

**【ENGLISH】**

**Summary of Research Project Results  
(Projects launched in JFY1998)**

**Japan Society for the Promotion of Science (JSPS)**

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# **【 Physical and Engineering Sciences】**

# 1. Innovation in Energy Generation, Conversion, Materials and Systems for the Future

## (1) Research Promotion Committee Members

- Ichiro TANASAWA (Nihon University)
- Yoshiaki OKA (The University of Tokyo)
- Itsuro KIMURA (Institute of Nuclear Safety System, Incorporated)
- Tadashi SAITOH (Tokyo University of Agriculture and Technology)
- Katsuto NAKATSUKA (Tohoku University)
- Satoru YANABU (Tokyo Denki University)

○ : Committee Chairperson

## (2) List of Research Project

No.	Research Project	Project Leader
1	Basic Research on the Development of New Materials Used under Strong Radiation Fields	Yosuke KATSUMURA (The University of Tokyo)

## Basic Research on the Development of New Materials Used under Strong Radiation Fields

### Project Leader:

**Yosuke Katsumura** Professor, Graduate School of Engineering,  
The University of Tokyo



### 1. Objective:

A new concept of supercritical water-cooled reactor has been proposed by a group of the University of Tokyo more than 10 years ago. Since this reactor employs supercritical water as a coolant, much higher energy conversion efficiency more than 44 %, which is 30 % higher than those of conventional light water reactors. The reactor has compact and smaller volume. Much experience of light water reactors and supercritical fossil plants will be applied to the development the reactor. Thus, this reactor is highly ranked in economics. In fact, this concept has been selected as one of the forth generation reactors by the Department of Energy in USA.

However, to realize the supercritical water-cooled reactor, there are many problems to be solved. Therefore, in the present project, fundamental subjects related to the development of supercritical water-cooled reactor has been selected: (1) Quantum chemical modeling of the radiation damage induced by irradiation ion beam, (2) Visualization of the thermal hydraulics behavior in the supercritical fluid and its numerical simulation, (3) Radiolysis of supercritical water and water chemistry, and (4) Development of highly energy resolved X-ray detector.

### 2. Summary

#### 2.1 Quantum chemical modeling of radiation damage induced by ion beam (1), (2)

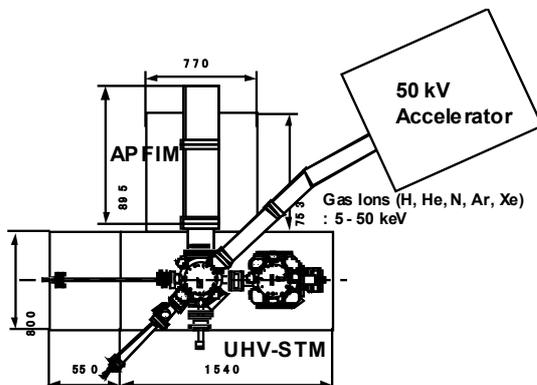


Fig. 1 An AFM combined with a 50 kV accelerator.

A tunneling microscope combined with a 50 kV ion accelerator has been newly constructed and *in situ* measurement of the radiation damage on the first layer of high purity silicone substrate induced ion irradiation was observed. In order to explain the primary processes of the radiation damage, simulation on quantum chemical modeling has been done for the (1) point defect formation, (2) swelling behavior of the material, and (3) dose rate effect for the change of the physical properties of material.

#### 2.2 Visualization of the thermal hydraulics behavior in the supercritical fluid and its numerical simulation (3), (4)

Using a supercritical carbon dioxide as a test fluid, the forced convection heat transfer inside the upward rectangular channel with one side heating was visualized by the shadowgraph and shlieren technique. The images were the world first images of the supercritical forced convection heat transfer. The transient velocity distributions were measured from the images using the high-speed camera and high-speed pulsed lasers. The visualized images for the pseudo-critical condition of the supercritical carbon dioxide reveals the existence of the relatively larger non-equilibrium fluid structures (1.5mm in width and 2.5mm in height) under higher heat flux.

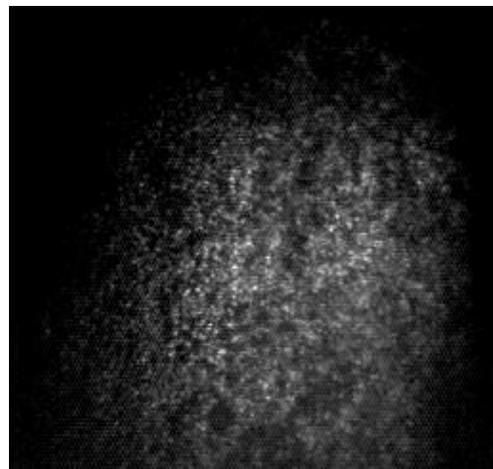


Fig. 2 A shlieren image in supercritical  $\text{CO}_2$ .  $T=42^\circ\text{C}$ ,  $q=210\text{ kW/m}^2$ .  
A non-equilibrium fluid structure.

### 2.3 Radiolysis of water at high temperature and high pressure and water chemistry (5), (6)

Since no radiation chemical study of supercritical water has been done, an experimental method has been established. By use of this method radiolysis study on supercritical water has been done and characteristics of the radiolysis of supercritical water has been revealed. The measurement of the hydrated electron in supercritical water has been done for the first time. Temperature dependence of the absorption band the decay behaviors of the transient radicals such as  $(\text{SCN})_2^{\cdot-}$ , carbonate radical,  $\text{Ag}^0$  &  $\text{Ag}_2^{2+}$ , benzophenone ketyl & anion radicals have been observed. Gamma radiolysis study of supercritical water was also done.

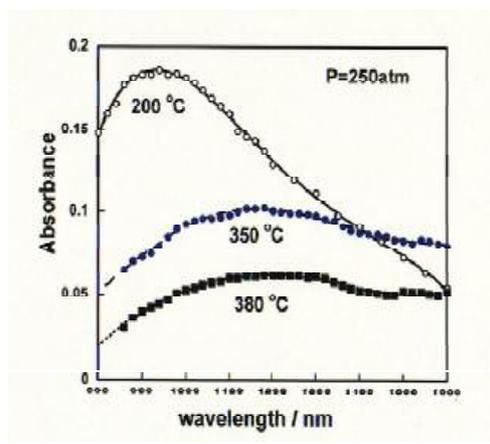


Fig. 3 Temperature dependent spectrum change of hydrated electron.

### 2.4 Development of highly energy resolved X-ray detector (7), (8)

A new generation X-ray detector by use of micro-calorimeter based on the transition edge between super conducting state and normal one (TES) has been developed in order to detect the radiation damage induced by irradiation with high accuracy.

A micro-calorimeter with double layered structure of Ir and Au has a transition temperature of 110 mK and response of  $\alpha > 300$ . From the measurement of  $^{55}\text{Fe}$  X-ray source, energy resolution of  $9.4 \pm 0.5$  eV was derived and the response time was evaluated to be 34  $\mu\text{s}$ .

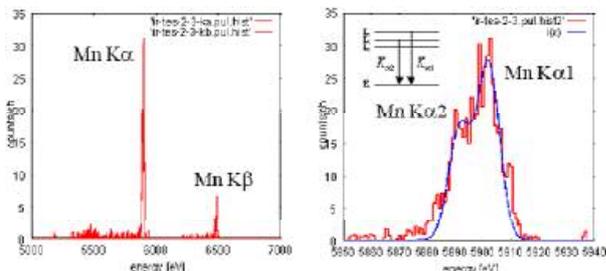


Fig. 4 Energy spectrum of Mn  $K\alpha$  and  $K\beta$  (left). Fine structure of  $K\alpha$  (right).

### 3. Concluding Remarks

- (1) By the use of a newly constructed tunneling microscope combined with an ion accelerator, *in situ* measurement of the radiation damage induced by ion beam irradiation becomes available. The modeling of the radiation damage in the material under radiation field has been carried out. The multi-scale modeling as a function of space and time has been developed.
- (2) At the pseudo-critical point in supercritical carbon dioxide, visualization of the flow of the fluid has been done. Non-equilibrated fluid structure of the bulk liquid has been detected. Through the image processing a quantitative two-dimensional flow pattern was obtained.
- (3) Hydrated electron in the supercritical water has been detected for the first time and it was found that absorption spectrum is strongly dependent on the pressure (density). The temperature dependent spectra of various kinds of transient radicals have been measured. The G-values of hydrated are also pressure dependent.
- (4) The X-ray spectroscopy by using TES micro-calorimeter has been fabricated and its high energy resolution has been verified.

### Primary Publications

- (1) N. Sekimura, Y. Shirao, H. Yamaguchi, S. Yonamine and Y. Arai, "Defect Cluster Formation in Vanadium Irradiated with Heavy Ions", *Journal of Nuclear Materials* **271&272** 63-67 (1999).
- (2) N. Sekimura, T. Morioka and K. Morishita, "Modeling of Cascade Damage Interactions by Monte-Carlo Method", *Journal of Nuclear Materials*, **283-287** 758-762 (2000).
- (3) K. Okamoto, J. Ota, K. Sakurai and H. Madarame, "Transient velocity distributions for the supercritical carbon dioxide forced convection", *J. Nuclear Science and Technology*, (Submitted).
- (4) K. Kitoh, S. Koshizuka and Y. Oka, "Refinement of Transient Criteria and Safety Analysis for a High-temperature Reactor Cooled by Supercritical Water," *Nucl. Technol.*, **135**, 252-264 (2001).
- (5) G. Wu, Y. Katsumura, Y. Muroya, X. Li and Y. Terada, "Hydrated Electron in Supercritical and Supercritical Water: A pulse radiolysis study", *Chem. Phys. Lett.*, **325**, 531-536 (2000).
- (6) M. Mostafavi, M. Lin, G. Wu, Y. Katsumura, Y. Muroya, "Pulse Radiolysis Study of Absorption Spectra of  $\text{Ag}^0$  and  $\text{Ag}_2^+$  in Water from Room Temperature up to 380 °C", *J. Phys. Chem. A*, **106**, 3123-3127 (2002).
- (7) D. Fukuda, H. Takahashi, M. Ohno, M. Nakazawa, "Ir TES for X-ray microcalorimeters," *Nucl. Instrum and Meth. A*, **444**, 241-244 (2000).
- (8) N. Yasuda, K. Uchikawa, K. Amemiya, N. Watanabe, H. Takahashi, M. Nakazawa and et al., "Estimation of the latent track size of CR-39 using atomic force microscope", *Radiat. Meas.*, **34**, 45-49 (2001).

## 2. Highly Efficient Use of Energy and Reduction of its Environmental Impact

### (1) Research Promotion Committee Members

- Yoshikazu NISHIKAWA (Osaka Institute of Technology)
  - Junzo KAWAKAMI (TOKIKO LTD.)
  - Shunsuke KONDO (The University of Tokyo)
  - Hideo SHINGU (Kyoto Energy-Environmental Research Association)
  - Taketoshi TANIGUCHI (Central Research Institute of Electric Power Industry)
  - Akira TOMITA (Tohoku University)
  - Koichi WATANABE (Keio University)
- : Committee Chairperson

### (2) List of Research Project

No.	Research Project	Project Leader
1	Synthesis of Ecological High Quality Transportation Fuels	Muneyoshi YAMADA (Tohoku University)

# Synthesis of Ecological High Quality Transportation Fuels

## Project Leader:

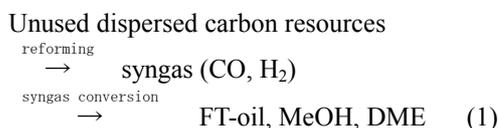
**Muneyoshi Yamada**

Professor, Graduate School of Engineering,  
Tohoku University



## 1. Objective:

This project aims to develop new-generation catalysts to ensure a compact productive process to synthesize the high-quality and environmentally benign diesel fuels from distributed untapped non-petroleum hydrocarbon resources via syngas. Synthetic fuels such as Fischer-Tropsch oil (FT-oil) and dimethyl ether (DME) have recently become known that the tail gas from the combustion of DME or FT-oil in diesel engines contains much less PM and NO<sub>x</sub> than that from the combustion of ultra-clean gas-oil. The thermal efficiency of diesel engine is, in principle, higher than that of the gasoline engine. Therefore, by using FT-oil or DME instead of gas-oil as diesel fuel, the simultaneous reduction of CO<sub>2</sub>, NO<sub>x</sub>, PM, and SO<sub>x</sub> is expected. On the basis of these new findings and the expectations, we have planned the following scheme (1).



To achieve the scheme (1), targets of each elemental step were decided as follows:

- (1) Catalytic formation of syngas: Syngas production from distributed untapped non-petroleum hydrocarbon resources such as small-scale natural gas fields, coal bed methane, and biomass without coke deposition.
- (2) FT synthesis: High one-pass conversion with higher selectivity to diesel fraction than those predicted by so-called Anderson-Schulz-Flory distribution using mesoporous material as shown in Fig. 1.
- (3) Methanol and DME synthesis: High one-pass conversion to eliminate the gas recycle.
- (4) Sulfur-tolerant catalysts: High tolerance to sulfur to simplify or omit the desulfurization unit.
- (5) Computational chemistry: Estimation and designing of an active catalysts employing an innovative molecular design technology, which combines combinatorial computational chemistry and a new accelerated quantum chemical molecular dynamics algorithm.
- (6) Process analysis: LCA for synthetic fuels and clean-energy cars.

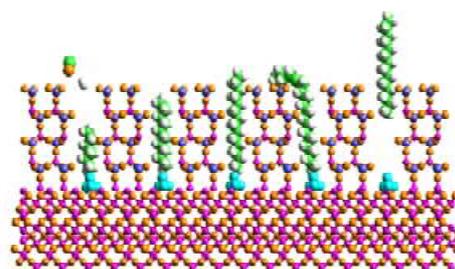


Fig. 1 Control of chain growth by mesoporous support

## 2. Summary

The industrial, target and achieved processes are compared in Fig. 2. Performance of the achieved processes is superior because they are ready for small scale carbon resources. The details are listed below.

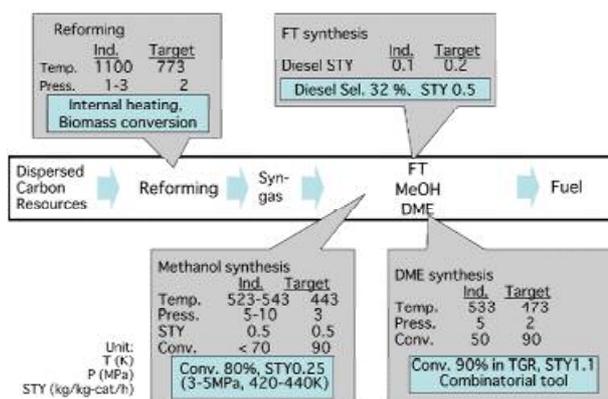


Fig. 2 Industrial, target and achieved processes

- (1) A new catalytic gasification process of biomass at low temperature under 873 K was developed. We found that a combination of Rh/CeO<sub>2</sub>/SiO<sub>2</sub> with the fluidized bed reactor yields high conversion of biomass to synthesis gas.
- (2) FT synthesis with Co catalysts supported on SBA-15 was newly carried out. Active catalysts achieve very high STY of 500 g-C/kg-cat/h for C<sub>10</sub>-C<sub>20</sub> hydrocarbons as the main fraction of diesel fuels at 503 K, 2 MPa. Supported Co catalysts prepared by the alkoxide method were also effective.

