skeletons and through-pores. The silica monolith has some advantages over the polymer monolith, such as good mechanical strength, well-controlled pore structure and high column efficiency especially for small molecules. On the other hand, polymer monolith is robust over a wide range of pH and it is easy to prepare.

Silica monolith is normally prepared via thermal initiation, while polymer monolith could be prepared via thermal- and photo-polymerization. Photo-polymerization is more popular recently because it has some advantages over the traditional thermal-polymerization, while microwave irradiation had been reported as an attractive alternative in preparing polystyrene-based monolith and it could achieve fast and localized polymerization as in photo-polymerization [1]. Microwaves are electromagnetic waves with frequencies ranging from 300 MHz to 300 GHz, and it has been widely used and applied in the field of organic synthesis reactions such as radical reactions, organometallic reactions, rearrangement, metathesis, alkyl/aryl coupling, and so on [2]. We thus focused our attention in using the advantage of this electromagnetic wave dielectric heating in fabricating both silica- and organic polymer-based monoliths for capillary liquid chromatography.

Monolithic silica- and organic polymer-based capillary columns were fabricated using a MWO-1000S (Wave Magic) microwave device, which has a controllable output range from 50 to 500 W, and a controllable temperature range between room temperature + 10-250℃. The reaction solution was filled into fused-silica capillary tubing and the column was then irradiated at different time under various temperature and output control of the microwave device. The morphology of the monoliths was observed with a scanning electron microscope (SEM) and was compared to those monoliths that were fabricated under normal thermal conditions. The results showed that the degree of output of the microwave device had direct influence on the morphology as well as size of the skeleton backbone of the monoliths. In addition, when a methacrylate-based polymer was used, the capillary column could be fabricated within less than 15 min, which is ca. 100-fold faster than the conventional thermal polymerization in a water-bath, and the theoretical plate number, i.e. N is calculated to be approx. 50,000 plates/m for a normal liquid chromatographic separation.

References
[1] Y-P Zhang, X-W Ye, M-K Tian, L-B Qu, S-H Choi, A. I. Gopalan, K-P Lee, J. Chromatogr. A, 1188, 43–49 (2008).
[2] P. Lidström, J. P. Tierney, B. Wathey, J. Westman, Tetrahedron, 57, 9225–9283 (2001).

L1002A
[ASAS講演]

Evaluation of chiral recognition ability of quinoline-based oligoamide foldamers with one-handed helical structure

(Kumamoto Univ.[1]・Univ. of Bordeaux[2]・CNRS[3]・Kumamoto Inst. for Photo-electro Organics (PHOENICS)[4]) ○Hiroki Noguchi[1]・Victor Maurizot[2,3]・Ivan Huc[2,3]・Makoto Takafuji[1]・Hirotaka Ihara[1,4]

The quinoline oligoamide foldamer (Q_n) is bio-inspired oligomer that forms helical structure in solution.[1] We reported that the handedness of the Q_n helix could be controlled by introducing chiral source such as camphanyl group at the one terminal of foldamer.[2] In this study, we demonstrate to develop chiral stationary phase for HPLC using the Q_n helix. To stabilize the Q_n helix on the surface of porous silica particles, new quinoline oligoamide tetramer (Q_4-Cmp) was synthesized, which a (S)-camphanyl moiety and a trimethoxysilyl group were introduced to both termini of Q_4. Q_4-Cmp was immobilized onto the silica surface through siloxane bonds (Sil-Q_4-Cmp). Elemental analysis indicated that the amount of Q_4-Cmp on the silica surface was 0.63 μmol/m^2. The chiral recognition ability of Sil-Q_4-Cmp was evaluated with various enantiomers. Sil-Q_4-Cmp showed enantio-selectivity for axially chiral molecules (e.g. $\alpha_{trans-\text{stilben oxide}}$=1.04, $\alpha_{1-1'-\text{Bi (2-naphthol)}}$ = 1.08). Sil-Q_4-Cmp showed remarkable recognition of quinoline oligoamides (Q_n) with same handedness ($\alpha_{P/M}$=10.36). On the other hand, (S)-camphanyl group-immobilized porous silica (Sil-Cmp) showed no chiral recognition for all enantiomers used in this study. These results suggested that the chiral recognition ability of Q_n was based on the helical structure of Q_4-Cmp.

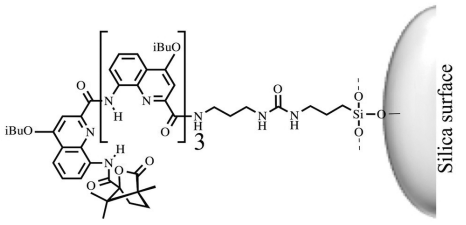


Fig.1 Chemical structure of quinoline oligoamide tetramer immobilized silica (Sil-Q_4-Cmp).

[1] T. Qi, T. Deschrijver, I. Huc, Nat. Protc., 8, 2013.
[2] A. M. Kendhale, L. P. Zeyuan, K. Laxmi-Reddy, B. Kauffman, Y. Ferrand, I. Huc, J. Org. Chem., 76, 2011.

L1003A*
[ASAS講演]

Applications of Functional Metabolomics in Investigating the Biological Functions of Small Molecules

(Dalian Institute of Chemical Physics, CAS) ○Guowang Xu・Peng Gao・Peiyuan Yin・Xin Lu

Small molecules are the intermediates or end products of metabolism, they play important roles in a biological system (cell, tissue, or organism) with various functions including structure unit, fuel, hormone, signaling, defense in a biological system or biomarkers for disease diagnosis and drug R & D. To understand the life process, functions of small molecules should be shed light on.
Metabolomics is a very useful tool for studying the small molecules under a given condition by using NMR or mass spectrometry (MS) techniques. It can be used to define the differential metabolites, further, novel metabolic markers based on the metabolic profiling data and multi/mono-variate analysis of two or more groups. Therefore it can be used to investigate the functions of small molecules.
Over the last 10 years the targeted and non-targeted metabolomics studies have been greatly intensified. However, up to now most of them are of a descriptive nature mainly based on literatures and only in several cases the missing link between important differential metabolites and their (patho) physiological function has been elucidated.
In this lecture, we shall report a functional metabolomics platform including stable isotope-labelled cell-based dynamics metabolomics, target, non-target and pseudotarget analyses based on the various chromatography - mass spectrometry methods[1)] and molecular biology. By using this platform we can study not only the potential of the small molecules as potential disease biomarkers[2)], but also the basic biological functions[3)].

References
1) (a) J. Li, M. Hoene, X. Zhao, S. Chen, H. Wei, H. Häring, X. Lin, Z. Zeng, C. Weigert, R. Lehmann, G. Xu, *Anal. Chem*. **85**, 4651–7 (2013), (b) P. Yin, G. Xu, *J. Chromatogr. A*, **1374**, 1–13 (2014).
2) (a) P. Gao, C. Yang, H. Liu,C. L. Swallows, H. Chu, F. Yang1, L. Tang , J. Tian, S. Zhao, G. Li, J. D. Heiss, Z. Zhuang, G. Xu, *Cancer Research*, (2015) submitted. (b) M. Wolf, S. Chen, X. Zhao, M. Scheler, M. Irmler, H. Staiger, J. Beckers, M. H. de Angelis, A. Fritsche, H. Häring, E. D. Schleicher, G. Xu, R. Lehmann, C. Weigert, *J. Clin. Endocrinol. Metab*., **98**, E1137–E1142 (2013), (c) R. Lehmann, H. Franken, S. Dammeier, L. Rosenbaum, K. Kantartzis, A. Peter, A. Zell, P. Adam, J. Li, G. Xu, A. Konigsrainer, J. Machann, F. Schick, M. H. de Angelis, M. Schwab, H. Staiger, E. Schleicher, A. Gastaldelli, A. Fritsche, H. Haring, N. Stefan, *Diabetes Care*, **36**, 2331–2338 (2013).
3) R. Wu, Z. Wu, X. Wang, P. Yang, D. Yu, C. Zhao, G. Xu, L. Kang, *Proc Natl Acad Sci USA*, **109**, 3259–63 (2012).

