2.3.9 Laboratory: Bio-analytical and Physical Chemistry

Professor Kenji Kano Member: **Associate Professor** Osamu Shirai **Assistant Professor** Seiya Tsujimura Doctor's program 2 9 Master's Program 4 Undergraduate Post-Doctoral fellow 1 2 Researcher

A. Research Activities (2009.4-2010.3)

A-1. Main Subjects

a) Oxidation-reduction reactions relevant to biological phenomena

Structure and function of fructose dehydrogenase from acetic acid bacterirum (molecular cloning, structural analysis of active site, thermochemical and dynamic properties, electrode reaction, etc.). Single mutation of multicopper oxidaze and its function analysis. Interaction between the enzymes and various electrode materials.

- b) Fundamental study of bioenergy conversion system and its application to biofuel cell Multi-copper oxidases as very efficient catalysts for electrocatalytic reduction of dioxygen to water based on mediated and direct electron transfer mechanisms. Bioelectrocatalytic oxidation of saccharide using dehydrogenase. Bioelectrocatalytic oxidation of saccharide using saccharide dehydrogenase (mediated and direct electron transfer-type bioelectrocatalysis). Electron transfer at an enzyme-adsorbed and modified carbon and gold electrodes. Development of biofuel cell using enzymes and microbes.
- c) Construction of electrochemical biosensing systems

Development of a method of complete electrolysis micro-coulometry for multi-purpose use. Development of blood glucose sensor.

d) Redox chemistry of antioxidants

Autooxidation of catechins concerning ferrous/ferric compounds. Redox chemistry of poly-phenols.

- e) Fundamental study on charge (ion and electron) transfers across biomembranes

 Electrochemical analysis on ion transport across planar lipid bilayers in the presence of
 hydrophobic ions and ionophores such as ion channels, carrier compounds, etc. Ion transport
 across liposomal membranes. Electron transfer at the surface of supported BLM. Function
 of ion channels using planar bilayer lipid membranes (Effect of coexisting ions, Reaction
 mechanism of accelerator and inhibitor).
- f) Fundamental study of transmission processes on biological electric signals Mechanism on nervous transmission by use of liquid membrane cells. Electrochemical study on anesthetic functions.

A-2. Publications and presentations

a) Publications

Books

- Tsujimura, S. and K. Kano: Biofuel cells, Application technologies and new trends in industrial enzymes. pp.246-256, CMC Publishing CO.,LTD., Tokyo, 2009 (in Japanese)
- Tsujimura, S. and K. Kano: Biofuel cell. Electrochemical measurements. pp.391-400, Joho-Kikou, Tokyo, 2009 (in Japanese)
- Ohsaka, T., M. Atobe, S. Ishiwata, K. Kano, S. Kuwabata, T. Tachima, T. Momma: Electrochemical methods. Igakuhyoronsya Inc., Tokyo, 2009 (in Japanese)
- Fujiwara, M., T. Okada, K. Kano, H. Hisamoto, T. Toyoda: Analytical Chemistry. Igakuhyoronsya Inc., Tokyo, 2009 (in Japanese)
- Tsujimura, S. and K. Kano: Electrochemical characterization of enzymes, Enzyme-Conpediums on Application and Technology-. pp.106-111, STS Publishing CO.,LTD., Tokyo, 2009 (in Japanese)

Original Papers

- Tsutsumi, M., S. Tsujimura, O. Shirai and K. Kano: Direct electrochemistry of histamine dehydrogenase from Nocardioides simplex. J Electroanal Chem 625; 144-148, 2009
- Sakai, H., T. Nakagawa, A. Sato, T. Tomita, Y. Tokita, T. Hatazawa, T. Ikeda, S. Tsujimura and K. Kano: A High-power Glucose/oxygen Biofuel Cell Operating under Quiescent Conditions. Energy Environ Sci 2; 133-138, 2009
- Miura, Y., S. Tsujimura, K. Kurose, Y. Kamitaka, K. Kataoka, T. Sakurai and K. Kano: Direct Electrochemistry of CueO and Its Mutants at Residues to and near Type I Cu for Oxygen-Reducing Biocathode. Fuel Cells 9; 70-78, 2009