It was claimed that direct production of iso-paraffins as a multipurpose fuel for IC/FC engine from syngas could be realized by a hybrid reaction comprising the conventional FT reaction and successive hydrocracking and hydroisomerization of FT products.

- (3) The preparation method of copper-based catalysts was carefully studied for a new low-temperature synthesis method of methanol via formic ester. 80-90% one-pass conversion was achieved at 423-443 K and 3-5 MPa. Both high STY (1100 g-MeOH/kg-cat/h) and 90% CO conversion were achieved at 3 MPa in temperature-gradient reactor for DME synthesis from syngas.
- (4) CO hydrogenation catalysts with sulfur tolerance that work without desulfurization of the feed were developed. Some metal sulfides were newly found to show methanol synthesis activities even in the presence of H<sub>2</sub>S. Pd sulfide was modified and the methanol yield at 593-613 K reached 50-60% of that with conventional Cu/Zn/Al catalyst.
- (5) Combinatorial computational chemistry approach assisted by "Accelerated Quantum Chemical Molecular Dynamics" program with improved accuracy and "Design Rule of Promoter" using computational physics approach enables us to perform high-throughput screening of the catalyst model including active metals, supports, and additives. Thereby, collaboration with experimental groups in this project was advanced significantly and successfully (Fig. 3).
- (6) Evaluation of a new process for ecological high quality transportation fuels was conducted. Thermal efficiency and CO<sub>2</sub> emission of the simple process developed in this project is equal or superior to the conventional large-scale process. It is possible to reduce CO<sub>2</sub> emission by application of clean fuels using the new process for a diesel engine offering higher thermal efficiency than a gasoline engine.

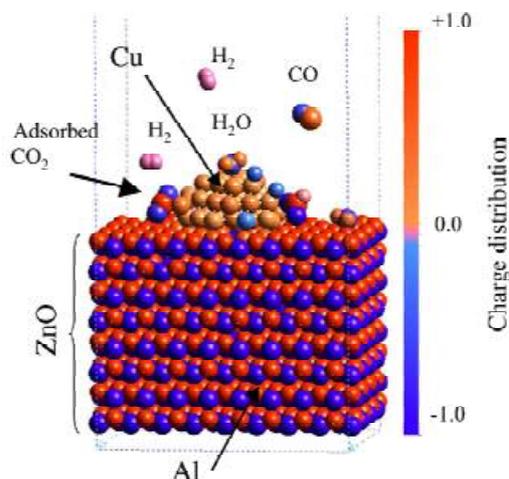


Fig. 3 Catalyst design by computational chemistry

### 3. Concluding Remarks

Our project not only produced very important fruits as ecological high-quality transportation fuels, but also brought large spin-off benefits to the chemical industry, basic catalyst and surface chemistry. Elemental technologies newly found in the project also have possibilities for extensive development and flexibly deal with various energy demands in future. Since the process can treat various kinds of carbon resources, it is applicable in a hydrogen energy society and in a society ensuring sustainable development with biomass.

### Primary Publications

- (1) M. Yamada: "High-Quality Transportation Fuels", *Energy & Fuels*, 17[4] (2003) 797 - 798.
- (2) G. Bian, T. Mochizuki, N. Fujishita, H. Nomoto and M. Yamada: "Activation and Catalytic Behavior of Several Co/SiO<sub>2</sub> Catalysts for Fischer-Tropsch Synthesis", *Energy & Fuels*, 17[4] (2003) 799 - 803.
- (3) Y. Ohtsuka, T. Arai, S. Takasaki, and N. Tsubouchi: "Fischer-Tropsch Synthesis with Cobalt Catalysts Supported on Mesoporous Silica for Efficient Production of Diesel Fuel Fraction", *Energy & Fuels*, 17[4] (2003) 804 - 809.
- (4) B. Li, X. Li, K. Asami and K. Fujimoto: "Low-Pressure Hydroformylation of Middle Olefins over Co and Rh Supported on Active Carbon Catalysts", *Energy & Fuels*, 17[4] (2003) 810 - 816.
- (5) P. Reubroycharoen, T. Yamagami, T. Vitidsant, Y. Yoneyama, M. Ito and N. Tsubaki: "Continuous Low-Temperature Methanol Synthesis from Syngas Using Alcohol Promoters", *Energy & Fuels*, 17[4] (2003) 817 - 821.
- (6) K. Okabe, M. Wei, and H. Arakawa: "Fischer-Tropsch Synthesis over Cobalt Catalysts Supported on Mesoporous Metallo-silicates", *Energy & Fuels*, 17[4] (2003) 822 - 828.
- (7) N. Koizumi, K. Murai, S. Tamayama, T. Ozaki and M. Yamada: "Promoting Effects of Some Metal Additives on the Methanol Synthesis Activity of Sulfided Pd/SiO<sub>2</sub> Catalyst from Syngas Containing H<sub>2</sub>S", *Energy & Fuels*, 17[4] (2003) 829 - 835.
- (8) K. Omata, T. Ozaki, T. Umegaki, Y. Watanabe, N. Nukui and M. Yamada: "Optimization of the Temperature Profile of a Temperature Gradient Reactor for DME Synthesis Using a Simple Genetic Algorithm Assisted by a Neural Network", *Energy & Fuels*, 17[4] (2003) 836 - 841.
- (9) M. Asadullah, T. Miyazawa, S. Ito, K. Kunimori and K. Tomishige: "Catalyst Performance of Rh/CeO<sub>2</sub>/SiO<sub>2</sub> in the Pyrogasification of Biomass", *Energy & Fuels*, 17[4] (2003) 842 - 849.
- (10) M. Kubo, T. Kubota, C. Jung, K. Seki, S. Takami, N. Koizumi, K. Omata, M. Yamada and A. Miyamoto: "Combinatorial Computational Chemistry Approach to the High-Throughput Screening of Metal Sulfide Catalysts for CO Hydrogenation Process", *Energy & Fuels*, 17[4] (2003) 857 - 861.

### 3. Computational Science and Engineering

#### (1) Research Promotion Committee Members

- Genki YAGAWA (The University of Tokyo)
- Yasuyoshi INAGAKI (Aichi Prefectural University)
- Yoshio OYANAGI (The University of Tokyo)
- Shoichi KOBAYASHI (Fukui University of Technology)
- Nobuyuki SATOFUKA (Kyoto Institute of Technology)
- Daiichiro SUGIMOTO (The University of the Air)
- Kiyoyuki TERAOKA (National Institute of Advanced Industrial Science and Technology)
- Masatake MORI (Tokyo Denki University)

○ : Committee Chairperson

#### (2) List of Research Project

No.	Research Project	Project Leader
1	Development of Simulation Algorithms for the First-Principles Prediction of Three-Dimensional Structures of Proteins	Yuko OKAMOTO (Okazaki National Research Institutes)

# Development of Simulation Algorithms for the First-Principles Prediction of Three-Dimensional Structures of Proteins

**Project Leader:**

**Yuko Okamoto**

Associate Professor, Institute for Molecular Science,  
Okazaki National Research Institutes



## 1. Objective:

There is a close relationship between the three-dimensional structures of proteins and their biological functions. The study of protein structures is thus aimed at the understanding of how proteins carry out their functions. The research in this field is ultimately led not only to drug design and *de novo* design of artificial proteins with specific functions but also the elucidation of the pathogenic mechanism for the disease that is caused by misfolding of proteins (such as mad cow disease and Alzheimer's disease).

It is widely believed that the three-dimensional structures of proteins are determined solely by their amino-acid sequence information. However, the prediction of protein structures by computer simulations with the input of only the amino-acid sequence (prediction from the first principles) has yet to be accomplished. The goal of the present project is to develop an effective simulation algorithm for the first-principles prediction of three-dimensional structures of small proteins.

There are two elements of the difficulty. One is that the number of internal degrees of freedom of protein systems is extremely large, and there exist a huge number of local minima in the energy function. It is a very challenging problem to find the global-minimum state in free energy, which corresponds to the native protein structure, since simulations by conventional algorithms will get trapped in a few of the local-minimum states. The other element for the difficulty is the problem of accuracy of the energy function for the protein system including the solvent. It is obvious that if the energy function is wrong, then one cannot expect to have the right prediction.

We try to establish a simulation algorithm that allows the prediction of the three-dimensional structures of proteins from the first principles by overcoming the above-mentioned difficulties (namely, by developing a simulation algorithm that can avoid getting trapped in states of energy local minima and a numerical method that can evaluate the energy function both accurately and efficiently).

Before starting this project, we had already introduced the so-called generalized-ensemble algorithm to this field and shown its effectiveness. However, generalized-ensemble algorithms that are easy to implement even with the system with explicit water molecules are yet to be developed. The project consisted of the following two efforts:

- (1) the development of a simulation algorithm that can overcome the above-mentioned multiple-minima problem (Simulation Algorithm Group; Group Leader: Okamoto),
- (2) the development of a numerical method that can evaluate the energy function both accurately and efficiently (Solvation Theory Group; Group Leader: Fumio Hirata, and Electronic Structure Group; Group Leader: Mutsumi Aoyagi).

We tried to encourage collaborations among groups in order to meet our goal. In Figure 1 a schematic picture of the present project is shown.

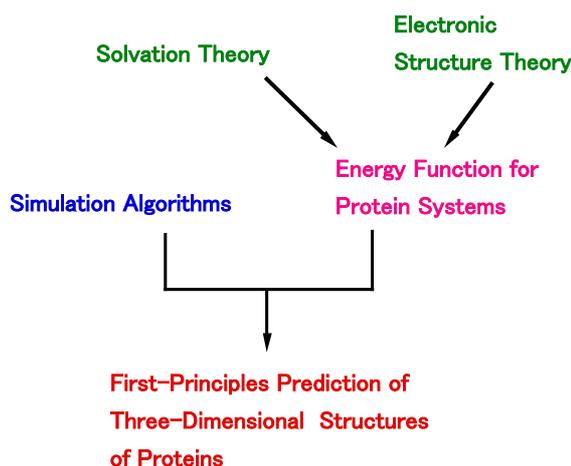


Fig. 1 Schematic picture of the project.

## 2. Summary

In this project we succeeded in increasing accuracy of solvation theory by improving the RISM theory. In particular, we can now treat effects of alcohol with atomistic details<sup>1)</sup>. We have also combined the RISM theory and multicanonical algorithm, which is one of

generalized-ensemble algorithms<sup>2)</sup>. As for simulation algorithms, we have first developed the replica-exchange molecular dynamics method<sup>3)</sup>. Because it is very easy to implement this method to the protein systems, the method was immediately accepted widely upon the publication of our paper, and now many leading groups abroad as well as in Japan use this method. However, the replica-exchange method had one problem in that when the system in question becomes very complicated, much more computation time will be required than other generalized-ensemble algorithms such as multicanonical algorithm. In order to overcome this difficulty, we have newly developed the replica-exchange multicanonical algorithm<sup>4)</sup>, multicanonical replica-exchange method<sup>4)</sup>, replica-exchange simulated tempering<sup>5)</sup> and multi-dimensional replica-exchange method<sup>6)</sup>. We believe that these new simulation algorithms are “ultimate generalized-ensemble algorithms” that are effective in realistic protein systems (for a review, see Ref. (7)). Hence, the goal of the present project has been achieved.

Given effective simulation algorithms, we can now discuss the accuracy of the energy function (force field) for the protein systems. We have applied the replica-exchange multicanonical algorithm to the C-peptide of ribonuclease A and the multicanonical replica-exchange method to the G-peptide of protein G and compared the commonly used energy functions such as AMBER parm94, parm96, parm99, CHARMM, OPLS-AA/L, and GROMOS<sup>8)</sup>. By experiments it is known that C-peptide forms  $\alpha$ -helix structure and G-peptide a  $\beta$ -hairpin structure. We judged the accuracy of the energy functions by whether or not these secondary structures ( $\alpha$ -helix and  $\beta$ -hairpin) are reproduced as the global-minimum free energy states from generalized-ensemble simulations of these peptides in aqueous solution with random initial conformations. These peptides were placed in a sphere of radius of about 26 angstroms (the number of water molecules was about 2300). In Figure 2 we show the “native structures” ( $\alpha$ -helix and  $\beta$ -hairpin structures) of these peptides.

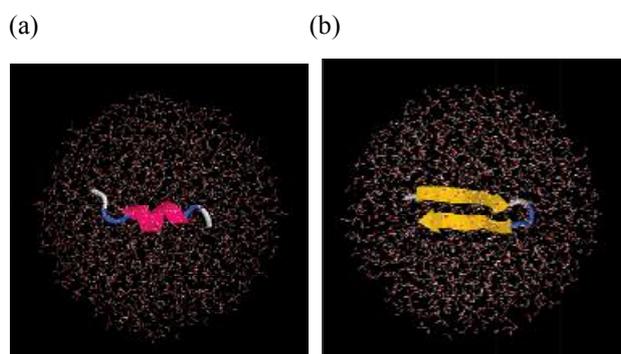


Fig. 2 The “native structure” of C-peptide (a) and that of G-peptide (b).

To our surprise, the results turned out that completely different tendencies of secondary structure contents were obtained depending on the energy functions (from that forming too much  $\alpha$ -helix to that forming too much  $\beta$ -hairpin). Hence, the development of a more accurate energy function is required. We have just proposed a new method for the development of accurate energy functions for protein systems<sup>9)</sup>.

### 3. Concluding Remarks

Because effective simulation algorithms have been developed in the present project, many people are now starting to believe that the first-principles prediction of the three-dimensional structures of proteins may finally become possible. It was April, 2003 that a timely announcement was made in the media, which said that the reading of the human genome has been essentially completed. This means that the amino-acid sequences of all the proteins that human cells synthesize are now known. In the coming post-genomic era, the most important problem is to predict the three-dimensional structures of proteins and their biological functions. The Protein3000 project that aims to determine protein structures by experiments is now underway as international collaborations. The achievements of the present project will allow one to do the same (hopefully more efficiently) by computer simulations. We expect that our methods will be important tools of research in the post-genomic era.