L2001A*

Specific Liquid Chromatographic Separations by a C_{60}-fullerene Bonded Silica-monolithic Capillary Prepared via Perfluorophenyl azide

(Kyoto Univ.[1]・UMASS, Lowell[2]) ○KUBO, Takuya[1]・TSUZUKI, Madoka[1]・NAITO, Toyohiro[1]・YAN, Mingdi[2]・OTSUKA, Koji[2]

Carbon nano-materials such as a fullerene, graphene, and nanotube have been widely studied in the fields because of their excellent thermal and/or electrical conductivity, physical strength, and specific surface area. These carbon materials consist of a π-conjugated system from sp2 carbons and π electrons that are diffusely distributed within the molecule. The π electrons provide the dipole interaction based on the London dispersion force and allow the π–π interaction in regard to the aromatic compounds. According to these advantages of the nano-carbon materials in separation fields, we previously reported the preparation of a C60-fullerene bonded open-tubular capillary (1). In present study, we report the preparation procedure of a C60-fullerene bonded silica-monolithic capillary using a photo/thermal active agent, perfluorophenyl azide (PFPA) and its specific separation ability in liquid chromatography. C60-fullerene conjugated PFPA with N-hydroxysuccinimide (NHS) was successfully synthesized in advance. The C60-PFPA-NHS was thermally reacted with a silica-monolithic capillary, which was modified by (3-aminopropyl) trimethoxysilane. After the optimization of the preparation procedures, the density of the bonded C60-fullerene onto the surface of a silica monolith was reached to 32.0 μmol g − 1.

As results of liquid chromatographic analyses for the C60-fullerene bonded silica-monolithic capillary, specific separations based on effective π–π interaction were achieved for polycyclic aromatic hydrocarbons, such as naphthalene, anthracene, triphenylene, and benzo [a] pyrene using n-hexane as the mobile phase. Furthermore, several aromatic compounds with various functional groups were also separated due to the differences of π–π interaction strength. Interestingly, the C60-fullerene bonded silica-monolithic capillary provided specific separation between coronene and corannulene. In general, the retention of coronene is much higher than that of corannulene in the separation with π stacking based LC column because coronene has more π-conjugations, whereas corannulene has cup-shape structure. However, corannulene was more retained than that of coronene in the C60-fullerene bonded silica-monolithic capillary. The result strongly suggested that spherical surface of C60-fullerene contributed to the specific retention of corannulene.

References
1) T. Kubo, Y. Murakami, Y. Tominaga, T. Naito, K. Sueyoshi, M. Yan, K. Otsuka, J. Chromatogr. A, 1323, 174–178 (2014)

L2002A　講演中止

E1012A*
〔ASAS講演〕

Multi-functional Nanopipette Probe for Single-cell Analysis of Tissue Models

(Tohoku Univ.[1]・Tohoku Univ. WPI-AIMR[2]) ○Hitoshi Shiku[1]・Yuji Nashimoto[1]・Hidenori Ito[1]・
Yuanshu Zhou[2]・Yasufumi Takahashi[2]・Kosuke Ino[1]・Tomokazu Matsue[2]

Recently, high-throughput RNA analysis at single-cell level has become available and applied to various biological fields including embryogenesis, organogenesis, and tumor progression. However the methods to link these comprehensive transcript data with positioning were not established. In conventional methods, dissociation of tissue for single-cell sorting by fluorescence-activated cell sorting (FACS) is required, that causes the loss of the spatial information of the individual single-cell samples. We have reported that the cellular function linking its nanoscale address using various multi-functional probe, and the mRNA analysis by direct collection of cytoplasm in situ.

We have developed a platform for comprehensive single-cell gene-expression analysis and other ormic spaces of early embryos, embryonic stem (ES) cells, and other cellular aggregates based on scanning probe microscopy (SPM). We prepared a double barrel carbon probe (DBCP) to electrically lyse single cells and collect mRNA. The cells were collected from the beating or non-beating areas of the ES-derived myocardium. The expression level of the myocardium marker Actc-1 at the beating area was significantly higher than that at non-beating area.[1)] ES cells-derived angiogenesis model was also evaluated. First, we demonstrated the local cellular collection from live tissue in hydrogel was possible to quantify mRNA. Second, we found that vascular marker genes (Pecam1, Dll4, Robo4) expressed higher in sprouting vessel than in central core of the embryoid body. The expression of housekeeping gene (Actb) had no difference between the sprouting vessel and the central core of the embryoid body.

The probe was also applied for high-resolution multi-functional SPM imaging. Differentiation status of single ES cells were quantified by monitoring the activity of alkaline phosphatase using scanning electrochemical microscopy (SECM). The current responses for single ES cells were significantly higher than those for differentiated ES cells. The result was consistent with the mRNA expression level of Oct-3/4, a pluripotent marker gene. Scanning ion conductance microscopy (SICM) was applied for tip-sample distance regulation on the basis of feedback control to maintain the ion current through a nanopipette filled with electrolyte solution. A nanopipette/nanoring electrode probe was used for simultaneous imaging of topography and electrochemical responses of immobilized enzyme membranes and single living cells. [2)] Double barrel carbon nanoprobe (CBCNP) was also fabricated with one barrel for carbon electrode to obtain electrochemical response and another for pipette filled with electrolyte to maintain the tip-sample distance in constant.[3)]

Finally, simultaneous nano-imaging and subcellular collection was developed by combining the double barrel nanopipette as scanning ion conductance microscopy (SICM) and electrochemical syringe. Simultaneous nano-imaging and subcellular collection system was demonstrated. The electrochemical syringe using organic-phase barrel could collect minute amounts of cytoplasm by controlling the charge at the oil-water interface. It was confirmed that collected cytoplasm was enough to judge the cellular differentiation status of mouse embryoid bodies from the subcellular volume of the sampling. The precise imaging could be achieved using the water phase current based on SICM. This system was applied to the Actb mRNA localization in single cell. The present system will be applicable for spatially resolved gene expression analysis with intracellular positioning.

References

1) Y. Nashimoto et al. Anal. Bioanal. Chem. 406 (1), 275–282 (2014).
2) Y. Takahashi et al. J. Am. Chem. Soc. 132, 10118–10126 (2010).
3) Y. Takahashi et al. Angew. Chem. Int. Ed. 50, 9638–9642 (2011).

E1013A*
〔ASAS講演〕

A Sputtered Nanocarbon Film Electrode as a Platform for Detecting Biomolecules

(Biomedical Research Institute, AIST) ○Dai KATO

We have been studying nanocarbon film electrodes formed by an electron cyclotron resonance (ECR) sputtering or an unbalanced magnetron (UBM) sputtering method. [1)]The film provides a nanocrystalline sp^2 and sp^3 mixed bond structure with an atomically flat surface (surface roughness of 0.05–0.1 nm) and high conductivity without doping. The film electrode has excellent properties including a low background current, a wide electrochemical potential window, and little surface fouling, while maintaining relatively high electrode activity for various biomolecules. [2)]These characteristics allow the detection of biomolecules with slower electron transfer rates, such as pyrimidine bases. As a result, this film electrode can measure all DNA bases (including DNA base derivatives e.g., 5′-methylcytosine and 8′-hydroxy 2′-deoxyguanosine) quantitatively. [3)]Moreover, due to their good electrochemical stability and low background current, the nanocarbon film electrode is suitable for long-term analysis including as the electrode of an HPLC detector for detecting cerebral gliotransmitter from real samples. [4)]