- Kubota, S., S. Ozaki, J. Onishi, K. Kano and O. Shirai: Selectivity on Ion Transport across Bilayer Lipid Membranes in the Presence of Gramicidin A. Anal Sci 25; 189-193, 2009
- Fujieda, N., M. Mori, T. Ikeda and K. Kano: Silent Form of Quinohemoprotein Amine Dehydrogenase from Paracoccus denitrificans. Biosci Biotechnol Biochem 73; 524-529, 2009
- Kurose, S., K. Kataoka, N. Shinohara, Y. Miura, M. Tsutsumi, S. Tsujimura, K. Kano and T. Sakurai.: Modification of Spectroscopic Properties and Catalytic Activity of Escherichia coli CueO by Mutations of Methionine 510, the Axial Ligand to the Type I Cu. Bull Chem Soc Jpn 82; 504-508, 2009
- Nagai, T., A. Uehara, T. Fujii, O. Shirai, M. Myochin, H. Yamana: Redox Equilibria of Pu4+/Pu3+ and PuO22+/Pu4+ Couples in Molten NaCl-CsCl Eutectic as Measured by Absorption Spectrophotometry. Radiochim. Acta, 97, 209-212 (2009).
- Uehara, U., O. Shirai, T. Nagai, T. Fujii, H. Yamana: Electrochemical Redox Reactions of Chromium and Iron Ions in Molten NaCl-2CsCl Eutectic for Pyro-reprocessing of Nuclear Fuels. J. Appl. Electrochem., 39, 827-835 (2009).
- Kontani. R., S. Tsujimura, K. Kano: Air Diffusion Biocathode with CueO as Electrocatalyst Adsorbed on Carbon Particle Modified Electrodes. Bioelectrochemistry,76 (1/2), 10-13 (2009).
- Freguia, S., M. Masuda, S. Tsujimura, K. Kano: Lactococcus lactis Catalyses Electricity Generation at Microbial Fuel Cell Anodes via Excretion of a Soluble Quinone. Bioelectrochemistry, 76 (1/2), 14-18 (2009).
- Shirai, O., S. Ozaki, J. Onishi, N. Kozai, T. Ohnuki and K. Kano: Ion Transport Across a Bilayer Lipid Membrane in the Presence of Hydrophobic Ions. Chem. Lett., 38 (11), 1038-1039 (2009).
- Tsujimura, S., Nishina, A., Kamitaka, Y., and Kano, K.: Coulometric D-fructose biosensor based on direct electron transfer using D-fructose dehydrogenase. Anal. Chem., 81, 9383-9387 (2009).
- Freguia, S., S. Tsujimura, K. Kano: Electron Transfer Pathways in Microbial Oxygen Biocathodes. Electrochimica Acta, 55 (3), 813-818 (2010).
- Masuda, M., S. Freguia, Y.-F. Wang, S. Tsujimura, K. Kano: Flavins Contained in Yeast Extract are Exploited for Anodic Electron Transfer by Lactococcus lactis. Bioelectrochemistry, 78 (2), 173-175 (2010).
- Tsutsumi, M., N. Tsuse, N. Fujieda, K. Kano: Site-directed Mutation at Residues near the Catalytic Site of Histamine Dehydrogenase from Nocardioides simplex and Its Effects on Substrate Inhibition. J. Biochem., 147(2), 257-264 (2010)

- Onishi, J., O. Shirai, K. Kano: Electrochemical Elucidation of the Facilitated Ion Transport across a Bilayer Lipid Membrane in the Presence of Neutral Carrier Compounds. Electroanalysis, 22 (11), 1229-1238 (2010).
- Tsujimura, S., A. Nishina, Y. Hamano, K. Kano, S. Shiraishi: Electrochemical Reaction of Fructose Dehydrogenase on Carbon Cryogel Electrodes with Controlled Pore Sizes. Electrochem. Commun.,12 (3), 446-449 (2010).
- Tsutsumi, M., S. Tsujimura, O. Shirai, K. Kano: Stopped-flow Kinetic Studies on Reductive Half-reaction of Histamine Dehydrogenase from Nocardioides simplex with Histamine. J. Biochem., in press.
- Noda, T., K. Hamamoto, M. Tsutsumi, S. Tsujimura, O. Shirai, K. Kano: Bioelectrocatalytic Endpoint Assays Based on Steady-state Diffusion Current at Microelectrode Array. Electrochem. Commun., 12 (8), 839-842 (2010).
- Li, S.-L., S. Freguia, S.-M. Liu, S.-S. Cheng, S. Tsujimura, O. Shirai, K. Kano: Effects of Oxygen on Shewanella decolorationis NTOU1 Electron Transfer to Carbon Felt Electrodes. Biosens. Bioelectron., in press.
- Mizutani, K., M. Toyoda, K. Sagara, N. Takahashi, A. Sato, Y. Kamitaka, S. Tsujimura, Y. Nakanishi, T. Sugiura, S. Yamaguchi, K. Kano, B. Mikami: X-ray Crystal Analysis of Bilirubin Oxidase from Myrothecium verrucaria at 2.3 Å Resolution using a Twin Crystal. Acta Crystallogra. F, in press.