### Primary Publications

- (1) M. Kinoshita, Y. Okamoto, and F. Hirata, *Journal of the American Chemical Society* **122** (2000) 2773-2779.
- (2) A. Mitsutake, M. Kinoshita, Y. Okamoto, and F. Hirata, *Chemical Physics Letters* **329** (2000) 295-303.
- (3) Y. Sugita and Y. Okamoto, *Chemical Physics Letters* **314** (1999) 141-151.
- (4) Y. Sugita and Y. Okamoto, *Chemical Physics Letters* **329** (2000) 261-270.
- (5) A. Mitsutake and Y. Okamoto, *Chemical Physics Letters* **332** (2000) 131-138.
- (6) Y. Sugita, A. Kitao, and Y. Okamoto, *Journal of Chemical Physics* **113** (2000) 6042-6051.
- (7) A. Mitsutake, Y. Sugita, and Y. Okamoto, *Biopolymers (Peptide Science)* **60** (2001) 96-123.
- (8) T. Yoda, Y. Sugita, and Y. Okamoto, *Chemical Physics Letters* (2004), in press.
- (9) Y. Sakae and Y. Okamoto, *Chemical Physics Letters* **382** (2003) 626-636.

## 4. Photoscience

### (1) Research Promotion Committee Members

- Gin-ya ADACHI (College of Analytical Chemistry, Japan)
- Chiaki HIROSE (Tokyo Institute of Technology)
- Seizo MIYATA (Tokyo University of Agriculture and Technology)
- Keitaro YOSHIHARA (Japan Advanced Institute of Science and Technology)
- : Committee Chairperson

### (2) List of Research Projects

No.	Research Project	Project Leader
1	Development and Application of High Performance Lasers and Spectrometers	Hajime KATO (Kobe University)
2	Molecular Knife—Control of Chemical Reactions by Core Excitation—	Kenichiro TANAKA (Hiroshima University)
3	Development of Sub-nanosized Aggregates Having Novel Optical Properties	Atsushi NAKAJIMA (Keio University)

# Development and Application of High Performance Lasers and Spectrometers

**Project Leader:**

**Hajime Kato**

Professor, Molecular Photoscience Research Center,  
Kobe University



## 1. Objective:

- (1) A laser to use high-resolution molecular spectroscopy must satisfy the following conditions: the line width is narrower than a spectral line width, the wave length is continuously tunable, the out-put power is stable. Such high performance lasers and high resolution spectrometers will be developed.
- (2) By using the developed high performance lasers and spectrometers, molecular structure, dynamics of excited molecules, and fundamental processes in chemical reactions will be studied. By collecting new and accurate data on quantized states of molecules, interaction between levels, dynamics in the excited states, and chemical reaction dynamics, the mechanism and fundamental rules are studied.

## 2. Summary

### 2.1 Construction of high performance laser and spectrometers

A part of output beam of a single mode tunable laser was passed through a temperature stabilized confocal etalon, and the jitter was reduced by using the error signal. A scanning single mode laser tunable in the visible region and the line width less than  $0.00003 \text{ cm}^{-1}$  was constructed.

A crystal, which produces the second harmonic light, was installed in an external optical resonator. A scanning single mode laser tunable in the ultraviolet region was constructed, and the line width was less than  $0.00006 \text{ cm}^{-1}$ .

The wavelength of a cw Nd:YAG laser pumped by a diode laser was locked to a hyperfine line of iodine molecule. The jitter of wavelength of the stabilized laser was less than  $0.00003 \text{ cm}^{-1}$ .

The cavity length of a reference etalon was maintained to be constant by locking to satisfy a resonance condition (see Fig. 1.) against the wavelength of the stabilized cw Nd:YAG laser.

A fraction of the scanning laser beam was introduced into the reference etalon, and the transmitted beam intensity was detected by a photo-diode. The incident beam of the scanning laser was modulated at a frequency of 30 MHz. Thus, it

was possible to obtain transmission peaks of the interval of every 30 MHz. This transmission peaks were used as a measure of the wave length change of a scanning laser.

In order to calibrate the absolute wave number, we have constructed a system to measure a Doppler free absorption spectrum of iodine molecule by using a fraction of the scanning laser beam.

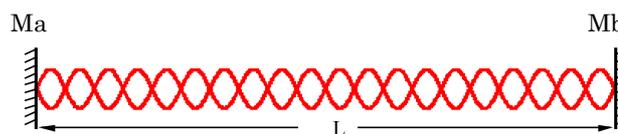


Fig. 1. Optical resonator. Resonance condition:  $L = M\lambda/2$ , where  $L$  is the distance between mirrors  $Ma$  and  $Mb$ ,  $\lambda$  is wave length,  $M$  is integer

### 2.2 Publication of the Doppler-Free High Resolution Spectral Atlas of Iodine Molecule 15000 to 19000 $\text{cm}^{-1}$

In order to calibrate an absolute wave length in visible and ultraviolet regions, the *M.I.T Wavelength Tables* (M.I.T. Press, 1939 and 1969 editions, the Accuracy is  $0.02 \text{ cm}^{-1}$ ) and the *Atlas du Spectre D'absorption de la Molecule D'iode 14800-20000  $\text{cm}^{-1}$*  (CNRS, 1978, accuracy is  $0.01 \text{ cm}^{-1}$ ) have been used over the world. The resolution of molecular spectroscopy has been increased drastically by the application of single mode tunable lasers (the resolution of our laser spectrometer is  $0.00003 \text{ cm}^{-1}$ ). Accordingly, there were demands for more accurate spectral atlas in order to calibrate the absolute wave number.

We have measured the absorption spectrum of the iodine molecule by applying the technique of Doppler-free laser spectroscopy. The results were published as *Doppler-Free High Resolution Spectral Atlas of Iodine Molecule 15000 to 19000  $\text{cm}^{-1}$*  (JSPS, 2000, accuracy is  $0.0001 \text{ cm}^{-1}$ ): 4 vol. (4000 pp) of printed document and CDROM (see Fig. 2). Each individual spectrum (approximately  $0.52 \text{ cm}^{-1}$  in range) consists of a) The frequency marks of the etalon. b) The excitation spectrum of  $\text{I}_2$  broadened by the Doppler effect. c) The Doppler-free

absorption spectrum of I<sub>2</sub>. Using CDROM, it is possible to obtain the absolute wave numbers of all the hyperfine components in the atlas.

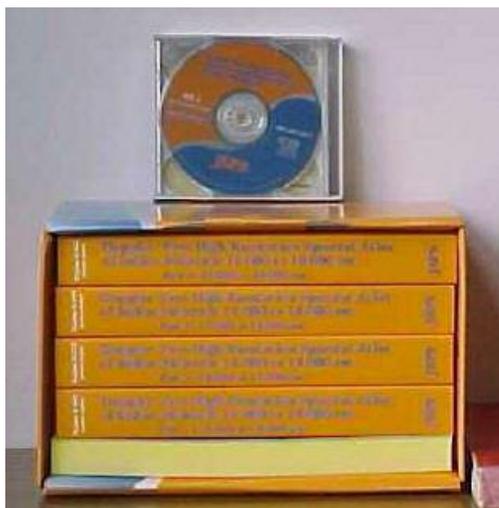


Fig. 2, Spectral atlas produced by this project.

### 2.3 Opening of a new research field in the study on molecular structure of polyatomic molecules and the dynamics

Doppler-free high resolution spectrum, where line broadening due to Doppler effect is deleted, can be measured by using the monochromatic and straight advance properties. In this project, the Doppler-free molecular beam crossed laser beam excitation spectrometer (MLCE), the Doppler-free two-photon absorption fluorescence excitation spectrometer (DFTAE), the Doppler-free laser polarization labeling spectrometer (DFPL), the Doppler-free optical-optical double resonance laser polarization labeling spectrometer (DFOOPL) etc. were constructed and developed. MLCE is free from collision and the spectrum is simple because the temperature is extremely low by the supersonic expansion in making molecular beam. DFTAE can be very high in resolution. DFPL can be used even the fluorescence is weak, and DFOOPL can be useful to assign the spectral lines even the density of lines is high.

DFTAE spectra of glyoxal, benzene, and naphthalene were measured, and the world record in the resolution and in the accuracy of absolute wave number could be achieved in the field of molecular electronic spectroscopy. Accordingly, the molecular constants were determined with the highest accuracy. By the increase of resolution, we could measure a small splitting of lines and Zeeman splitting even in diamagnetic molecules. Perturbations between singlet and triplet states could be identified easily and accurately.

By using the scanning laser operated in the ultraviolet region, we could measure rotationally resolved MLCE spectra of carbon disulphide, benzene, and naphthalene. The effect of an external magnetic field could be studied, and very important results could be obtained.

The DFLP and DFOOPL spectra of naphthalene and phenol were measured. This is the first application of this method to a polyatomic molecule. This could be achieved by constructing a very stable scanning laser and an optical system for the measurements. These studies demonstrated that these techniques can be applied for a number of polyatomic molecules and are very useful to study very complicated spectra.

### 3. Concluding Remarks

Scanning single mode laser tunable in visible and ultraviolet, which can be useful for ultra high resolution molecular spectroscopies, were developed. A system which can measure the wavelength of the scanning laser with accuracy better than 0.00003 cm<sup>-1</sup> was constructed. A spectral atlas for calibration of the absolute wave number with accuracy better than 0.0001 cm<sup>-1</sup> in the 15000 – 19000 cm<sup>-1</sup> was constructed and published.

Laser spectrometers for ultra high resolution molecular spectroscopies: MLCE, DFTAE, DFLP, DFOOPL et. ct., were constructed and applied to various molecules, and these were demonstrated to be useful.

It is demonstrated that a) these techniques of high resolution laser spectroscopies can resolve the rotational structure of naphthalene at room temperature, b) the rotational lines can be assigned, c) Zeeman splitting can be measured even in the singlet state. Accordingly, these techniques were demonstrated to be able to apply on a number of molecules, and a new and very important research field is opened.

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- (3) M. Misono, J. G. Wang, H. Katô and M. Baba: "Zeeman spectra of the  $\tilde{A}^1A_u \leftarrow X^1A_g$  transition of trans-glyoxal studied by Doppler-free two photon fluorescence excitation spectroscopy," *J. Chem. Phys.* 118, 5422-5430 (2003).
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# Molecular Knife—Control of Chemical Reactions by Core Excitation

## Project Leader:

**Kenichiro Tanaka**

Professor, Graduate School of Science, Hiroshima University



## 1. Objective:

Realization of active control of chemical-bond scission, "molecular knife", using the unique site-specific chemical reactions by core excitation.

- (1) Active control of Site-specific photochemical reactions of surface molecules. (K. Tanaka, T. Sekitani, S. Wada, N. Ueno and K. Mase)
- (2) New types of photochemical reactions for core-excited gaseous molecules. (A. Hiraya, H. Yoshida, K. Okada, T. Ibuki and S. Nagaoka)
- (3) Development of new theoretical procedure for the molecular Auger decay and the core-excited chemical reaction (K. Saito, S. Iwata, O. Takahashi and M. Mitani)

## 2. Summary

### 2.1 Active control of site-specific photochemical reactions of Surface molecules

A lot of site-specific ion desorption reactions have been discovered for various surface molecules and correlations between desorbed ions and core-excited and Auger final states were examined in detail<sup>1,2)</sup>, in intimate cooperation with a theoretical group. With the aim of the improvement in the controllability of site-selective bond scission, the systematic research using the linearly polarized synchrotron radiation (SR) has been carried out on self-assembled monolayer (SAM) which can be prepared with highly oriented functional group on the topmost surface. In the research of SAM, we chose MHDA-SAM which has

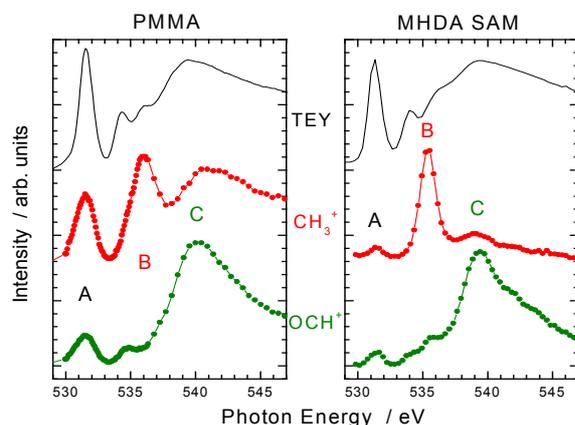


Fig. 1 Comparison of site-selectivity in chemical bond scission between MHDA-SAM and PMMA.

the same methyl-ester functional group (-COOCH<sub>3</sub>) as a side chain of PMMA polymer.

As shown in Fig. 1, we have succeeded in improving the site-selectivity of chemical bond scission ( $\gg 95\%$ ) in comparison with previous PMMA results, where CH<sub>3</sub><sup>+</sup> ion is observed almost only at peak B after O-CH<sub>3</sub> bond scission, while OCH<sup>+</sup> ion is observed at peak C after C-OCH<sub>3</sub> bond scission. This selective bond scission has also been examined by changing the incidence angle of exciting SR light. As the result, as shown in Fig. 2, a yield of CH<sub>3</sub><sup>+</sup> product ion increases with decreasing the incidence angle. These results indicate, for the first time, that site-selective chemical bond scission can be actively controlled by adjusting an electric field vector of the incident SR light to the direction of transition dipole moment of a particular resonant core excitation<sup>3,4)</sup>.

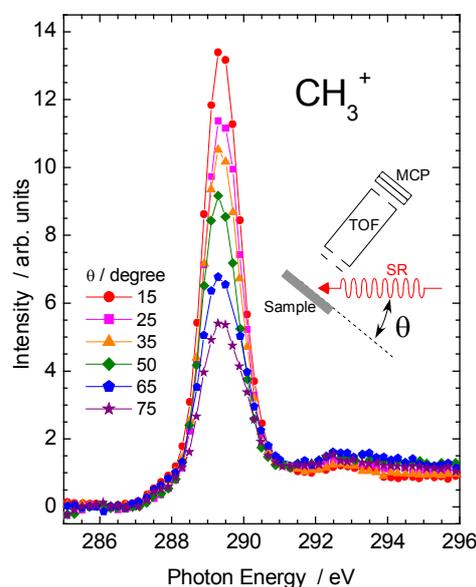


Fig. 2 CH<sub>3</sub><sup>+</sup> ion yield spectra of MHDA-SAM at different incidence angles of SR light.

### 2.2 New types of photochemical reactions for core-excited gaseous molecules

A lot of new types of excited-state and Auger final-state selective reactions have been discovered for various gaseous molecules. Mechanism of such unique reactions was successfully elucidated by using newly

developed coincidence measurements. One of the new types of state-selective reactions for core-excited molecules was identified for methanol molecules. As shown in Fig. 3, pronounced state-selective dissociation processes of O1s excited methanol were observed; 1) decrease of  $\text{OH}^+$  and  $\text{COH}^+$  while increase of  $\text{CD}_3^+$  and  $\text{DCO}^+$  at the  $3s\text{a}'$  resonance, 2) slight increase of  $\text{CD}_3^+$  while decrease of  $\text{DCO}^+$ ,  $\text{COH}^+$ , and  $\text{CO}^+$  at the  $3p\text{a}''$  resonance. These state-selective reactions were interpreted, for the first time, to be initiated by “ultra-fast bond scissions”, that compete with fast Auger decay, of O-H at the  $3s\text{a}'$  and C-O at the  $3p\text{a}''$ . Another new type reaction is vibrational state-selective  $\text{H}_2^+$  formation of the core-excited water molecule<sup>5)</sup>.