Recently, we developed an electrochemical lipopolysaccharides (LPS) detection system using the nanocarbon film electrode because the atomically flat surface of the nanocarbon film electrode can be expected to achieve electrochemical measurement of LPS with low concentration (pg/mL–ng/mL). The detection system constitute an LPS-affinity microparticle, a zinc complex-based probe for LPS detection (LPS probe), and our nanocarbon film electrode. LPS sample was captured on the microparticles modified with poly-ε-lysine with a high affinity to LPS, and then the LPS probes consisting of zinc complex parts and LPS-affinity molecules such as cetylpyridinium (CP) were captured on the LPS adsorbed microparticles via the LPS-CP affinity interaction. The adsorbed LPS probe was treated with acid solution, and finally we obtained zinc ion (Zn^{2+}) containing solution. Anodic stripping voltammetry (ASV) was carried out using our nanocarbon film electrode to measure Zn^{2+} concentration. Our nanocarbon film electrode allowed us to detect Zn^{2+} with lower concentration compared with a conventionally available glassy carbon electrode. This was due to (1) the efficient accumulation of Zn^{2+} on the nanocarbon film, and (2) the very low noise made possible by the ultraflat surface. The current response was dependent on the amount of captured LPS (LOD = 0.2 ng/mL), which was superior result to our previous reports. [5)]

References

1) (a) O. Niwa, J. Jia, Y. Sato, D. Kato, R. Kurita, K. Maruyama, K. Suzuki, S. Hirono, *J. Am. Chem. Soc.*, **128**, 7144 (2006), (b) J. B. Jia, D. Kato, R. Kurita, Y. Sato, K. Maruyama, K. Suzuki, S. Hirono, T. Ando, O. Niwa, *Anal. Chem.*, **79**, 98 (2007), (c) T. Kamata, D. Kato, H. Ida, O. Niwa, *Diamond. Relat. Mater.*, **49**, 25 (2014).
2) (a) A. Ueda, D. Kato, R. Kurita, T. Kamata, H. Inokuchi, S. Umemura, S. Hirono, O. Niwa, *J. Am. Chem. Soc.*, **133**, 4840 (2011), (b) D. Kato, M. Sumimoto, A. Ueda, S. Hirono, O. Niwa, *Anal. Chem.*, **84**, 10607 (2012).
3) (a) D. Kato, N. Sekioka, A. Ueda, R. Kurita, S. Hirono, K. Suzuki, O. Niwa, *Angew. Chem. Int. Ed.*, **47**, 6681 (2008), (b) D. Kato, N. Sekioka, A. Ueda, R. Kurita, S. Hirono, K. Suzuki, O. Niwa, *J. Am. Chem. Soc.*, **130**, 3716 (2008), (c) D. Kato, K. Goto, S. Fujii, A. Takatsu, S. Hirono, O. Niwa, *Anal. Chem.*, **83**, 7595 (2011), (d) D. Kato, M. Komoriya, K. Nakamoto, R. Kurita, S. Hirono, O. Niwa, *Anal. Sci.*, **27**, 703 (2011).
4) S. Yamamura, M. Hoshikawa, D. Kato, H. Saito, N. Suzuki, O. Niwa, M. Okada, *Br. J. Pharmacol.*, **168** 1088 (2013).
5) (a) D. Kato, S. Iijima, R. Kurita, Y. Sato, J. Jia, S. Yabuki, F. Mizutani, O. Niwa, *Biosens. Bioelectron.*, **22**, 1527 (2007), (b) S. Iijima,. D. Kato, S. Yabuki, O. Niwa, F. Mizutani, *Biosens. Bioelectron.*, **26**, 2080 (2011), (c) D. Kato, A. Oda, M. Tanaka, S. Iijima, T. Kamata, M. Todokoro, Y. Yoshimi, O. Niwa, *Electroanalysis,*, **26**, 618 (2014).

E1014A*
〔ASAS講演〕

Ferrocenylnaphthalenediimide-based Electrochemical telomerase assay（ECTA）as oral cancer screening

(Kyutech[1]・Kyushu Dental Univ.[2]) ○Shinobu SATOU[1]・Mana Hayakawa[2]・
Kazuhiro Tominaga[2]・Tatsuji Nishihara[2]・Shigeori Takenaka[1]

Telomerase is expected to serve as a new tumor marker and its activity has been detected by Telomerase Repeat Amplification Protocol (TRAP), which contains some laborious manipulations such as PCR and gel electrophoresis.
We have been developing an electrochemical gene detection method with ferrocenylnaphthalene diimide (FND) as an electrochemical hybridization indicator. The advantages of this method lie in the speed and high sensitivity. In fact, it is possible to detect telomerase activity electrochemically without PCR. Where telomerase activity is present in a sample solution, a telomerase substrate (TS)-primer immobilized on the electrode is elongated to yield a telomeric repeat sequence and the resulting products can form a tetraplex DNA. FND binds to the tetraplex formed on the electrode to give rise to an electrochemical signal whose magnitude reflects telomerase activity. Herein, we tested telomerase activity in sexfoliated oral cells, and solid tumor for diagnosis of oral cancer.
On the basis of this difference individual clinical samples were judged telomerase positive, ambiguous or negative. The positive rate in the cancerous tissue and exfoliated cells of the patients was 85 and 80%, respectively, whereas the corresponding values were 50 and 10% by the Telomerase Repeat Amplification Protocol as an existing method. Furthermore, the positive rate amounted to 100% in early tumors smaller than 2 cm by ECTA. Likewise, 95 and 80% of biopsy and exfoliated cells of healthy individuals were judged negative properly. The electrochemical method yielded high hit rates for cancerous and normal cells, especially in exfoliated cells, making this low invasive test suitable for oral cancer diagnosis.

H1012A*
[ASAS講演]

Development and Application of ICP-MS Techniques for Elemental Analysis in Chemical Metrology

（National Metrology Institute of Japan, AIST）○Yanbei（やんべい） Zhu（しゅ）

Development and Application of ICP-MS Techniques for Elemental Analysis in Chemical Metrology
Yanbei Zhu
Research Institute for Material and Chemical Measurement, National Metrology Institute of Japan,
National Institute of Advanced Industrial Science and Technology
E-mail : yb-zhu@aist.go.jp

Inductively coupled plasma mass spectrometry（ICP-MS）is a powerful instrument with an extreme high temperature ionization source（6000 to 10000 K）as well as the capabilities of simultaneous multi elemental analysis and isotope analysis. The high temperature resulted ionization ratios over 90% for most metals and lower detection limits under ng mL-1 or even pg mL-1. However, accuracy and precision are especially required for the application of ICP-MS in chemical metrology. For such purpose, matrix effects and spectral interferences should be removed effectively. Spectral interferences could be removed by chemical separation, high-resolution ICP-MS or collision/reaction cell ICP-MS. Matrix effects could be cancelled by isotope-dilution（ID）analysis, matrix matching, and standard addition. The present author had developed a gravimetric standard addition method coupled with internal standard correction, along with other multiple techniques for elemental analysis in chemical metrology.

In the presentation, the discussion will be focused on the standard addition method with the operation, calculation, uncertainty estimation, validation, and application in real sample as well as in international comparison. The calculation equations were given for measurement of the element based on its ratios to the internal-standard in the spiked sample and the non-spiked sample. The relative quantity of standard solution added to make the spiked sample was optimized to improve the precision of measurement. The results showed that high-precision measurement could be obtained when the concentration of the measurand in the spiked sample were over 2-fold of that in the non-spiked sample. Uncertainty estimation in the present method was estimated based on a spreadsheet approach considering all of the parameters concerning the calculation equations. Based on the present method, the elemental analysis could be carried out with an expanded relative uncertainty close to or lower than 1%, which is comparable to the precision of ID analysis. Multiple elements, the constitute of a sample or added element, could be used as internal standard element for the measurement and the results neither depended on the atomic number nor depended on the ionization rate of the internal standard element.