Reviews

- Tsujimura, S. and K. Kano: Enzymatic Cathodes for Fuel Cells. Function & Materials 29 (9); 44-57, 2009 (in Japanese)
- Tsujimura, S. and K. Kano: Bioelectrocatalytic reduction of O2 to water using Multi-copper oxidases. The journal of fuel cell technology 9(1); 62-71, 2009 (in Japanese)
- Tsujimura, S.: Research and development of Biofuel cells. KagakuKogyo 61 (2); 9-14, 2010 (in Japanese)
- Kano, K., H. Matsumoto, M. Ishii: Potentiality of Electrochemical Biotechnology Fe-BioJ. OHM; 2010 (5); 8-9, 2010 (in Japanese)
- Tsujimura, S. and O. Shirai: Electrochemical Methods-Bioelectrochemistry. Electrochemistry 77(6); 486-492, 2009 (in Japanese)

b) Conference and seminar papers presented

- The 49th Seminar on Electrochemistry of Kansai Branch of the Electrochemistry

Society of Japan: 1 report

- Workshop on Fuel Cell of the Catalysis Society of Japan: 1 report
- The Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry in 2010: 4 reports
- The 90th Annual Meeting of the Chemical Society of the Japan: 1 report
- The 25th Meeting of the Chemical Battery Materials: 1 report
- The 77th Annual Meeting of the Electrochemical Society of Japan: 5 reports
- The Autumn Meeting of the Electrochemical Society of Japan in 2009: 7 reports
- The 36th Annual Meeting of the Carbon Society of Japan: 1 report
- The 6th Basic Course of Analytical Chemistry: 1 report
- The presymposium on the 70th Annual Meeting of the Japan Society for Analytical Chemistry: 1 report
- The 70th Annual Meeting of the Japan Society for Analytical Chemistry: 5 reports
- The 55th Annual Meeting on Polarography and Electroanalytical Chemistry: 3 reports
- 42nd Heyrovsky Discussion: 1 report
- Dr. George Wilson's 70th Birthday Symposium: 1 report
- The 216th Annual Meeting of Electrochemical Society: 1 report
- The 217th Annual Meeting of Electrochemical Society: 2 reports
- CPC society: 1 report

A-3.Off-campus activities

Membership in academic societies

- Kano, Kenji, D.Agric.Sci: The Electrochemical Society of Japan (a director, the chief of Kansai Branch), The Japan Society for Analytical Chemistry (a council member, an organizer of Kinki Branch), The Japan Society for Bioscience, Biotechnology, and Agrochemistry (a director), The Polarographic Society of Japan (a director)
- Shirai, Osamu, D.Sci: The Electrochemical Society of Japan (the secretary-general of Kansai Branch), The Japan Society for Analytical Chemistry (an organizer of Kinki Branch), The Polarographic Society of Japan (the accountant director, a secretary)
- Tsujimura, Seiya, D.Agric.Sci: The Polarographic Society of Japan (a council)

Research grants

1. Grants-in-aid for Scientific Research(KAKENHI)

- Scientific Research (B): Kano, Kenji: Biocatalysis on energy conversion
- Scientific Research (C) : Shirai, Osamu : Analytical Method on ion transport across biomembranes by use of ion channels
- 2.Other Research Grants
- NEDO: Kano, Kenji: Strategic Development of PEFC Technologies for Practical Application. Development of Technology for Next-generation Fuel Cells. Research and Development of Novel Anode Catalysts Based on Multi-copper Oxidases
- Excellent Young Researcher Overseas Visit Program: Tsujimura, Seiya: Development of porous functional enzyme electrodes modified by redox polymers and their application

A-4.International cooperation and overseas activities

Membership in academic societies

- Kano, Kenji, D.Agric.Sci: The Electrochemical Society (Member), Analytical Biochemistry (Excective Editor), Journal of Electroanalytical Chemistry (Editorial Board)
- Tshujimura, Seiya, D.Agric.Sci: The Electrochemical Society (Member)

<u>International meetings(country,roles)</u>

- Kano, Kenji: The 42nd Heyrovský Discussion (Czech Republic, Invited Lecture), George Wilson's 70th Birthday Symposium U.S.A., (Keynote Lecture), The 216th ECS Meeting (Austria, Contributed)

Visiting Research Scholars

- Post Doctoral fellow 1 (Italy)
- Visiting Research Scholar 1 (Taiwan)

B.Educational Activities(2009.4-2010.3)

B-1.On-campus teaching

a) Courses given

- Undergraduate level: Biophysical Chemistry I (Kano), Biophysical Chemistry II (Kano),

Introduction to Applied Life Science I (Kano and others), Analytical Chemistry (Shirai), Laboratory Course in Analytical Chemistry

(Shirai, Tsujimura), Laboratory Course in Biophysical Chemistry

(Shirai, Tsujimura)

- Graduate level: Bio-Analytical and Physical Chemistry (Kano, Shirai),

Bio-Analytical and Physical Chemistry (advanced course) (Kano,

Shirai, Tsujimura), Experimental Course of Bio-Analytical and Physical Chemistry (Kano, Shirai, Tsujimura).

B-2.Off-campus teaching etc.

Part-time lecturer

- Kano, Kenji: The University of Tokushima, Faculty of Engineering(Special Lecture on Chemical Science and Technology 2), Osaka University, Global COE Program:(Dynamics of Biological Systems)

C.Other Remarks

- Kano, Kenji, D.Agric.Sci: NARO Examining Committee Member, NEDO Research Fellow, JST Screening Committee Member