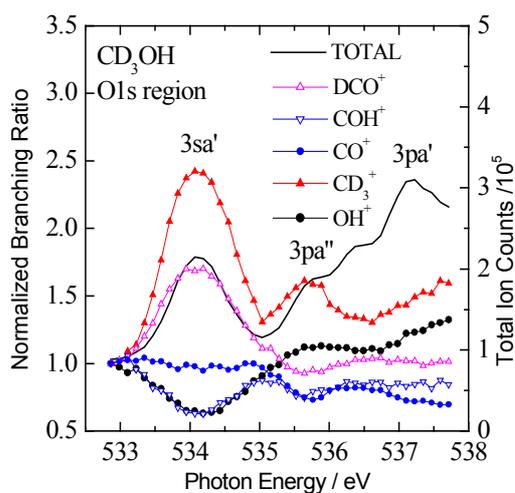


Fig. 3 Total ion-yield spectrum and normalized branching ratio spectra of  $\text{CD}_3\text{OH}$  at O1s region.

### 2.3 Development of new theoretical procedure for the molecular Auger decay and the core-excited chemical reaction

New types of theoretical procedure for the resonant core excitation, the resonant Auger decay and the following chemical reaction have been successfully developed in order to explore the origin of site-selectivity in core-excited reactions.

First, theoretical calculations for core excitation process were performed by the density functional theory, and core-excited states for various molecules were quantitatively identified. Second, new theoretical procedure for Auger decay process originated by electron population analysis was developed, and it succeeded in well reproducing the experimental results for relatively large molecules<sup>6)</sup>. For a typical example, the resonant Auger decay spectrum of acetone molecule is shown in Fig. 4. Finally, “bond dissociation factor”, which can estimate the degree of chemical bonding variance between the Auger initial and the Auger final states, was introduced for the first time, and the site-selectivity after Auger decay and the correlation between molecular size and the position of the primary excitation can be successfully explained by using this factor.

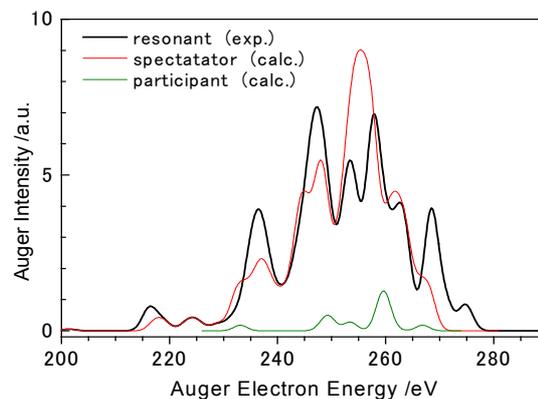


Fig. 4 Resonant Auger decay spectra of acetone at the excitation of  $\text{C1s}(\text{C}=\text{O}) \rightarrow \pi^*(\text{C}=\text{O})$ .

### 3. Concluding Remarks

Whole pictures of the site-specific photo-chemical reactions by core excitation and the fundamental aspects for realizing site-selective bond scission utilizing this unique reaction were clearly elucidated. By choosing the energy and incident angle of the incident SR light, it succeeded in remarkably improving the controllability and efficiency of the site-selective bond scission. Finally, a scenario for realizing “molecular knife” was proposed.

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- (1) K. Tanaka, E. O. Sako, E. Ikenaga, K. Isari, S. A. Sardar, S. Wada, T. Sekitani, K. Mase and N. Ueno, “Control of chemical reactions by core excitation” *J. Electron Spectrosc. Relat. Phenom.* **119**, 255-266 (2001).
- (2) E. Ikenaga, K. Isari, K. Kudara, Y. Yasui, S. A. Sardar, S. Wada, T. Sekitani, K. Tanaka, K. Mase and S. Tanaka, “Study of ion desorption induced by carbon core excitation for poly-methylmethacrylate thin film using electron-ion coincidence spectroscopy” *J. Chem. Phys.* **114**, 2751-2759 (2001).
- (3) S. Wada, E. O. Sako, R. Sumii, S. Waki, K. Isari, T. Sekiguchi, T. Sekitani and K. Tanaka, “Active control of site specificity in ion desorption by core excitation” *Nucl. Instr. Meth. Phys. Res. B*, **199**, 361-365 (2003).
- (4) S. Wada, R. Sumii, K. Isari, S. Waki, E. O. Sako, T. Sekiguchi, T. Sekitani and K. Tanaka, “Active control of chemical bond scission by site-specific core excitation” *Surf. Sci.* **528**, 242-248 (2003).
- (5) A. Hiraya, K. Nobusada, M. Simon, K. Okada, T. Tokushima, Y. Senba, H. Yoshida, K. Kamimori, H. Okumura, Y. Shimizu, A. -L. Thomas, P. Millie, I. Koyano and K. Ueda “ $\text{H}_2^+$  formation from  $\text{H}_2\text{O}^+$  mediated by the core-excitation-induced nuclear motion in  $\text{H}_2\text{O}$ ” *Phys. Rev. A*, **63**, 042705 (2001).
- (6) M. Mitani, O. Takahashi, K. Saito and S. Iwata, “Theoretical estimation of molecular Auger spectra by electron population analysis” *J. Electron Spectrosc. Relat. Phenom.* **128**, 103-117 (2003).

## Development of Sub-nanosized Aggregates Having Novel Optical Properties

### Project Leader:

**Atsushi Nakajima**

Professor, Faculty of Science and Technology,  
Keio University



### 1. Objective:

In our project, we have aimed at the creation of sub-nanosized aggregates having novel optical properties on the basis of fundamental understanding about electronic and geometrical structures to develop new optoelectronic materials, and we have investigated three following systems.

- (1) luminescent semiconductor nano-cluster/nano-crystal
- (2) creation of novel organometallic nano-cluster
- (3) networked nano-cluster formed by hydrogen bonding

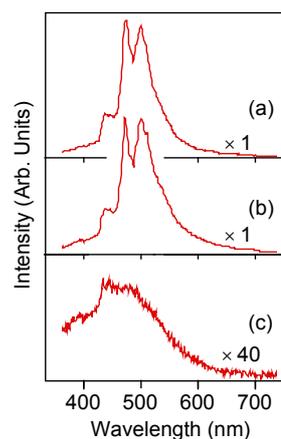
### 2. Summary

#### 2.1 luminescent semiconductor nano-cluster/nano-crystal

After the finding of visible luminescence from electro-chemically treated silicon/germanium substrate, many experimental and theoretical studies have been reported for decades, but no definite conclusions have been obtained. In order to elucidate fundamental understandings towards optical properties of nano-clusters/nano-crystal of silicon/germanium, two different approaches of gas phase and matrix were taken to evaluate their optical properties quantitatively. These works have successfully revealed fundamental understanding of quantum size effect on luminescence and the substantial contribution of silicon/germanium oxides.

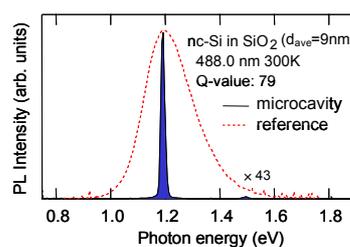
Photoelectron spectroscopy for germanium cluster anions has size-selectively revealed electronic structure regarding photoemission bands. The energy gaps oscillates with the cluster size at the cluster size of 5-20, and converge around 1 eV at 30 atoms, which clearly manifests that germanium nano-clusters cannot emit visible emissions themselves. On the other hand, germanium oxide clusters exhibit larger energy gap of 2-3 eV in the photoelectron spectra, and this result shows that germanium oxide clusters having Ge:O = 1:1 composition are promising function units for visible luminescence. Indeed, when germanium oxide clusters were deposited onto a substrate, the deposited substrate exhibit visible photoluminescence around 400-600 nm (Figure 1).

Based on the luminescence mechanism of nano-sized semiconductors, we have proposed two concepts for optical devices: (a) emission enhancement with a microcavity structure by silicon nano-crystals and (b) doping of rare earth atoms for efficient energy transfer or for formation of a novel caged structure.



**Figure 1.** photoluminescence spectra of deposited germanium oxides onto (a) silicon and (b) gold substrates. Exposure of oxygen molecules during the deposition reduced the intensity drastically (c). Excitation wavelength was 325 nm.

In the microcavity structure, silicon nano-crystals were embedded in thin SiO<sub>2</sub> layer which plays a role of emitting layer, and the SiO<sub>2</sub> layer was sandwiched by dielectric multi-layers of Si/SiO<sub>2</sub>. Figure 2 shows luminescence from silicon nanocrystals embedded in the microcavity structure. Very sharp emissions were observed at 1.2 eV (near infra-red region) with Q-value of 79 for the microcavity structure, while broad emissions were observed with Q-value of 6.1 without the micro-structure. The intensity of the luminescence in the direction of normal vector of the layered structures was 40 times enhanced, showing that this is a favorable nano-structure to create an efficient optoelectronic device based on silicon materials.



**Figure 2.** luminescence from silicon nanocrystals embedded in the microcavity structure (solid line).

## 2.2 creation of novel organometallic nano-cluster

In gas phase reactions, we have discovered various geometrical structures of organometallic nano-cluster, such as low-dimensional sandwich and core-shell ball. Their electronic and magnetic properties were quantitatively evaluated by photoionization/photoelectron spectroscopy or by Stern-Gerlach experiments, showing that the organometallic clusters are promising function units of optoelectronic/magnetic properties. For example, vanadium(V)-benzene organometallic clusters were formed efficiently in gas phase, resulting in the composition of  $V_n(C_6H_6)_{n+1}$  exclusively. Figure 3 shows a typical mass spectrum for the  $V_n(C_6H_6)_{n+1}$  clusters, which take a multiple decker sandwich structure.

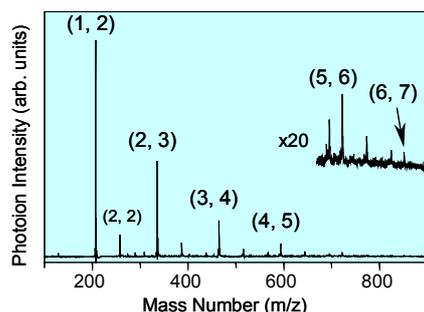


Figure 3. Mass spectrum of vanadium-benzene clusters

From the quantitative measurements on their electronic and magnetic properties, it has been revealed that d electrons are delocalized along the molecular axis, and that electron spin on each V atom exhibits ferromagnetic alignment. These results clearly show that the organometallic clusters are promising candidates for optoelectronic/magnetic function units.

We have successfully established the patented soft-landing technique of size-selective deposition upon a substrate covered with low-temperature argon matrix (figure 4) or with self-assembled monolayer of alkylthiol. This methodology enables us to create and design new nano-scaled functionalized substrate with decoration of gas-phase clusters.

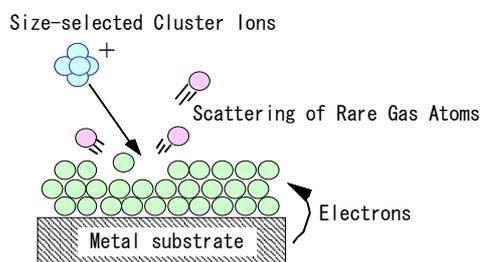


Figure 4. Soft-landing with Ar matrix thin layers

## 2.3 networked nano-cluster formed by hydrogen bonding

We have developed a new production method to generate networked molecular clusters through hydrogen bondings up to 500 molecules, and electronic properties and hydrogen transfer dynamics in the electronic excited states were revealed spectroscopically. Figure 5 shows mass spectrum for

acetonitrile nano-cluster anions (up to a few nanometer diameter) which take two different electronic states with an excess electron. Bridging between an isolated molecule and bulk liquid/solid was successfully built for fundamental concepts and applications based on molecular nano-clusters.

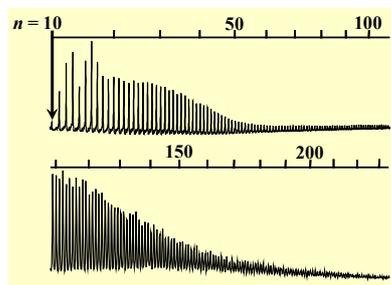


Figure 5. Mass spectrum of acetonitrile cluster anions

## 3. Concluding Remarks

We have successfully established several new methodologies to generate sub-nanosized aggregates having novel optical properties, and revealed fundamental properties of their electronic and geometrical structures as well as molecular aggregates bridging between gas and liquid/solid phases. New areas of “gas-phase organometallic chemistry” have been developed to create and to design new optoelectronic materials with our proposed soft-landing method.

This project was started by Prof. Koji Kaya (Director General, Institute for Molecular Science) at Keio University, and was continued as a collaborator after his moving.

## 4. Primary Publications

- (1) M. Mitsui, N. Ando, S. Kokubo, A. Nakajima, and K. Kaya: “Coexistence of solvated electrons and solvent valence anions in negatively charged acetonitrile clusters,  $(CH_3CN)_n^-$  ( $n = 10-100$ )” *Phys.Rev.Lett.* **91**, 153002 (2003).
- (2) M. Mitsui, A. Nakajima, and K. Kaya: “Negative ion photo-electron spectroscopy of  $(benzene)_n^-$  and  $(toluene)_n^-$ : Solvation energetics of excess electron in size-selected nanoclusters” *J. Chem. Phys.* **117**, 9740 (2002).
- (3) K. Judai, K. Sera, S. Amatsutsumi, K. Yagi, T. Yasuike, S. Yabushita, A. Nakajima, and K. Kaya: “A soft-landing experiment of organometallic cluster ions: infrared spectroscopy of  $V(benzene)_2$  in Ar matrix” *Chem. Phys. Lett.* **334**, 277 (2001).
- (4) Y. Negishi, S. Nagao, Y. Nakamura, A. Nakajima, S. Kamei, and K. Kaya: “Visible Photoluminescence of the Deposited Germanium-Oxide Prepared from Clusters in the Gas Phase” *J. Appl. Phys.* **88**(10), 6037-6043 (2000).
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- (6) M. Fujii, A. Mimura, S. Hayashi, Y. Yamamoto, and K. Murakami: “Hyperfine structure of electron spin resonance of phosphorus doped Si nanocrystals” *Phys. Rev. Lett.* **89**, 206805 (2002).