Validation of the sample was confirmed by analyzing multiple certified reference materials（CRMs）issued by the National Metrology Institute of Japan, the National Institute of Advanced Industrial Science and Technology. The method was applied to the internal comparisons of samples with various matrixes, such as ethanol, human serum, shrimp powder, bio-diesel, and so on.

References

1）Y. Zhu, T. Narukawa, M. Numata, et. al, *Fuel*, **103**, 736–741（2013）.
2）Y. Zhu, T. Narukawa, S. Miyashita, et. al, *Anal. Sci.*, **29**, 247–253（2013）.
3）T. Zhou, E. Kakoulides, Y. Zhu, et. al, *Metrologia*, **51**, 08103（2014）.
4）Y. Zhu, K. Chiba, *J. Anal. At. Spectrom.*, **27**, 1000–1006（2012）.

H1013A*
[ASAS講演]

Determination method for measurement of methylmercury in soils and sediments

(Kagoshima University) ○Hitosi KODAMATANI・Takashi TOMIYASU

Although mercury (Hg) is known as a toxic metal, the toxicity is highly dependent on its chemical form. Methylmercury (MeHg) is more toxic than other mercury species in environment. The main MeHg exposure pathway for humans is marine food consumption. The source of marine food contamination by MeHg is generally considered to be bioaccumulation through the food chain, where the primary producers of MeHg are microorganisms in marine sediments. Therefore, accurate determination of MeHg in sediments is critical to understanding the mercury cycle in the environment. In addition, a simpler determination method for mercury speciation is highly desired in developing countries in order to accommodate the Minamata Convention on Mercury.

Recently, we proposed a novel sensitive HPLC-chemiluminescence system for detecting mercury species in water and biological samples.[1, 2] Our method involves the formation of mercury complexes with the emetine-CS_2 ligand, HPLC separation, and $Ru(bpy)_3^{3+}$ chemiluminescent reaction detection. The absolute detection limit was $<$6 pg (as Hg). However, determining MeHg content in soil and sediment is difficult, because of an interference of inorganic Hg; the concentration of inorganic mercury in these samples are over 100–1000 times higher than that of MeHg. Therefore, a simple sample preparation method for selective organo-mercury determination was also established.[3] A leaching solution of 5 M HCl containing 0.1 M $CuSO_4$ and 5 mM $PdCl_2$ was added to the sample. The mixture was shaken to elute the Hg species. The supernatant was decanted for solid-liquid separation, and toluene was added for selective transfer of organo-mercury species to the toluene phase as chlorides. An aliquot of the toluene phase was transferred to a centrifuge tube and EDTA solution was added for back-extraction of organo-mercury into the aqueous phase as EDTA complexes. An aliquot of the aqueous phase was transferred to an auto-sampler vial, and emetine-CS_2 was added for complexation with organo-mercury. An aliquot was injected into the HPLC-CL system. As a result, the calibration curve for MeHg, determined using peak height, was linear up to 250 ng (as Hg). The method detection limit for MeHg in soil/sediment samples was 0.07 ng/g.

For soils and sediments, sample preparation procedures such as drying and grinding are also necessary before analysis. The influence of various drying conditions on the MeHg concentrations in various soils and sediments was investigated. The ratio of the MeHg concentrations in 40℃ oven-dried subsamples obtained by dividing by the concentrations in freeze-dried subsamples ranged from 0% to 336%. Hg^{2+} was added at 15 mg/kg to paddy soil and stored at 30℃ to ensure the generation of MeHg. Four days after spiking with Hg^{2+}, MeHg concentrations were 36.0$\pm$5.0 μg/kg (n=3). The MeHg concentration four days after Hg^{2+} spiking and sterilization (121℃, 30 min) was 1.8 μg/kg. These results indicated that bacterial Hg-methylation may occur within a few days. Thus, the best way to preserve MeHg in soil and sediment samples was to freeze the samples immediately after collection, followed by freeze-drying, grinding, homogenization, and freezer storage until measurement.

It is generally believed that sediment is the major source of MeHg. However, it was confirmed that forested soils also had high MeHg concentrations and ratios (MeHg/total-Hg) in the process of above experiments. The highest MeHg concentration observed in forested soil was 0.4 mg/kg (dry-base). We also confirmed that the soil concentration of MeHg was affected by the total Hg concentration, organic carbon content, and the environment from which they were collected.

Acknowledgment
This work was supported by Grants-in-Aid for Scientific Research (No.22404002) from the Japan Society for the Promotion of Science, the Environment Research and Technology Development Fund (5RF-1303) of the Ministry of the Environment, Japan and the Steel Foundation for Environmental Protection Technology (2013, 2014).

References

1) H. Kodamatani, R. Kanzaki, T. Tomiyasu, K. Saito, Y. Kono, *Anal. Lett.*, **44**, 2769–2779 (2011).
2) H. Kodamatani, A. Matsuyama, K. Saito, Y. Kono, R. Kanzaki, T. Tomiyasu, *Anal. Sci.*, **28**, 959–965 (2012)
3) H. Kodamatani, T. Tomiyasu, *J. Chromatogr. A*, **1288**, 155–159 (2013).

H1014A
[ASAS講演]

Development of dendrimer modified magnetic chitosan for magnetic separation of Cu(II) ion and humic acid in water

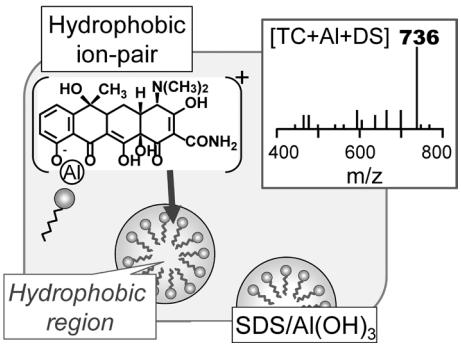
(Grad. Sch. Env. Sci. Hokkaido. Univ) ○Satya Candra Wibawa Sakti・Yasuyuki Narita・Shunitz Tanaka

Among the many methods to treat wastewater, adsorption is the most popular method due to its effective removal performance. However, the conventional adsorbents are difficult to be recollected and reused after releasing water pollutants. There is a possibility of secondary pollution with the adsorbent itself if it is not recollected. The aim of this study is to develop modified magnetic chitosan based adsorbents, which can adsorb pollutants in water and be recollected easily using a magnet. In order to increase the number of amine group on the surface of magnetic chitosan (MC), dendrimer moiety was introduced to the MC. The preparation of dendrimerized magnetic chitosan (DMC) was conducted by using ethylenediamine or diethylenetriamine in the presence of methyl acrylate as a bridging agent. The quaternarization of dendrimer modified magnetic chitosan (QDMC) was performed by introducing glycidyltrimethylammonium chloride to the DMC. The dendrimer and quaternarized dendrimer magnetic chitosan were characterized by using a Fourier Transform Infra-Red spectrophotometer (FTIR), an X-ray Diffractometer (XRD) and a Thermo Gravimetric Analyzer (TGA). The morphology and the amount of nitrogen were examined by using a Scanning electron microscope (SEM) and Elemental (CHN) analyzer. The magnetic property was investigated by using the Magnetic Property Measurements System (MPMS). The magnetic separation of Cu (II) by the DMC was very fast and reached the equilibrium within 5 min. The DMC could be reused for magnetic separation of Cu (II) for 10 times. The QDMC was employed for magnetic separation of humic acid in the water. Humic acid in the water could be separated magnetically within 10 min by using a magnet. The QDMC showed high regeneration, which could be reused for magnetic separation of humic acid for 10 times without any significant lost. In addition to its fast kinetics in removal, the easiness on recollection and the feature of high regeneration make the dendrimer modified magnetic chitosan economical and effective magnetic materials for magnetic separation of Cu(II) and also humic acid in water.