# **【Life Sciences】**

*1 . Genetic and Environmental Factors in Diseases  
Prevalent in Adults and the Elderly: Molecular and  
Cellular Mechanism of Vascular Complications*

( 1 ) Research Promotion Committee Members

- Kiyoshi KUROKAWA      (Tokai University)
- Etsuro OGATA            (Japanese Foundation for Cancer Research)
- Hiroshi OKAMOTO        (Tohoku University)
- Toru KITA                (Kyoto University)
- Hisayuki MATSUO        (Miyazaki Medical College)

○ : Committee Chairperson

( 2 ) List of Research Projects

No.	Research Project	Project Leader
1	<a href="#">Molecular Elucidation of Significance of Cardiovascular Hormones in Vascular Protection and Regeneration</a>	Kazuwa NAKAO (Kyoto University)
2	<a href="#">In Vivo Molecular Studies on Cardio-renal Remodeling</a>	Iekuni ICHIKAWA (Tokai University)

## Molecular Elucidation of Significance of Cardiovascular Hormones in Vascular Protection and Regeneration

**Project Leader:**

**Kazuwa Nakao** Professor, Graduate School of Medicine, Kyoto University



### 1. Objective:

Recent progress on the search for new vasoactive substances has elucidated that the heart and the blood vessels themselves secrete several bioactive materials (cardiovascular hormones). It has been recognized that cardiovascular homeostasis is maintained through complex interactions among a variety of cardiovascular hormones, such as natriuretic peptides; atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP).

We have been investigating the physiological and pathophysiological significance of cardiovascular hormones and demonstrated that ANP and BNP are secreted mainly from the atrium and the ventricle of the heart, respectively to act as cardiac hormones, whereas CNP is secreted from the endothelial cells to act as an endothelium-derived vasorelaxing peptide, sharing the signal transduction pathway, guanylate cyclases (natriuretic peptide receptors themselves)/cGMP/cGMP-dependent protein kinase (cGK), with nitric oxide (NO).

In this research project, in order to delineate the clinical relevance of cardiovascular hormones in the control of circulatory homeostasis, we developed several gene-engineered animals (transgenic and knock-out mice) related to natriuretic peptide system and examined the phenotypic alteration in physiological and pathophysiological conditions. In addition, using adenoviral gene transfer method, we examined the effect of over-expression of CNP gene in vascular walls *in vivo* for vascular protection and regeneration. We also investigated the molecular mechanisms for natriuretic peptides' actions on cardiovascular remodeling, especially focusing upon the molecular interaction of cGK and small GTPase Rho and its downstream effector molecule, Rho-kinase.

### 2. Summary

#### 2.1 Establishment of BNP knock-out mice and elucidation of BNP as a cardiocyte-derived antifibrotic factor

In BNP knock-out mice, no signs of systemic hypertension and ventricular hypertrophy were noted.

However, histologic examinations revealed multifocal fibrotic lesions in the subendocardial regions of ventricles from male BNP knock-out mice at 15 wks of age and thereafter.

Northern blotting analysis revealed in the ventricle from BNP knock-out mice the increased expression of mRNAs for TGF- $\beta_3$  and angiotensin converting enzyme (ACE), which are shown to be involved in cardiac fibrosis. Therefore, BNP might prevent the development of cardiac fibrosis through the inhibition of cardiac ACE and TGF- $\beta_3$  production. Our data suggest that BNP constitutes a novel intracardiac counterregulatory mechanism to prevent the progression of cardiac fibrosis *in vivo*; it may serve as a cardiomyocyte-derived anti-fibrotic signal to cardiac fibroblasts during the process of ventricular remodeling.

#### 2.2 Establishment of CNP knock-out mice and elucidation of the role of CNP in endochondral ossification

To investigate the physiological significance of CNP *in vivo*, we generated mice with targeted disruption of CNP. CNP knock-out mice were all viable perinatally, but less than half could survive during postnatal development. Moreover, CNP knock-out mice showed severe dwarfism. The naso-anal lengths of CNP knock-out mice were 60 to 70% of those of wild-type mice. Soft X-ray analysis revealed that the longitudinal growth of vertebrae, tail and limb bones was affected in CNP knock-out mice. These observations indicate that loss of CNP affects endochondral ossification but not membranous ossification *in vivo*. To explore whether local expression of CNP in the bone can rescue the dwarfism of CNP knock-out mice *in vivo*, they were crossed with mice with transgenic expression of CNP in the growth plate chondrocytes (Tg mice). The genetically "rescued" animals (or CNP knock-out mice with the transgene expression (CNP Tg/KO mice)) were of normal appearance, and their skeletons were indistinguishable from those of wild-type mice. This study demonstrates that CNP acts locally as a positive regulator of endochondral ossification *in vivo* and

suggests its pathophysiological and therapeutic implication in some forms of skeletal dysplasia.

### 2.3 Adenoviral gene transfer of CNP into vascular walls and elucidation of the role of CNP in endothelial regeneration/its molecular mechanism

We constructed an adenovirus containing CNP cDNA, which we cloned (Ad.CNP) and infected injured blood vessel walls in several animal models. In rabbit femoral artery balloon-injury model, the infection of Ad.CNP at the injury site significantly reduced neointimal formation with alteration of vascular smooth muscle phenotype (from SM-2 negative synthetic phenotype to SM-2 positive contractile phenotype), compared to the infection of control adenovirus containing  $\beta$ -galactosidase gene (Ad.LacZ). In addition, re-endothelialization of the injured site was accelerated with the recovery of endothelium-dependent vasorelaxation.

In rabbit jugular vein-grafting model, in which the jugular vein was interposed to the ipsilateral carotid artery, the infection of Ad.CNP suppressed intimal thickening and also significantly potentiated re-endothelialization, compared to control adenovirus infection. Accompanied with the acceleration of re-endothelialization, thrombi-formation was remarkably suppressed in Ad.CNP-treated group. In vitro cultured endothelial cells, natriuretic peptides at physiological concentration ( $10^{-9}$ ~ $10^{-11}$ M) in low serum culture condition significantly enhanced proliferation and migration, which was suppressed with a cGK inhibitor.

### 2.4 Significance and therapeutic potential of natriuretic peptides /cGMP/cGMP-dependent protein kinase (cGK) pathway in vascular regeneration

A murine model of operatively induced hindlimb ischemia was used to investigate the effect of NPs/cGMP/cGK pathway on angiogenesis. In transgenic mice that overexpress BNP in the liver (BNP-Tg), which we previously generated (*J Clin Invest.* 1994 93: 1911-21), serial blood flow measurements by laser Doppler perfusion imager revealed that accelerated limb perfusion improvement was observed for up to 12d in BNP-Tg mice compared to non-Tg mice. Ischemia-induced angiogenesis was also significantly potentiated in cGK type I (cGKI: one of cGK isoforms and present in endothelial cells and smooth muscle cells) transgenic mice, and conversely attenuated in cGKI knockout mice. These findings reveal the novel action of NPs to stimulate angiogenesis and suggest that NPs can be used for new strategy of therapeutic angiogenesis in patients with tissue ischemia.

### 2.5 Elucidation of molecular interaction of natriuretic peptides/cGMP/cGK pathway and Rho/Rho kinase pathway in vascular remodeling

Rho-associated kinase (ROCK), an effector of small GTPase Rho, is known to enhance vascular tone via a calcium sensitization and plays a key role in the pathogenesis of hypertension. We investigated the role of Rho/ROCK pathway in the regulation of vascular smooth muscle cells (VSMCs) growth and vascular remodeling *in vivo*. We revealed that mitogen-induced DNA synthesis of cultured VSMCs was suppressed by

Y-27632, a specific ROCK inhibitor, and the overexpression of dominant-negative ROCK. Y-27632 also suppressed the chemotaxis of VSMCs. The neointimal formation of balloon-injured carotid arteries was significantly suppressed in Y-27632-treated rats compared with vehicle-treated rats. These results indicate the essential roles of ROCK in the control of VSMC proliferation *in vitro* and vascular remodeling *in vivo*. We prepared mutant RhoA with substitution of the residue Ser188 to Ala (A188RhoA), where protein kinase A (PKA) was reported to phosphorylate RhoA. We incubated the RhoA mutant with cGK and demonstrated that cGK phosphorylated RhoA at Ser188 *in vitro*. In HeLa cells, we revealed that stress fiber formation induced by lysophosphatidic acid or V14RhoA was blocked by transfection with constitutively active cGK (C-cGK). In contrast, C-cGK failed to inhibit stress fiber formation in cells transfected with the mutant RhoA with substitution of Ser188 to Ala. Collectively, our findings suggest that cGK phosphorylates RhoA at Ser188 and inactivates RhoA signaling in cultured cells.

### 3. Concluding Remarks

Our data provide new insight into the pathophysiological and therapeutic implication of natriuretic peptides in the process of vascular protection and regeneration.

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## *In Vivo* Molecular Studies on Cardio-renal Remodeling

**Project Leader:**

**Iekuni Ichikawa** Professor, School of Medicine, Tokai University



### 1. Objective:

Diseases of the cardiovascular system, such as heart diseases and cerebrovascular accidents, account for 1/3 of mortality in Japan. In addition, the number of people, who have lost the function of the kidney and are in need of dialysis therapy, has reached approximately 200, 000. Eight thousand more are added every year to the dialyzed population, creating a serious medical-economical problem. It is well known that these cardiovascular diseases are aggravated by high blood pressure. Therefore, decreasing blood pressure has been the most important therapy for these diseases. Blood pressure in humans is regulated by a number of hormones and nerve transmitters. In particular, the hormone called “angiotensin” is known to be most influential. At present, drugs that inhibit the generation of angiotensin or block the action of angiotensin are widely used. Interestingly, when cardiovascular and renal diseases are treated with these drugs, the beneficial effects exceed those that are induced by other anti-high blood pressure drugs. These observations suggest that in addition to its role in blood pressure regulation, angiotensin has un-identified direct actions on the heart, blood vessels, and the kidney independently of its blood pressure regulating effects. Thus, we began our studies to identify the not-yet-described effects of angiotensin in the hope to find the clue to better remedies for cardiovascular and renal diseases.

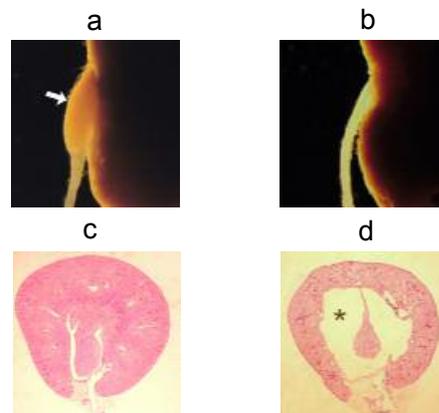
### 2. Summary

Often, we find the value of money, when we lose it. Likewise, roles of angiotensin can be revealed when angiotensin is lost or its actions are inhibited. We generated mice in which we destroyed the genes necessary for the action of angiotensin. By comparing these mice with normal mice, we looked for the unknown roles of angiotensin. For the first time, our studies showed that angiotensin is essential for the formation and function of the ureter. The ureter is a narrow duct that carries urine from the kidney to the bladder. The ureter is well known as the site where stones (“ureteral stones”) get stuck, creating tremendous pain. The ureter is not just a duct. Similar to the heart and the intestine, the ureter functions autonomously; it

pushes down urine actively to the bladder. In mice that lack actions of angiotensin, the ureter was not formed properly so that urine was not carried to the bladder, creating the upward pressure and causing damage to the kidney (Figure 1). When inhibitors of angiotensin are administered to pregnant women, there is a risk that babies would be born with problems of the kidney, suggesting that the inhibitors of angiotensin interfere with the formation of the ureter. These findings indicate that angiotensin is necessary for fetuses and newborns. Next question is “is angiotensin not necessary once humans matured?” In this regard, we also found that in adult mice, too, inhibitors of angiotensin suppress the activity of the ureter. Angiotensin has often been regarded as a culprit that increases blood pressure or aggravates renal diseases. After all, angiotensin also plays beneficial roles by activating the function of the ureter to carry urine to the bladder, thereby protecting the kidney from damage.

#### Figure 1. Angiotensin is essential for formation of the ureter.

a. In normal mice, the beginning portion of the ureter is bulky (an arrow). b. In mice lacking the action of angiotensin, no bulky portion is found. c. Cross section of the kidney of normal mice. d. In mice lacking the action of angiotensin, the calyx (\*), where urine accumulates, is markedly enlarged.

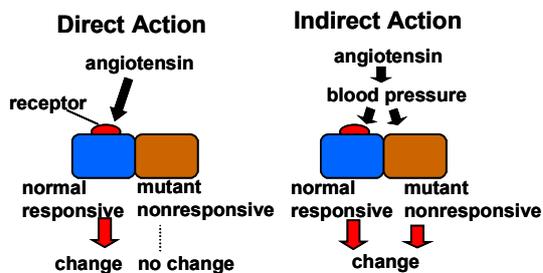


Blood pressure is lower in mutant mice lacking angiotensin activity than that in normal mice. Although these mutant mice are resistant to develop heart diseases as fast as in normal mice, we cannot tell if it is because of the low blood pressure or the lack of the activity of

angiotensin. In order to address this question, we generated mice in which only some of the cells can respond to angiotensin. In these mice, some cells have the receptor for angiotensin that is essential for transmitting the stimulus by angiotensin, and others do not have the receptor. Since these two types of cells exist in the same body, they are both exposed to the identical systemic environment, such as blood pressure. In comparing the two types of cells in a given mouse, phenomena that are common to the two types of cells must have been caused by the systemic changes, whereas those that are different between these cell types must have been induced by direct actions of angiotensin (Figure 2).

#### Figure 2. Direct and indirect actions of angiotensin.

Angiotensin has potent effects to increase blood pressure. If angiotensin directly affects individual cells independently of its action on blood pressure, there would be differences between the cells that can respond to angiotensin and those that cannot (left). If angiotensin affects cells through its systemic effects such as that on blood pressure, both types of cells would show identical changes (right).



When angiotensin was administered into mice continuously, blood pressure was elevated, and the heart became enlarged. At the same time, fibrous materials accumulated in the heart (a disease called “fibrosis”), and the wall of the blood vessels became thicker. When we administered angiotensin into mice in which parts of the body can respond to angiotensin, blood pressure increased greatly in those that had many normal cells, while blood pressure increased little in those that had few normal cells. Examinations of the heart demonstrated that those mice mostly have little fibrosis, and there were more cells (called “fibroblasts”) that produce the fibrous materials in the vicinity of normal cells. Thus, independent of the action on blood pressure, angiotensin, in fact, acts upon the heart directly and increases the production of fibrous materials. However, more influential is through the action on blood pressure. We made similar findings in the blood vessels, too. Abnormal thickening of the vessel wall was determined primarily by blood pressure, and there was no evidence that angiotensin directly induced thickening of the blood vessels.

In patients who have lost the kidney function due to diabetic nephropathy or nephritis, the kidney develops common lesions called “glomerulosclerosis” regardless of the primary diseases. Glomerulosclerosis cannot be induced in mice by angiotensin administration or any other experimental manipulations that can induce glomerulosclerosis efficiently in other animal species such as rats. Since it is important to create a mouse

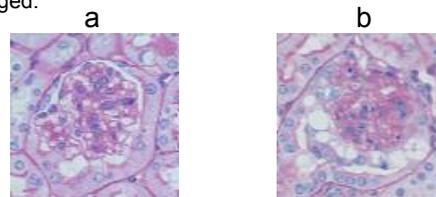
model for glomerulosclerosis, we put much of our effort into the generation of mice in which we can induce glomerulosclerosis. The kidney consists of a number of different types of cells. Among the most critical kidney cells in the pathogenesis of glomerulosclerosis is “glomerular epithelial cells” (also called “podocytes”). We generated mutant mice in which only podocytes can be injured. As we expected, when injury was induced in podocytes, mice lost a large amount of proteins into urine. Furthermore, kidney cells other than podocytes were also gradually damaged, glomerulosclerosis developed and, finally, the function of the kidney was rapidly lost (Figure 3). In this unique model, a single administration of small amount of toxin can induce irreversible damage to podocytes, and kidney diseases can readily be reproduced. Our model of glomerulosclerosis will be highly instrumental in advancing our understanding of how kidney diseases are induced and how they can be treated.

### 3. Concluding Remarks

As described above, this project revealed new roles of angiotensin, and succeeded in generating an animal model that will contribute to a great extent to the progress of the studies on kidney diseases.

#### Figure 3. The kidney disease model we have developed.

a. Before induction of podocyte injury, the glomerulus (an important structure of the kidney) appeared normal. b. After inducing podocyte injury, the glomerulus was markedly damaged.



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## 2. Regulation Networks of Eukaryotic Gene Expression

### (1) Research Promotion Committee Members

- Yoshiaki FUJII (University of Tsukuba)
  - Masaki IWABUCHI (National Institute of Agrobiological Sciences)
  - Masanao MIWA (University of Tsukuba)
  - Masami MURAMATSU (Saitama Medical School)
- : Committee Chairperson

### (2) List of Research Projects

No.	Research Project	Project Leader
1	<a href="#">Regulation of Gene Expression in Cell Differentiation</a>	Toru NAKANO (Osaka University)
2	<a href="#">Regulatory Mechanisms of Gene Expression for Environment Adaptation</a>	Masayuki YAMAMOTO (University of Tsukuba)
3	<a href="#">Regulation of Gene Expression in the Nervous System</a>	Ryoichiro KAGEYAMA (Kyoto University)
4	<a href="#">Nuclear Structure and Gene Expression</a>	Masatoshi HAGIWARA (Tokyo Medical and Dental University)

# Regulation of Gene Expression in Cell Differentiation

**Project Leader:**

**Toru Nakano**

Professor, Research Institute for Microbial Diseases,  
Osaka University



## 1. Objective:

Regulation of gene expression governs many aspects of biological phenomena such as cell differentiation from immature cells to functional cells or development of new functions in mature cells. In this study, the following three distinct biological systems were chosen for analyzing molecular mechanisms of cell differentiation and proliferation.

- (1) Cell fate determination process in hematopoietic differentiation (T. Nakano, T. Kimura, K. Kitajima, et al)
- (2) Regulation of gene expression in cell differentiation of immune responses (T. Fujita, M. Yoneyama, N. Watanabe, et al)
- (3) Gene expression network controlling development and differentiation of nervous system (M. Nakafuku, R. Mizuguchi and M. Nagao)

## 2. Summary

### 2.1 Cell fate determination process in hematopoietic differentiation

In vitro hematopoietic differentiation from mouse embryonic stem cells (ES cells) is a fascinating method to analyze the development and differentiation process of hematopoiesis. Quite a lot of transcription factors controlling gene expression are involved in the commitment of hematopoietic cells. Functions of various transcription factors were analyzed by expressing them or their derivatives with conditional gene expression method. GATA-2, a zinc finger transcription factor expressed in immature blood cells, turned out to enhance the proliferation of hematopoietic progenitors<sup>1)</sup>.

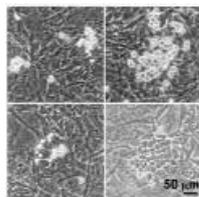


Fig. 1 Enhancement of hematopoiesis by GATA-2. right: effects of GATA-2. (ref. 1)

Molecular mechanisms maintaining the pluripotency of immature cells were analyzed as well.

PTEN is a tumor suppressor that dephosphorylates phosphatidylinositol triphosphate (PIP3) to PIP2. Germ cell specific PTEN null mice were examined. The mutant mice show significantly high incidence of “de-differentiation” from primordial germ cells to the pluripotent embryonic germ cells<sup>2)</sup>. In addition PTEN plays crucial roles in B and T cell differentiation<sup>3)</sup>.

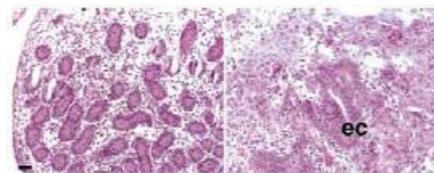


Fig. 2 “De-differentiation” by the null mutation of PTEN. left: control, right: PTEN null. (ref 2)

### 2.2 Regulation of Gene Expression in Immune System

Interferon system plays a crucial role in innate immune responses to viral infection. Interferon system is normally shut off, however viral infection/replication triggers a signal that results in the production of type I interferon, then the secreted interferon secondarily activates a set of genes responsible for the antiviral activities. The transcription factor interferon regulatory factor 3 (IRF-3) functions as a molecular switch for the primary activation of interferon genes.

We elucidated that IRF-3 undergoes phosphorylation at specific serine residues, dimerizes, then associates with coactivator p300 or CBP to become fully active holocomplex in the nucleus. More recently we determined X-ray crystal structure of IRF-3 dimer. The determined structure and the pinpoint mutagenesis revealed the mechanism of phosphorylation-induced dimerization and the interaction interface with the coactivators. Thus the mechanism of IRF-3 activation is elucidated at molecular level. Furthermore, the overall structure of IRF-3 activation domain resembles that of Smad, a signal transducer and activator of transcription functioning in TGF- $\beta$  signaling. Thus it is strongly suggested that IRF-3 evolved from Smad and acquired its new function in innate immune system.

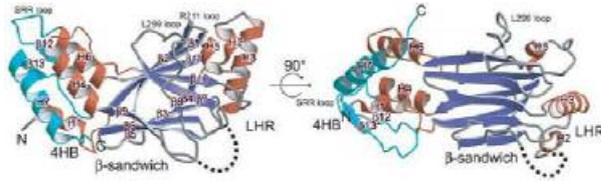


Fig. 3 Crystal structure of IRF-3. (ref 6)

### 2.3 Gene expression network controlling development and differentiation of nervous system

Neural stem cells with the capacity of extended self-renewal and multi-lineage differentiation serve as the ultimate origin of diverse types of neurons and glia during development of mammalian central nervous system. We have identified multiple homeodomain and helix-loop-helix classes of transcription factors that show spatially and temporally restricted expression patterns in the developing spinal cord. We have proposed a transcriptional code model in which specific combinations of these factors direct neural stem cells to adopt particular cell fates.

Along the course of these studies, we also identified that cells expressing the similar set of transcription factors in the adult nervous system. Observations in many lines of studies supported the idea that these cells are neural stem/progenitor cells resident in the adult brain and spinal cord, and also that similar molecular control mechanisms operate in both embryonic and adult neural stem cells. The majority of these cells remain quiescent in the intact brain except two well-known neurogenic regions, subventricular zone of the forebrain and dentate gyrus of the hippocampus. However, we found that they are induced to proliferate under various pathological conditions. Furthermore, we could induce massive regeneration of new neurons in the damaged hippocampus by intraventricular infusion of FGF and EGF, potent mitogens for adult neural stem cells. These results opened a new avenue to develop novel strategies for regenerating damaged nervous system with aid of the regenerative capacity of endogenous adult neural stem cells.

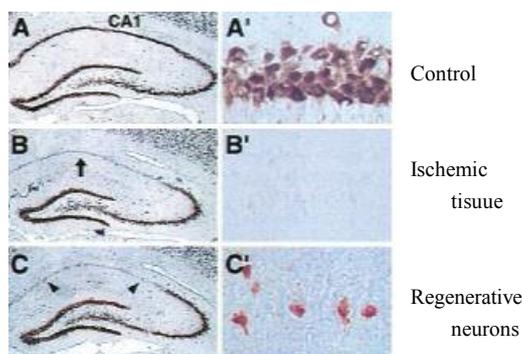


Fig. 1 Regeneration of neurons after ischemic damage. (ref. 8)

### 3. Concluding Remarks

Molecular mechanisms of cell differentiation among hematopoietic, immune and nervous system were examined from molecular structure to *in vivo* function. These data would be useful not only for the basic analysis of cell differentiation but also for the clinical application of regenerative medicine or immuno-regulation.

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- (8) H. Nakatomi, T. Kuriu T, S. Okabe, S. Yamamoto, O. Hatano, N. Kawahara, A. Tamura, T. Kirino and M. Nakafuku: "Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors," *Cell*, **110** [4] (2002) 429-441.

## Regulatory Mechanisms of Gene Expression for Environment Adaptation

**Project Leader:**

**Masayuki Yamamoto**

Professor, Institute of Basic Medical Sciences,  
University of Tsukuba



### 1. Objective:

Clarification of the molecular basis of cellular response to environmental signals and development of mouse models for the human diseases using this response system.

- (1) Molecular mechanisms of how induction of phase II enzymes is regulated by the oxidative stresses and electrophiles. (M. Yamamoto)
- (2) Molecular mechanisms of how phase I enzymes are regulated during the induction by dioxins. (M. Yamamoto and K. Sogawa)
- (3) Molecular mechanisms of how animals adapt to hypoxia. (M. Yamamoto and K. Sogawa)

### 2. Summary

#### 2.1 Molecular mechanisms of how induction of phase II enzymes is regulated by the oxidative stresses and electrophiles.

The mechanisms to respond against electrophilic intermediates within dietary chemicals and reactive oxygen species represent one of the most important cellular defense mechanisms against environmental toxins. This electrophilic response has been observed in the rat, mouse and human, in the form of a group of detoxification enzymes and antioxidant proteins. Transcription of these defense enzymes/ proteins is coordinately induced through the antioxidant responsive element (ARE). Using gene-knockout techniques in mice, we demonstrated that transcription factor Nrf2 is essential for ARE-mediated induction of the phase II enzyme genes, and is also critical for cytoprotection against various environmental stresses. A newly identified actin-binding protein Keap1 is revealed to regulate the activity of Nrf2 by two actions, nuclear shuttling and protein degradation. We have identified that Nrf2-Keap1-dependent phase II induction mechanism is also existed in zebrafish, suggesting that it is a highly conserved system among vertebrates.

Nrf2 requires a heterodimeric partner molecule for DNA binding. We have shown that small Maf proteins are *in vivo* partners for Nrf2. In addition, we revealed that quantitative balance of small Maf proteins is quite important for regulation of their target genes. This finding is considerable, since it was the first report demonstrating that amount of transcription factors is critical for their *in vivo* functions.

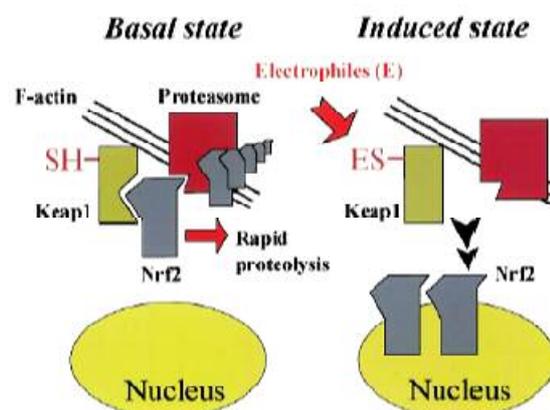


Fig. 1 Mechanism of Nrf2 activation

#### 2.2 Molecular mechanisms of how phase I enzymes are regulated during the induction by dioxins.

Polycyclic aromatic hydrocarbons including dioxins are ubiquitous environmental toxicants, and their toxicities are mediated by the activation of transcription factor AhR and induction of its targets, phase I detoxification enzyme genes. Despite strong conservation of the phase I system among vertebrates, there are wide interspecies differences in the toxicological responses to dioxins. To gain insight into the hazards to human health posed by dioxins *in vivo*, we generated a mouse model that harbors the human *AhR* gene instead of the mouse gene. Human *AhR* knock-in mouse showed weaker

response to dioxins than wild-type mouse. This humanized model mouse should serve as a better assessment system of the biological effects of environmental toxicants.

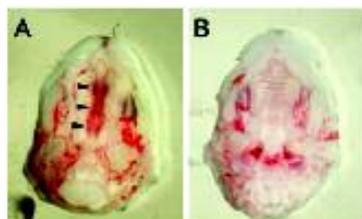


Fig. 2 Fetal teratogenesis in wild-type mice (A) after maternal administration of dioxins (cleft palate, arrowheads) were reduced in human *AhR* knock-in mice (B).

### 2.3 Molecular mechanisms of how animals adapt to hypoxia.

Oxygen is necessary for energy production in aerobic organisms, but it becomes toxic if levels are not maintained within limited concentrations. Mammalian cells adapt to insufficient oxygen levels, termed hypoxia, by switching on genes that encode proteins responsible for re-establishing conditions of normoxia. To elucidate the molecular mechanism of adaptation to hypoxia, we studied the transcriptional regulation of hypoxic-inducible genes using gene-manipulated mice. Transcription factors HIF-1 $\alpha$  and HLF are both important for hypoxia response, while their requirements vary among tissues and genes. We found that hypoxia-induced expression of erythropoietin gene in eye depends on HLF using HLF knock-down mice, which is responsible for experimental retinopathy of prematurity.

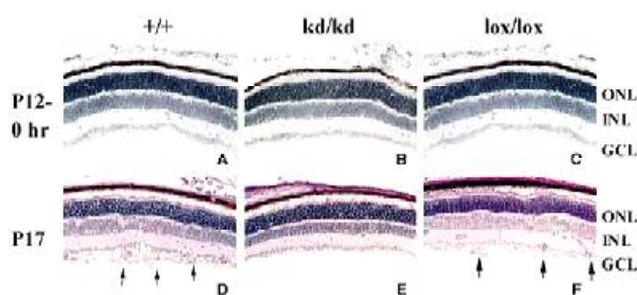


Fig. 3 Neovascularization in the retinas of mice was induced by hypoxia signals in wild-type mice (A, arrows) but not in HLF knock-down mice (B). Cre-mediated removal of the inserted gene recovered normal retinal neovascularization (C).