H1015A*
[ASAS講演]

Removal of Polar Organic Pollutants from Wastewaters by Surfactant-Assisted Coagulation

(Kitami Institute of Technology) ○Tohru (とおる), Saitoh (さいとう)



The speaker have reported that the addition of small amount of anionic surfactant assisted the coagulation of aluminum hydroxide and significantly increased the removal of hydrophobic organic pollutants from water.[1] This study reports the design of the surfactant-assisted coagulation system for the efficient removal of charged organic pollutants including cationic dyes[2] and antibiotics[3] that had hardly been eliminated from wastewaters by conventional coagulation-sedimentation methods. The coagulation occurred by the hydrolysis of aluminum(III) ions in the presence of an anionic surfactants, sodium dodecyl sulfate (SDS) far below the critical micelle concentration. Three cationic dyes; methylene blue, malachite green, and crystal violet, were nearly completely collected to the precipitate of SDS-impregnated aluminum hydroxide and therefore efficiently removed from water. A fluorometric study with a molecular probe, *N*-phenyl-1-naphthylamine, indicated the occurrence of hydrophobic region in the precipitate for the distribution of hydrophobic ion-pairs of cationic dyes with dodecyl sulfate ions.

The proposed method was also applied to the removal of tetracycline antibiotics from water. In the absence of SDS, tetracycline (m/z=445 [M+H]) was hardly collected to aluminum hydroxide precipitate because of the formation of positively charged aluminum chelate (m/z=471.4 [M+Al]) that can be excluded from positively charged precipitate. In the presence of SDS, however, it was well collected as the hydrophobic ion-pair (m/z=736 [M+Al+DS] with dodecyl sulfate ions. The use of 5 mg L^{-1} Al(III) ions and 80 mg L^{-1} allowed nearly complete (ca. 99%) removal of tetracycline. The tetracycline collected to the precipitate was readily photodegraded by a UV irradiation. Other tetracycline antibiotics such as oxytetracycline and chlortetracycline as well as fluoroquinolones including norfloxacin, ciprofloxacin, levofloxacin, and enrofloxacin were also removed from water. The effect of matrix components and the applicability to wastewater treatment were investigated.

[References] 1) (a) T. Saitoh, S. Matsushima, M. Hiraide, *Colloid Surf. A: Physicochem. Eng. Asp.* **299**, 88–92 (2007), (b) T. Saitoh, M. Yamaguchi, M. Hiraide, *Water Res.* **45**, 1879–1889 (2011). 2) T. Saitoh, M. Saitoh, C. Hattori, M. Hiraide, *J. Environ. Chem. Eng.* **2**, 752–758 (2014). 3) T. Saitoh, K. Shibata, M. Hiraide, *J. Environ. Chem. Eng.* **2**, 1852–1858 (2014).

H1016A*
[ASAS講演]

Triple-Quadrupole Inductively Coupled Plasma-Mass Spectrometry Combined with Selective Cs Adsorption and Ion Exchange Chromatographic Separation for 135Cs and 137Cs analysis

(NIRS, Japan[1] · Agilent, Japan[2]) ○Jian (じぇん) Zheng (つぇん)[1] · Wenting (おんてぃ) Bu (ぶ)[1] · Keiko (けいこ) Tagami (たがみ)[1] · Yasuyuki (やすゆき) Shikamori (しかもり)[2] · Kazumi (かずみ) Nakano (なかの)[2] · Shigeo (しげお) Uchida (うちだ)[1] · Nobuyoshi (のぶよし) Ishii (いしい)[1]

Since the Fukushima Daiichi nuclear power plant (FDNPP) accident in 2011, intensive studies on the distribution of released fission products, in particular 134Cs and 137Cs, in the environment have been conducted, and the activity ratio of 134Cs/137Cs has been widely used as a tracer for contamination source identification. However, due to the short half-life of 134Cs (2.06 y), this tracer will become unavailable in the near future.

In this work, we presents an analytical method for the determination of the long-lived 135Cs (half-life, 2 million years) and the atom ratio of 135Cs/137Cs, as a promising geochemical tracer in environmental samples. The analytical method involves ammonium molybdophosphate (AMP) selective adsorption of Cs and subsequent two-stage ion-exchange chromatographic separation followed by detection of isolated radiocesium isotopes by a triple quadrupole inductively coupled plasma mass spectrometry (ICP-MS/MS). The AMP selective adsorption of Cs and chromatographic separation system showed high decontamination factors (10e4–10e5) for interfering elements, such as Ba, Mo, Sb and Sn. Using ICP-MS/MS, only selected ions enter the collision/reaction cell to react with N2O, reducing the isobaric interferences (135Ba+ and 137Ba+) and polyatomic interferences (95Mo40Ar+, 97Mo40Ar+, 119Sn16O+ and 121Sb16O+) produced by sample matrix ions. The high abundance sensitivity (10e-9 for the 135Cs/133Cs ratio) provided by ICP-MS/MS allowed reliable analysis of 135Cs and 137Cs isotopes with the lowest detection limits ever reported by mass counting methods (0.01 pg/mL and 0.006 pg/mL, respectively).[1]

The developed analytical method was successfully applied to the analysis of heavily contaminated environmental samples (litter, lichen and soil) collected from Fukushima forests for the long-lived 135Cs (half-life, 2x106 y) and the 135Cs/137Cs isotopic ratio. For the first time, we obtained the isotopic composition of the 135Cs/137Cs isotopic ratio of the FDNPP-released radiocesium. We demonstrated that radiocesium was mainly released from the Unit 2 reactor. 135Cs/137Cs can be considered as a new tracer for source identification and long-term estimation of the mobility of released radiocesium in the environment.[2]

References
1) J. Zheng, W. Bu, K. Tagami, Y. Shikamori, K. Nakano, S. Uchida, N. Ishii, Anal. Chem. 86, 7103–7110 (2014).
2) J. Zheng, K. Tagami, W. Bu, S. Uchida, Y. Watanabe, Y. Kubota, S. Fuma, S. Ihara, Environ. Sci. Technol. 48, 5433–5438 (2014).

H1017A
[ASAS講演]

Measurement of translational and rotational energies for photodesorbed CO from CO-H_2O ice

(Kyushu Univ.[1]・Kyoto Univ.[2]) ○Shohei Matsuda[1]・Yusuke Kurotani[1]・Motoki Yamazaki[2]・Akihiro Yabushita[1]

[Introduction] Gaseous CO in interstellar space is used as tracers of density and temperature. While molecules including CO are predicted to be frozen as ice in high density region of interstellar clouds, measurable amount of gaseous CO is detected in cold region where thermal effect is negligible. Therefore, photodesorption of CO ice is suggested as a possible process which maintains gaseous CO in cold interstellar clouds. Thus far, photodesorption yields, wavelength-dependent photodesorption between 90 and 170 nm, and photodesorption mechanisms of CO from pure CO or CO-H_2O ice has been studied experimentally and theoretically. In this study, the translational and rotational energies for photodesorbed CO from CO-H_2O ice have been measured to investigate photodesorption dynamics, and in terms of which elucidation of photodesorption process has been attempted.

[Methods] The experiments were carried out in a ultrahigh vacuum chamber equipped with a 157 nm excimer laser and a dye laser. Amorphous solid water (ASW) was prepared on Pt substrate at 8 K, and then CO was deposited on ASW. The 157 nm laser was used for photodesorption of CO from CO-H_2O ice. Photodesorbed CO was ionized by (2+1) resonance enhanced multiphoton ionization. The time-of-flight (TOF) spectrum of CO (v=0) was measured and reproduced by Maxwell-Boltzman (M-B) distributions to determine translational temperature T_{trans}. In addition, the rotational spectrum of CO (v=0) was measured and fitted by spectral simulation.