### 3. Concluding Remarks

We discovered the important role of Nrf2 in the maintenance of animal health via protection against various chemical toxicities. Importance of other transcription factors, such as AhR and HLF, in

environment adaptation was also demonstrated. In addition, we developed the system generating and analyzing humanized model mouse to assess risk of chemical compounds for human.

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- (6) H. Motohashi, F. Katsuoka, J. A. Shavit, J. D. Engel and M. Yamamoto. (2000) Positive or negative MARE-dependent transcriptional regulation is determined by the abundance of small maf proteins. *Cell*, 103: 865-875.
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- (8) K. Itoh, N. Wakabayashi, Y. Katoh, T. Ishii, K. Igarashi, J. D. Engel and M. Yamamoto. (1999) Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev.*, 13: 76-86.

# Regulation of Gene Expression in the Nervous System

**Project Leader:**

**Ryoichiro Kageyama** Professor, Institute for Virus Research, Kyoto University



## 1. Objective:

It is essential to understand the basic mechanism of neural development for new diagnosis and therapy of brain diseases, which have been increasing in an aging society. Here, we analyzed (1) the transcription factors and (2) the promoter regions of neural-specific genes to elucidate the basic mechanism of neural development. During neural development, three steps, neural stem cell maintenance, neuronal differentiation, and glial differentiation, occur sequentially. In this research, we elucidated the transcription factor network that regulates these three steps. After differentiation, neurons extend their axons to the correct targets under the control of attractive and repulsive molecules. During these processes, gene expression should be controlled precisely. We elucidated the promoter regions that are required for spatial- and temporal-specific gene expression. The results of this research will be useful to understand the basic mechanism of neural development and develop the new diagnosis and therapy of brain diseases.

## 2. Summary

### 2.1 Transcription Factors for Neural Development

During neural development, three steps, (1) neural stem cell maintenance, (2) neuronal differentiation, and (3) glial differentiation, occur sequentially. In this study, we elucidated the transcription factor network that regulates these three steps (Fig. 1). We found that bHLH factors play an essential role in all the steps, as described below.

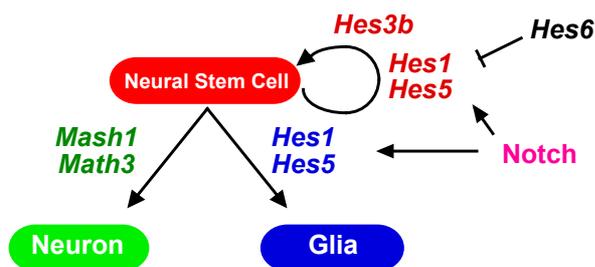


Fig. 1. The bHLH factor network that regulates neural development.

### (1) Neural stem cell maintenance

Forced expression of bHLH factors Hes1 and Hes5 with retrovirus inhibits neuronal differentiation and maintains neural stem cells while cells infected with the control retrovirus differentiate efficiently into neurons (Fig. 2). Conversely, in the absence of Hes1 and Hes5, neural stem cells are not maintained and neuronal differentiation is accelerated.

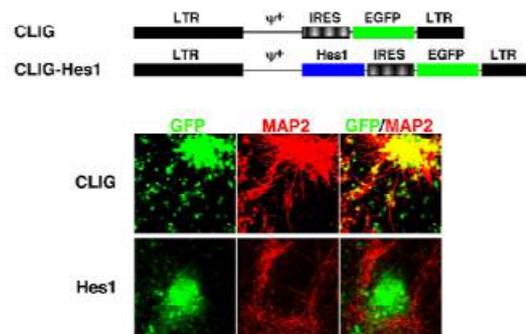


Fig.2. Forced expression of Hes1 with retrovirus inhibits neuronal differentiation.

### (2) Neuronal differentiation from neural stem cells

Forced expression of bHLH factors Mash1 and Math3 induces neuronal differentiation from neural stem cells. Conversely, in the absence of Mash1 and Math3, neurons are not formed, but instead glial cells are prematurely differentiated (Fig. 3). Thus, Mash1 and Math3 regulate neuronal versus glial fate determination.

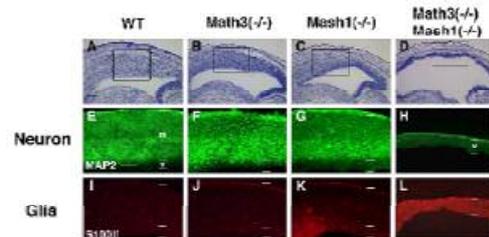


Fig. 3. In the absence of Mash1 and Math3, neurons are not formed, but instead glial cells are prematurely differentiated.

There are many subtypes of neurons. bHLH factors alone are not sufficient but homeodomain factors are additionally required for neuronal subtype specification.

(3) Glial differentiation from neural stem cells

bHLH factors Hes1 and Hes5 are expressed by glial cells at a later stage of development. Forced expression of Hes1 and Hes5 in neural stem cells at late stages increases glial cells. Thus, Hes1 and Hes5 regulate both neural stem cell maintenance and glial differentiation.

(4) Hes1 as a two-hour cycle biological clock

Expression of bHLH factor Hes1 oscillates in many cell types including neural cells. This oscillatory expression occurs autonomously by a negative feedback mechanism (Fig. 4).

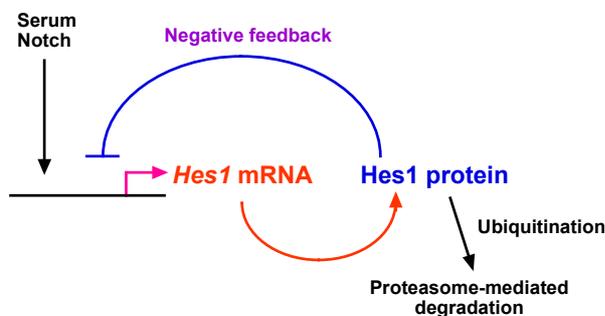


Fig. 4. Hes1 as a two-hour cycle biological clock. Hes1 oscillation occurs by a negative feedback mechanism.

### 2.2 Promoter Analysis for Neural-Specific Genes

We isolated floor plate-specific genes, SulfFP1, SulfFP2, and autotaxin, and elucidated their structure, expression and functions. We found that the upstream 12-kb region of SulfFP1 is sufficient for expression in the brain and that its expression is regulated by tissue-specific signals.



Fig. 5. Promoter analysis of SulfFP1. Expression of the GFP reporter is observed in bones and brain.

### 3. Concluding Remarks

In this project, we elucidated transcription factors and promoter regions that regulate neural development. We found that bHLH factors play a central role in all three steps of neural stem cell maintenance, neuronal differentiation and glial differentiation. In addition, we found that homeodomain factors are additionally required for neuronal subtype specification. These transcription factors may be applicable to procedures for expansion of neural stem cells and regeneration of specific neurons, which are useful for regeneration therapy. We also found that Hes1 functions as a two-hour cycle biological clock. It is suggested that this clock may control the timing of neuronal differentiation.

### Primary Publications

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# Nuclear Structure and Gene Expression

## Project Leader:

Masatoshi Hagiwara Professor, Medical Research Institute,  
Tokyo Medical and Dental University



## 1. Objective:

- (1) To find out the regulation mechanisms of transcription and alternative splicing, we investigate about the signaling pathways to phosphorylation of CREB and SR proteins (Masatoshi Hagiwara).
- (2) To find out the mechanisms of nucleosome assembly, we examine the effects of NAP-1 and ACF on nucleosome assembly (Takashi Ito).
- (3) To find out the role of SWI/SNF complex, we reconstitute cell free systems with highly purified general factors and cofactors (Tsutomu Ohta).

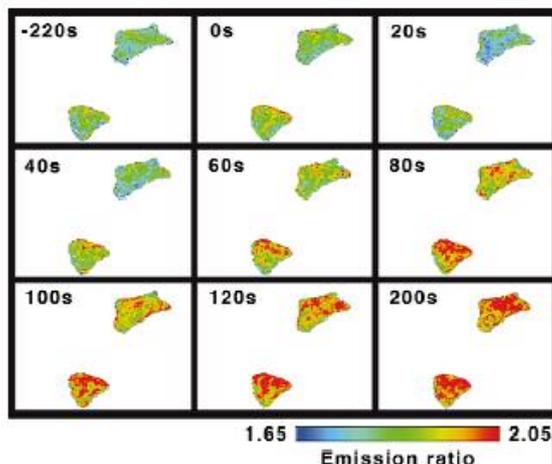
## 2. Summary

### 2.1 Detection of cAMP-induced phosphorylation with a novel fluorescent indicator

The phosphorylation process of proteins in living cells was visualized with a novel fluorescent indicator composed of two green fluorescent protein variants joined by the kinase-inducible domain (KID) of a transcription factor, CREB. Phosphorylation of KID catalyzed by cAMP-dependent protein kinase (PKA) decreased the fluorescence resonance energy transfer

### Figure 1: Imaging of FRET of ART in COS-7 cells.

Pseudocolor images reflect the emission changes detected through  $440 \pm 20$  nm and  $520 \pm 20$  nm bandpass filters at the indicated time points. Cells were treated with  $5 \mu\text{M}$  db-cAMP at time zero. Bar,  $30 \mu\text{m}$ .

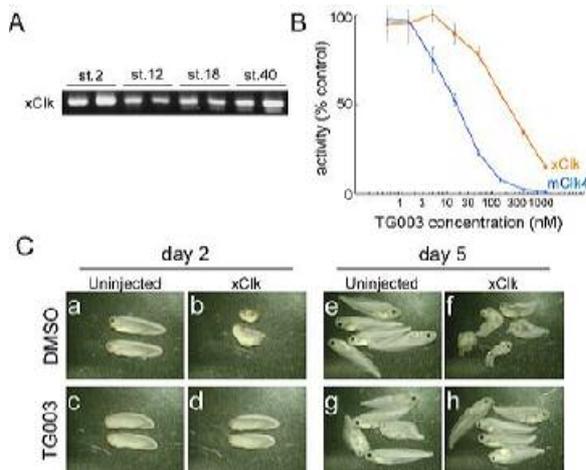


(FRET) between the flanking GFPs. We dubbed the indicator protein ‘ART (A-kinase Responsive Tracer)’ and visualized activation dynamics of PKA in COS-7 cells transfected with the expression vector of ART. This approach enabled the imaging of PKA-mediated phosphorylation in live cells and should be applicable to other kinases<sup>1</sup> (see Fig.1). In addition, we established *in vivo* reporter system of CREB activation using CRE::GFP and found that CaMKK-CaMKIV-CREB cascade is conserved in the sensory neurons of *C. elegans*<sup>2</sup>. CRE::GFP expressing nematoda is a useful tool as an “*in vivo* reporter assay” system to visualize CREB activation in the living animal body.

### 2.2 Detection of cAMP-induced phosphorylation with a novel fluorescent indicator

Transcription and Pre-mRNA splicing are closely coupled and catalyzed by a multimolecular complex including serine/arginine-rich (SR) proteins, which are thought to play crucial role in the spliceosome formation and in the regulation of alternative splicing. SR proteins are RNA-binding proteins which have both one or two RNA-recognition motifs (RRMs) and serine/arginine repeats (RS domain), and are involved in constitutive splicing as well as being specific modulators in alternative splicing. In living cells, SR proteins are phosphorylated, predominantly on serine residues in the RS domain. *Prp4* is a protein kinase of *Schizosaccharomyces pombe* identified through its role in pre-mRNA splicing, and belongs to a kinase family including mammalian SRPKs and Clks, whose substrates are SR proteins. We cloned human PRP4 (hPRP4) full-length cDNA and the antiserum raised against a partial peptide of hPRP4 recognized 170-kDa polypeptide in HeLa S3 cell extracts<sup>3</sup>. We also found that a novel compound TG003, (*Z*)-1-(3-ethyl-5-methoxy-2,3-dihydrobenzothiazol-2-ylidene) propan-2-one, inhibited AF2/ASF-dependent splicing *in vitro* by suppressing phosphorylation of SF2/ASF in HeLa S100 extract. *In vitro* kinase assay revealed that TG003 specifically inhibited phosphorylation of SF2/ASF by Clk1/Sty and Clk4 with the  $K_i$  value of 10 nM (unpublished data).

**Fig.2: TG003 suppressed xClk-induced abnormal development of *Xenopus* embryo.** A. RT-PCR analysis of *Xenopus* Clk (xClk) mRNA. RNA from two independent embryos at stage 2, 12, 18 and 40 was analyzed. B. xClk kinase activity is also inhibited by TG003 *in vitro* C. The effect of microinjected xClk mRNA. *Xenopus* embryos were incubated with 10  $\mu$ M TG003 (panels c-d and g-h) or DMSO (panels a-b and e-f) until 4-cell stage.



### 2.3 Multistep chromatin assembly by NAP-1 and ACF

The assembly of genomic DNA and histones into a fundamental process of eukaryotes that affects a broad range of biological phenomena, including DNA replication, DNA repair, gene expression and progression through the cell cycle. The basic structural unit of chromatin is the nucleosome, consisting of 145 base pairs (bp) of DNA wrapped around an octamer of the core histones H2A, H2B, H3, and H4. In contrast to previous studies using relaxed circular plasmids as templates, we have found that negatively supercoiled templates reveal distinct roles for NAP-1 and ACF in histone deposition and the formation of an ordered nucleosomal array. NAP-1 can efficiently deposit histones onto supercoiled plasmids. Furthermore, NAP1 exhibits a greater affinity for histones H2A and H2B than does naked DNA, but in their presence of H3 and H4, H2A and H2B are transferred from NAP-1 to the plasmid templates. Recombinant ACF, composed of ISW and Acf1, modulated nucleosomal spacing, and do so well after histone deposition by NAP-1. Atomic force microscopy, however, suggests that NAP-1 alone fails to accomplish the formation of nucleosomal core particles, which are only formed in the presence of ACF<sup>4</sup>. These results suggest a model for the ordered deposition of histones and arrangements of nucleosomes during chromatin assembly *in vitro*.

### 2.4 Role of human SWI complex in transcription regulation

The human SWI/SNF complex is partially purified with 4 ion-exchange columns (P11, DE52, HAP and Heparin) from HeLa cell nuclear extracts. Cell free systems reconstituted with highly purified general factors and cofactors show activation functions on naked DNA templates. The possible functions of the SWI/SNF complex in regulation through chromatin templates were analyzed by *in vitro* transcription system. We found that the SEI/SNF complex activated basal transcription but not activator dependent transcription in the reconstituted transcription systems. When HeLa cell nuclear extracts were used as the transcription systems, the SWI/SNF complex activated activator dependent transcription<sup>5</sup>. This suggests that cofactor(s) may be required for activation of transcription from chromatin templates by the SWI/SNF complex.