[Results and discussion] The reproduced TOF spectrum of CO from a thick CO on H_2O ice was composed of three different M-B distributions. These components were described by a fast component with T_{trans}=2700±200 K, a middle component with T_{trans}=530±50 K, and a slow component with T_{trans}=100±20 K. This result indicates that there are three possible processes of photodesorption. The TOF spectrum of CO from a thin CO on H_2O ice was also composed of three different M-B distributions, but translational temperature for fast and middle components were T_{trans}=1800±200 K and 450±50 K, respectively. In consideration of photodesorption, the CO-CO interaction is dominant in a thick CO on H_2O ice, and the CO-H_2O in a thin CO. This result implies that the hydrogen bridged CO-H_2O interactions are stronger than pure van der Waals interactions in solid CO.

P1101A
[ASAS講演]

Spectroscopic study of gold nanoparticles- protein mixture solutions

(Fukuoka Institute of Technology) ○XIN QIAO・NOBUYOSHI MIYAMOTO・XINGZHENG WU

Recently, gold nanoparticles are applied in diagnosing heart disease, cancer, and detection of the infectious pathogen biomarker. There are many studies on interaction of the surface modified gold nanoparticles-protein, but not so may reports on that of non-modified gold nanoparticles-protein. Here, we investigated spectroscopic property of gold nanoparticles-protein to try to understand the interaction of gold nanoparticles-protein.

Firstly, nanosized gold particles were prepared by reducing tetrachloro gold (Ⅲ) acids ($HAuCl_4$) with either sodium citrate or $NaBH_4$ in the existence of surfactant DDAB. Then, the prepared gold nanoparticles solution, myoglobin or albumin solution (0.001 g/ml), and the phosphate buffers of certain pH were mixed. Thirdly, the absorption spectra and fluorescence spectra of these mixtures were measured.

The maximum absorbance wavelengths of gold nanoparticles solution prepared by adding sodium citrate was dependent on pH. When pH was between 4.9–6.0, the maximum absorbance wavelength was about 530 nm. However, the absorbance spectrum became much broad when pH was smaller than 3.5 or larger 8.0 with maximum absorbance wavelength of about 600 nm. After addition of myoglobin into the gold nanoparticles solution, both the maximum absorbance wavelength and shape of the absorbance spectrum of the gold nanoparticle changed. On the other hand, the maximum absorbance wavelength of the gold nanoparticles/Albumin mixture was kept about 534 nm even with different pH. These results imply that the interaction of the gold particles with Albumin was different from that with myoglobin. On the other hand, background of absorbance spectrum of proteins-gold nanoparticles prepared by $NaBH_4$/DDAB was high because the mixture was a suspension above pH 3.0. Fluorescence spectra of proteins-gold nanoparticles prepared by adding sodium citrate were also investigated.

P1102A
[ASAS講演]

Combination of fluorescence and beam deflection method for monitoring materials movements across a plant surface

(Fukuoka Inst. of Technology[1]・National Inst. for Environmental Studies[2])
○Xiaoyan Wu[1]・Hironari Kubo[1]・Tomomi Inoue[2]・Xing-Zheng Wu[1]

Introduction In order to study material movements in plant activities, we have developed a beam deflection method for monitoring material movements across a plant surface. However, the deflection method could not tell us what kinds of materials moved across the plant surface. On the other hand, dissolved oxygen (DO), which is known to be detectable by fluorescence quenching method, plays an important role in various plant activities. Here we combined the beam deflection method and fluorescence quenching method together for building a novel optical detection system, which allows real-time continues monitoring of beam deflection and DO.

Experimental A piece of f model aqueous plant, *Ceratophyllum demersum L* (about 2 cm long) was put into a culture dish filled with a solution of 10^{-6} M Tris (2,2′-bipyridyl) ruthenium (II) chloride. A semiconductor laser (wavelength: 405 nm) provided a probe beam, which passed through a vicinity of the plant in the culture dish, and then was focused to a deflection detector. Fluorescence excited by the probe beam was detected by a PMT. A commercial DO sensor was placed into the dish to monitor DO concentration. A digital multimeter was used for recording signals from the PMT and the defection detector. Signals from DO sensor was recorded too.

Results and discussion Firstly effects of temperature and light illumination on fluorescence intensity and their corrections were investigated in detail. Secondly the Stern-Volmer constant K of the dissolved oxygen to Tris (2,2′-bipyridyl) ruthenium (II) chloride was determined to be 0.0529. Thirdly, the results of monitoring the aquatic plant in respiration process showed that both the deflection signal and DO concentration at vicinity of leaf changed more than at 1 cm away from the plant, suggesting more materials movement occurred at the vicinity. Moreover the deflection signal indicated that reverse materials movements occurred in photosynthesis process and respiration process.

P1103A
[ASAS講演]

Development of palm-sized ELISA system for the rapid and on-site diagnosis of infection disease

(Tokyo Metropolitan Univ.[1] • JSPS Research Fellow[2] • NIID[3] • Mebius Advanced Technology[4] •
Hue Univ. of Medicine and Pharmacy[5] • Univ. of Malaya[6])
○Kazuhiro Morioka[1,2] • Harpal Singh[1,3] • Hizuru Nakajima[1] • Akihide Hemmi[4] • Masami Sugamata[1,5] •
Le Van An[5] • Sazaly AbuBakar[6] • Hulie Zeng[1] • Shungo Kato[1] • Ming Yang[1] • Katsumi Uchiyama[1]

Introduction

In recent years, a pandemic outbreak of infection disease menaces our life with economic globalization and global warming. To prevent the pandemic outbreak, a prevention of infection disease by appropriate vaccination based on viral antibody test is very important. Enzyme-linked immunosorbent assay (ELISA) is commonly used for measuring antibody level. Conventional ELISA method, however, requires a complicated operation and a long analysis time. Moreover, it needs a large and expensive microplate reader as detector. Therefore, it is difficult to measure antibody level rapidly in the field.

In this study, a portable fluorescence microplate reader using LEDs and photodiodes was developed to overcome these drawbacks of ELISA. The developed microplate reader was successfully applied to the antibody test for measles.

Experimental

The developed portable microplate reader consists of nine LEDs, a short pass filter, a long pass filter, two light blocking plates with nine holes, nine photodiodes, an I/V conversion amplifier, an A/D converter, a CPU, a Bluetooth communication module and a lithium polymer battery. A 9-well microtiter plate, which has a light-blocking wall and a transparent bottom, was made of PDMS and carbon black.

The light beam from LED passed the short pass filter and then irradiated the sample in the microwell. The fluorescence from the sample passed the long pass filter and two light blocking plates and then detected by the photodiode. The fluorescence intensity was converted into photocurrent by the photodiode and then the photocurrent was converted into the voltage by the I/V conversion amplifier. The analog voltage signal was converted into the digital signal by the A/D converter. The digitized signal was transferred to an external PC by Bluetooth and then recorded.

Results and Discussion

An antibody test for measles by ELISA was performed using the developed 9-well microtiter plate and the portable microplate reader. The results obtained by the developed ELISA system were completely consistent with that by the conventional ELISA system. Compared to the conventional ELISA system, the present ELISA system shortened the analysis time to 1/2. The amounts of the reagent and sample were also reduced to 1/3. The ELISA system developed in this study would be useful for point of care testing since the system is palm-sized and inexpensive.

P1104A
[ASAS講演]

Ionic polymer-grafted porous silica particles for HPLC stationary phase

(Kumamoto Univ.[1] • Kumamoto Inst. of Photo-Electro Organics (PHOENICS) [2] • Daffodil International Univ.[3])
○Mohammad Shahruzzaman[1,3] • Yutaka Kuwahara[1] • Makoto Takafuji[1] • Hirotaka Ihara[1,2]

Ionic polymers are either organic or inorganic that contain both covalent and ionic bonds in its chain or network structure which differentiated them from other polymeric materials. In this paper, we report the synthesis of poly (alkylpyridinium) -grafted porous silica microspheres and evaluate their retention behavior as stationary phases in HPLC. We chose poly (*N*-alkyl pyridinium salt) as an ionic polymer, because alkyl chains were changeable through quaternization reactions with various alkyl halides.

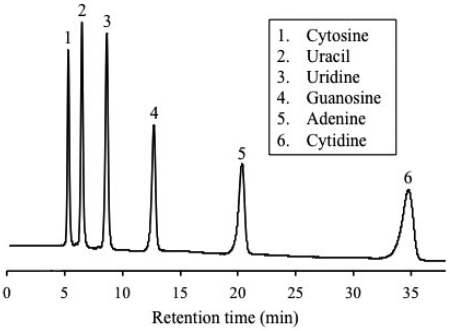


Fig. 1. Chromatogram for a mixture of 6 analytes containing nucleosides and nucleobases by using Sil-VPC1_{27} as a stationary phase. Mobile phase: 100 mM ammonium formate at pH 5.0 containing 90% ACN. Column temp.: room temperature (28 °C). Flow rate: 0.5 ml min^{-1}.

Poly (4-vinylpyridine) was synthesized by radical polymerization of 4-vinylpyridine using 3-mercaptopropyltrimethoxysilane as a telogen and the resulting polymer was grafted onto porous silica particles. Then the pyridyl side chains were quaternized with iodomethane (Sil-VPC1_{27}) and 1-bromooctadecane (Sil-VPC18_{27}). The prepared stationary phases were characterized by elemental analysis, DRIFT spectroscopy, SEM, TGA and BET analysis. The grafting amount (surface coverage) of Sil-VPC1_{27} and Sil-VPC18_{27} were found 40.17% (8.11 mol m^{-2}) and 38.82% (4.35 mol m^{-2}), respectively.

The Sil-VPC1_{27} stationary phase can separate nucleosides and nucleases indicating the typical characteristics of HILIC separation (Fig. 1). On the other hand, Sil-VPC18_{27} stationary phase can separate positional isomers, which means it can recognize the hydrophobicity of the sample. Development of these stationary phases opens the window for various applications as the side chains are changeable through quaternization reactions.

[1] W. Bicker, J. Wu, M. Lämmerhofer, W. Lindner, J. Sep. Sci. 31 (2008) 2971.

P1105A
[ASAS講演]

Development of hybrid certified reference material of ^{1}H and ^{19}F for quantitative NMR

(National Metrology Institute of Japan, AIST) ○Taichi Yamazaki・Sachiko Taniguchi・Nobuyasu Hanari・Toshiyuki Asakai・Ryoko Iwasawa・Masahiko Numata

【Introduction】NMR spectroscopy has been widely used for qualitative analysis in a molecule. Recently, some quantitative analysis using ^{1}H NMR has been reported. Quantitative NMR (qNMR) technique uses a different structural compound as a reference material. This technique was focused as the new approach for rapid purity determination, and came to practical use actually. The qNMR technique needs a reference material which has a high accurate value because the measurement value is calibrated by comparison with reference material. Some reference materials for qNMR were supplied, but certified reference material (CRM) as primary reference material is not supplied at all. In this research, we developed a CRM for ^{1}H qNMR and ^{19}F qNMR as a high accurate primary reference material for metrological traceability in qNMR.

【Purity evaluation of certified value】The certified value is the weighted mean of purities determined by mass balance approach (MBA), freezing point depression method (FPD) and coulometric acidimetric titration method (CT). The uncertainty of certified value was contained uncertainty of these methods, homogeneity, stability and between method. In the MBA, impurities were analyzed using HPLC-UV, GC-FID, a Karl-Fischer titrator, and a thermogravimeter. The purity of MBA was determined by subtraction of these impurity values from 1.000 kg/kg. For the FPD in a continuous scan mode, a differential scanning calorimeter was used. For the CT, a coulometric titrator was used.

【Result】The certified value of this CRM was determined to (0.9996±0.0006) kg/kg (k=2). The small expanded uncertainty of certified value was determined by high accurate primary methods. This uncertainty was small sufficiently to accuracy of the reported qNMR method. The impurity is not influenced on a NMR spectrum because this CRM is high pure material. Then, in purity measurement of reference materials by ^{1}H NMR and ^{19}F NMR with this CRM, the results were corresponded with the reference value in the range of uncertainty. From these results, this CRM can be used as the reference material of accurate qNMR with ^{1}H and ^{19}F.

P1106A
[ASAS講演]

Potential-dependent adsorption of water-soluble porphyrins at liquid|liquid interfaces studied by polarization-modulation total internal reflection fluorescence spectroscopy

(Kanazawa University) ○Sho Yamamoto・Hirohisa Nagatani・Kotaro Morita・Hisanori Imura

An interface between two immiscible electrolyte solutions (ITIES) is a two-dimensional specific reaction field, where charge transfer processes are significantly affected by the applied potential across the interface. It has been shown that several water-soluble porphyrins can be adsorbed strongly at the ITIES. The molecular orientation of porphyrins adsorbed at ITIES plays a crucial role in a variety of heterogeneous reaction systems.

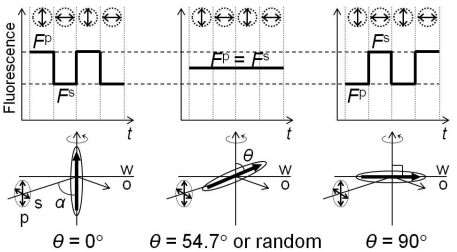


Fig. 1. Schematic representation of the fluorescence signal from an adsorbed molecule at an interface. F^p and F^s relate to the fluorescence intensities observed by p- and s-polarization, respectively.

Recently, we developed a novel *in situ* spectroscopic method, polarization-modulation total internal reflection fluorescence (PM-TIRF) spectroscopy, for ionic dye species adsorbed with a certain orientation at ITIES. In PM-TIRF experiments, the fluorescence signal from the interfacial region is analyzed as a function of periodic modulation of linear-polarizations (p and s polarizations) of excitation beams (Fig. 1), where the PM-TIRF signal is defined as $\Delta F^{p-s} = F^p - F^s$. PM-TIRF spectroscopy can effectively extract the fluorescence signals of interfacially oriented species from the signals arising throughout the optical path in the incident medium. In this study, PM-TIRF spectroscopy was applied to study the adsorption behavior of water-soluble porphyrins at the polarized water|1,2-dichloroethane interface. The PM-TIRF results clearly demonstrated that the *meso*-substituted free base porphyrins were adsorbed as relatively lying orientation with partial dehydration at the interface. Furthermore, an anionic porphyrin, protoporphyrin IX exhibited that its adsorption state was affected by the potential and J-aggregation at ITIES.

P1107A
[ASAS講演]

Gel-filtration separation of protein-coated gold nanoparticles

（Akita University）○Shuushi Tamura・Kazuhiko Fujiwara・Hirotoshi Matsumura・Masafumi Odaka・Nobuaki Ogawa

Development of general and simple methodology for functionalization of gold nanoparticles（AuNPs）surface is very important for applications of AuNPs. In AuNPs functionalization, chemical modification of AuNPs with thiols or proteins can be readily achieved, while removal of excess unreacted modifier from functionalized AuNPs is still need to make an improvement. Recently, we have investigated the more versatile and facile separation conditions for AuNPs preparation than the conventional method by centrifuge. In this study, we present the purification of BSA-coated AuNPs（BSA-AuNPs）from unreacted BSA by gel filtration chromatography（GFC）.

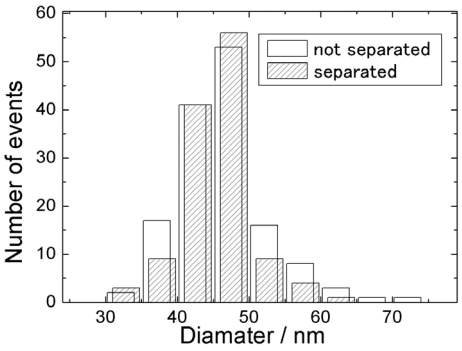


Figure 1. not separated and separated BSA-AuNPs size distribution

AuNPs were prepared by reduction of gold chloride with citric acid. The particle size was determined from TEM images. Bovine serum albumin（BSA）and AuNPs（46.3±6.2 nm particle size）were mixed for 30 min. To separate BSA-AuNPs and unreacted BSA, GFC with TOYOPEARL HW-65F（I.D. 1.6 cm×13 cm）was performed. The equilibration and elution buffer was 10 mM phosphate buffer at pH 6.8. The flow rate was 1.0 mL/min and the elution was monitored with absorbance at 200 nm.

BSA-AuNPs is completely separated from unreacted BSA by GFC. The particle size of the separated BSA-AuNPs is estimated as 45.4±5.0 nm from the TEM image（Fig. 1）, indicating that the size distribution of AuNPs can be also improved by the gel filtration column. We will also discuss the separation conditions of the AuNPs modified with different proteins that have different molecular weights or electrostatic surface potentials.

P1108A
[ASAS講演]

Effect of a Chemical Functionalization of Gold Nanoparticles on their Cytotoxicity

（Akita University）○Yuki Nagano・Kazuhiko Fujiwara・Hirotoshi Matsumura・Masafumi Odaka・Nobuaki Ogawa

Gold nanoparticles（AuNPs）have recently received great attention in the field of biomedical applications due to their chemical stability and unique optical properties derived from their localized surface plasmon resonance in combination with surface modification by self-assembled monolayers using thiol-molecules. Previously, we reported that antibody-modified AuNPs release the antibodies from their surface when they enter the cells. The observation implies a potential use of AuNPs in drug delivery systems. Although chemical functionalization of AuNPs surface is required for biomedical uses, the effect of the functionalization on cytotoxicity is still unclear. Here, we report that the cytotoxicity of AuNPs modified by a pentapeptide, CALNN, or mercaptoundecanoic acid（MUA）to HeLa cells.

Modification of AuNPs（46.8±8.1 nm）by CALNN or MUA were performed according to modified literature procedures. HeLa cells were incubated with CALNN and MUA modified AuNPs（CALNN-AuNPs, MUA-AuNPs）, respectively, for 24 hours. To evaluate cytotoxicities of the functionalized AuNPs to HeLa cells, viability assays were carried out by using CellTiter-Glo Luminescent Cell Viability Assay（Promega）with a luminescent scanner. Au ions were recovered from HeLa cells after the viability assays, and then their concentration were quantified by inductively-coupled plasma optical emission spectrometry in order to estimate the number of intracellular AuNPs.

In both CALNN-AuNPs and MUA-AuNPs, increasing the number of intracellular AuNPs has a substantial toxic impact to the HeLa cells. The profiles of the viability of HeLa cells to both CALNN-AuNPs and MUA-AuNPs show a characteristic dose-response curve in drugs and medicines. Comparison of the cell viability shows no significant change between MUA-AuNPs and non-coated AuNP. On the other hand, modification of AuNPs with CALNN gives impact on the cell viability at higher concentrations. The results suggest that functionalization of AuNPs surface affects the cytotoxicity to HeLa cells.

P1109A
[ASAS講演]

DNA quantification via nucleobase measurement by high performance liquid chromatography tandem mass spectrometry

(National Metrology Institute of Japan, AIST) ○Sachie Shibayama・Shin-Ichiro Fujii・Taichi Yamazaki・Akiko Takatsu

Introduction. Today, DNA quantification is very important for food analysis and clinical test. The method in which DNA is digested by enzyme and the components of DNA, such as deoxynucleotide or deoxynucleoside, are measured by liquid chromatography mass spectrometry (LC/MS) can quantify DNA with high accuracy. However, the enzymatic digestion method has the limitation of DNA size. In this study, we developed a DNA quantification method in which produced nucleobases by formic acid hydrolysis of DNA were quantified by LC/MS/MS.

Experimental. As a sample, we used λDNA solution (48,502 bp of double strand DNA, NIPPON GENE) and diluted with water into about 10 ng/μL. Deoxynucleotides (dNMPs) solutions were used as a standard for quantification and the ^{13}C and ^{15}N labeled deoxynucleotides (LdNMPs) solutions were used as internal standard. LC-10A series (Shimadzu) HPLC system with Kinetex XB-C18 column (4.6 mm i.d.×250 mm, 5 μm, Phenomenex) and LCMS-8030 (Shimadzu) was used. λDNA solution or dNMPs standard solution was mixed with LdNMPs solutions. Fifty μL of sample blend or standard blend and 200 μL of 88% formic acid were placed in crimp-top vial, then the vial was sealed with aluminium seal and heated at 150°C. After evaporating the solvent, 250 μL of water was added and the solution was subjected to analysis. The mass fraction of λDNA was calculated from the obtained mass fraction of nucleobases and molecular weight of λDNA.

Results and Discussions. When 0.1% acetic acid with 8% methanol was used as a mobile phase, four nucleobases were separated and measured with high sensitivity. Because more than 8 h at 150° C, the mass fraction of λDNA calculated from thymine was reached maximum, 8 h was chosen as optimum hydrolysis condition. By hydrolyzing λDNA under this condition, all of the mass fractions of λDNA calculated from four nucleobases were consistent within expanded uncertainty, and final mass fraction of λDNA was calculated as 12.7 μg/g ± 0.2 μg/g. Therefore, by using this quantification method, λDNA of small amount was quantified with an expanded uncertainty of 1.8% . This method could be concluded as an accurate quantification method for long DNA.

P1110A
[ASAS講演]

Resonance Raman spectroscopic studies for elucidating N_2O formation in nitric oxide reductase mechanism

(Akita Univ.[1]・Inst. of Environ. Health, OHSU[2])
○Hirotoshi Matsumura[1,2]・Pierre Moenne-Loccoz[2]・Nobuaki Ogawa[1]

The reduction of nitric oxide (NO) to nitrous oxide (N_2O) by denitrifying ground bacteria is an essential step of the global nitrogen cycle and of great significance to global warming since agricultural production leads to atmospheric emission of the powerful greenhouse gas N_2O. The biological reduction of NO is also of direct significance to human health since it allows pathogenic bacteria to fend off NO produced by the mammalian immune response. In the bacterial NO reduction system, nitric oxide reductases (NOR), which have heme/nonheme chromophore as their active site is a key protein to catalyze the 2-electron reduction of NO. Here, we present Resonance Raman (RR) analysis of NOR models containing heme/nonheme (heme/Fe_B) chromophore to elucidate the mechanism of N_2O emission. RR spectroscopy is a powerful technique to characterize molecular structures associated with catalytic chromophore.

Mechanistic studies continue to be limited by multiple experimental hurdles such as instability of NORs and the complexity from multiple configurations of oxidized NORs. Thus, simpler and better-behaved NOR models would be helpful to test the mechanistic routes. We have been offered engineering myoglobin model (Fe_BMb) that mimics the diiron site of NORs to investigate the mechanism of NO reduction in the unique active sites. Using time-resolved absorption and RFQ–EPR and –resonance Raman spectroscopy, we demonstrated the binding sequence of two NO molecules at the diiron center by determining the molecular structures of milli-second intermediates during NO reduction. Our findings shed light on N_2O generation by NOR reaction.