### 3. Concluding Remarks

- (1) Monitor systems of CREB phosphorylation and chemical probes of SR protein phosphorylation could revealed the phosphorylation-dependent regulation mechanisms of transcription and alternative splicing.
- (2) Our purified recombinant system is useful for the direct examination of detailed mechanisms of chromatin assembly and other phenomena, including chromatin remodeling coupled with transcription, repair, and recombination.
- (3) The SWI/SNF complex was not a component of nuclear matrix but strongly interacted with the nuclear matrix *in vitro*.

### Primary Publications

- (1) Nagai, Y., Miyazaki, M., Aoki, R., Zama, T., Inoue, S., Hirose, K., Iino, M. & **Hagiwara, M.** A fluorescent indicator for visualizing cAMP-induced phosphorylation *in vivo*. **Nat. Biotechnol.** 18, 313-316 (2000).
- (2) Kimura, Y., Corcoran, E.E., Eto K., Gengyo-Ando, K., Muramatsu, M., Kobayashi, R., Freedman, J.H., Mitani, S., **Hagiwara, M.**, Means, A.R., & Tokumitsu H. A CaMK cascade activates CRE-mediated transcription in neurons of *Caenorhabditis elegans*. **EMBO Rep.** 3, 962-966 (2002).
- (3) Kojima, T., Zama, T., Wada, K., Onogi, H. & **Hagiwara, M.** Cloning of Human PRP4 Reveals Interaction with Clk1. **J. Biol. Chem.** 276, 32247-32256 (2001).
- (4) **Ito, T.**, Levenstain M., Fyodorov D., Kutach, A.K., Kobayashi, R., and Kadonaga, J.T. The Acf1 and ISWI subunits of ACF function cooperatively in the ATP-dependent catalysis of chromatin assembly. **Genes & Dev.**, 13: 1529-1539 (1999).
- (5) Usui, T., **Ohta, T.**, Osiumi, H., Tomizawa, J., Ogawa, H., and Ogawa, T., Complex formation and functional versatility of Mre11 of budding yeast in recombination. **Cell** 95:705-716 (1998).

# **【Integrated Fields】**

# 1. Biomedical Observation and Control

## (1) Research Promotion Committee Members

- Toshiyuki FURUKAWA (Osaka National Hospital)
  - Tadashi KAWAI (International Clinical Pathology Center)
  - Satoshi KAWATA (Osaka University)
  - Ryoji SUZUKI (Kanazawa Institute of Technology)
  - Kunihiro CHIHARA (Nara Institute of Science and Technology)
- : Committee Chairperson

## (2) List of Research Project

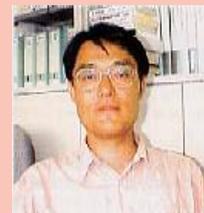
No.	Research Project	Project Leader
1	<a href="#">Creation of Generic Technology for Advanced Biosensors</a>	Eiichi TAMIYA (Japan Advanced Institute of Science and Technology, Hokuriku)

# Creation of Generic Technology for Advanced Biosensors

## Project Leader:

Eiichi Tamiya

Professor, School of Materials Science,  
Japan Advanced Institute of Science and Technology



## 1. Objective:

This project aimed at creation and development of generic technology for advanced biosensors which have high performance as follows. DNA aptamers combinatorial peptides and MIPs (Molecular imprint polymers) were studied for improvement of stability, sensitivity and selectivity. Microchip technology was employed for integration and miniaturization of biosensor total system. Nanoscopic tools such as AFM and SNOM contributed to single molecular bioanalyses.

## 2. Summary

We designed a sensor peptide based on structural change that takes place when MAPK (mytogen activated protein kinase) is phosphorylated by MAPKK (MAP kinase kinase) in the intracellular signal transduction. The imprinted polymer can strongly recognize an amine by the strong electrostatic interaction between the amino group of the amine and the sulfo group in the binding sites<sup>1)</sup>. This method provides an amine-recognizing polymer material by using a thiol derivative instead of the amine. We developed the construction of an artificial ructosyl amine dehydrogenase based on molecular imprinting technology and its application to the artificial enzyme sensor<sup>2)</sup>. We developed the molecularly imprinted catalyst (MIC). We have carried out to develop a high-through-put measurement system for the detection organic pollution (BOD) by using micro-chip immobilized luminous microorganisms. We made use of P19 as a model of stem cell and selected the peptides that bind with P19 from phage display library.

We screened the DNA aptamer inhibiting Taq DNA polymerase with novel method combining systematic evolution of ligands by exponential enrichment (SELEX) and in silico evolution. By adopting an alkaline phosphatase-linked aptamer that binds to bile acids a new analytical method for the detection of bile acids has been developed. As a method to estimate the activities of G-protein-coupled receptor, we developed a method to detect phosphate ion (Pi) using europium (III) and time-resolved fluorescence spectrometry.

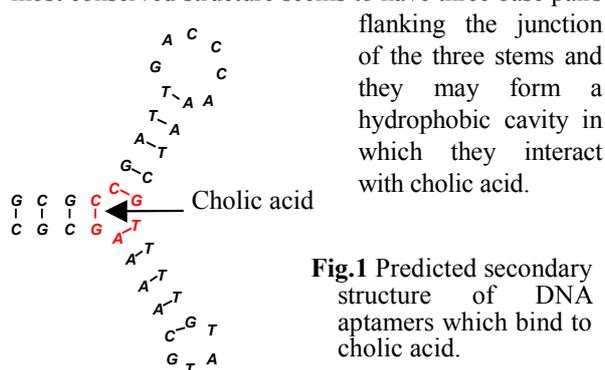
A micro optical cantilever made of thin silicon dioxide film has been applied to DNA imaging and provided a 100 nm resolution fluorescence image.

Imaging of single molecular fluorescence has been showed blinking and breaching phenomena that characterize the single molecular fluorescence.

We have developed miniaturized biotechnology tools both of flow-type and array-format. As a flow-type device, a hydrophobic valve structure has newly fabricated with switchable surface modification that can change its hydrophilicity. Microchemical sensors and microactuators which would become key components in micro analysis systems were fabricated using micromachining techniques. These include an analysis system that imitates a mosquito, an on-line sensing system for ammonia and creatinine, an integrated microfluidic transport system using bubbles as active elements for pumps and valves. A portable surface plasmon resonance (SPR) sensor system consisting of a miniature SPR sensor chip Spreeta<sup>TM</sup>, a hydrostatic pressure driven flow system using a Marionette bottle and a sample injector, was developed.

### 2.1 *In vitro* selection of DNA aptamers which bind to cholic acid

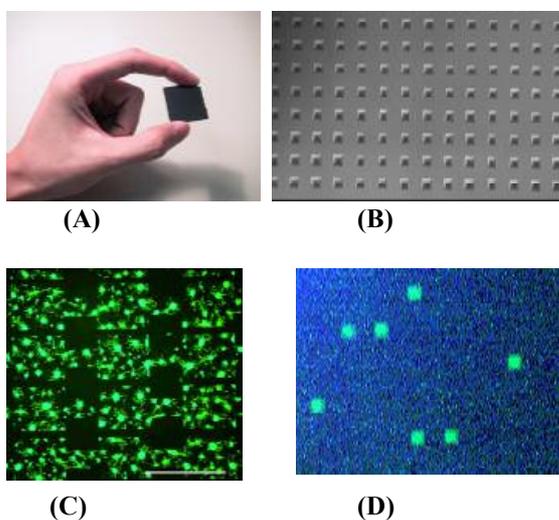
DNA aptamers which bind to cholic acid have been identified by *in vitro* selection from a pool of approximately  $9 \times 10^{14}$  DNA molecules<sup>3)</sup>. After 13 rounds of selection, 19 clones with 95-100 nucleotide length were sequenced. Deletion-mutant experiments and computational sequence analysis suggested that all clones contained cholic acid binding sequences which could fold into three-way junction structures. By comparing the sequences involved in the predicted three-way junction structure of these 19 clones, it was determined that the nucleotide sequences and lengths of three stem and loop regions have no similarity. The most conserved structure seems to have three base pairs



**Fig.1** Predicted secondary structure of DNA aptamers which bind to cholic acid.

## 2.2 Development of a microchamber array for picoliter PCR

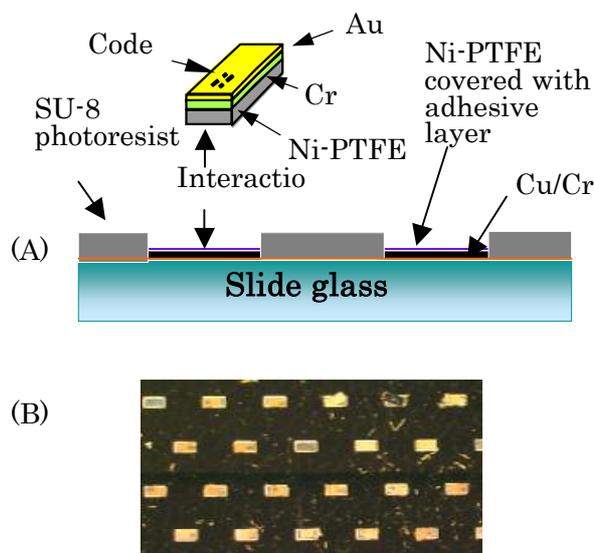
A microchamber array for PCR was developed by semiconductor microfabrication technology<sup>4)</sup>. The microchambers were designed to be of picoliter quantity. To optimize fluid retention, the surface states of the substrate and the inner walls were examined for four different types of microchamber. The substrate was silicon, while silicon dioxide was selected for the inner walls. PCR was performed in the microchamber array, and the amplification of DNA was detected using a technique based on the energy transfer of fluorescent dyes. The lower volume limit for PCR was investigated using various sizes of microchambers. Microchambers with volume greater than 86 pL gave successful PCR. In addition, the system was improved in order to take up the PCR product. To prevent mixing of the samples, the samples were dried after PCR using a membrane that permeates only vapor.



**Fig. 2** Microchamber array and applications (A) view of microchamber chip (B) scanning electron microscopy images of microchambers. (C) neuronal cell chip, (D) single cell PCR chip

## 2.3 Micromachining microcarrier-based biomolecular encoding for miniaturized and multiplexed immunoassay.

Micromachining techniques, which originated in the microelectronics industry, have been employed to manufacture microparticles bearing an engraved dot-type signature for biomolecular encoding<sup>5)</sup>. These metallic microstructures are photolithographically defined and manufactured in a highly reproducible manner. In addition, the code introduced on the particle face is a straightforward visible feature that is easily recognizable with the use of optical microscopy. The number of distinct codes theoretically could be many thousands, depending on the coding element numbers. Such microparticles are, thus, with appropriate surface organic functionalizations, ideal for encoding biomolecular libraries and serving as a platform for developing high-throughput multiplexed bioassay schemes based on suspension array.



**Fig. 3** Schematic representations of the encoded particles interacting with the microwell surface of the array template on a chip substrate (A). Particles assembled on the chip substrate as a form of ordered-array (B).

As proof of this statement, we demonstrated that encoded microparticles tagged with antibodies to human immunoglobulin classes are capable, using imaging detection as the interrogating approach, of high sensitivity and high specificity, as well as multiplexed detection of the respective antigens in a submicroliter-sample volume.

## 3. Concluding Remarks

We developed the biosensor to be high performance as described above. These achievements will contribute to practical biosensors for medical, environmental and food analyses and open new research areas based on biotechnology linked with micro-fabrication and nano-materials technology.

## Primary Publications

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- (3) Teru Kato, Taro Takemura, Kazuyoshi Yano, Kazunori Ikebukuro, Isao Karube, In vitro selection of DNA aptamers which bind to cholic acid, *Biochimica et Biophysica Acta.*, **1493**, 12-18 (2000).
- (4) Hidenori Nagai, Yuji Murakami, Yasutaka Morita, Kenji Yokoyama, and Eiichi Tamiya, Development of a microchamber array for picoliter PCR, *Anal. Chem.*, **73**, 1043-1047 (2001).
- (5) Zheng-liang Zhi, Yasutaka Morita, Quamrul Hasan, Eiichi Tamiya, Micromachining microcarrier-based biomolecular encoding for miniaturized and multiplexed immunoassay, *Anal. Chem.* **75**, 4125-4131 (2003).

## 2. Causes and Effects of Environmental Loading and its Reduction

### (1) Research Promotion Committee Members

- Motoyuki SUZUKI (The University of the Air)
- Kazuhiro UETA (Kyoto University)
- Toshio OJIMA (Waseda University)
- Tohru KODAMA (The University of Tokyo)
- Mitsuo KOBAYAKAWA (The University of Tokyo)
- Takeshi SHIGA (Osaka University)

○ : Committee Chairperson

### (2) List of Research Project

No.	Research Project	Project Leader
1	Development of Urban and Building Models for Densely Populated Area with Minimized Environmental Load in Hot and Humid Climate	Shinsuke KATO (The University of Tokyo)

# Development of Urban and Building Models for Densely Populated Area with Minimized Environmental Load in Hot and Humid Climate

**Project Leader:**

**Shinsuke Kato**

Professor, Institute of Industrial Science, The University of Tokyo



## 1. Objective:

This research proposes high-density housing models for densely populated urban area in Asia to minimize environmental loads in order to realize a future sustainable society, focusing on the following three basic subjects:

- Minimization of environmental load
- Maintenance/improvement of the quality of the living environment (hardware/software)
- Investigation of the density limit

The project was developed in cooperation with the following three specialized groups;

- Building & urban planning led by Hidekuni Magaribuchi
- Environmental design and evaluation by Shinsuke Kato and Shuzo Murakami
- Structural design and development by Koichi Takanashi

## 2. Summary

### 2.1 Development of porous-type housing models by the space block design method

This project proposes porous-type high-density housing models capable of minimizing environmental load to control environment by a space block design method that makes the best use of the natural potential for environmental control. The space block design method refers to a "space construction method that simultaneously considers substantial portions and voids of a building as the basic cubes (e.g., 2.5m cube)" (Fig. 1-a). The space block design method is also a tool that makes it easy to design buildings by making the space construction unit to a basic cube. This will make it easier to produce a housing form

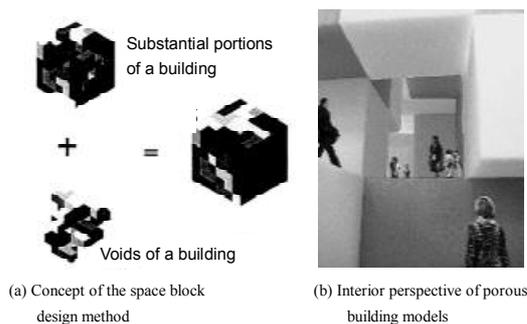


Fig. 1 Space block design method

according to regional characteristics and occupancy levels.

### 2.2 Development of a new environmental load-reductive structural system

A "non-frame SI system" was developed as a new environmental load-reductive structural system. A building frame must have long service life; therefore, the structural members used must not only be highly durable but also suitable for significant usage changes and partial repairs. Such consideration was given to the design of the core tower, utility, and pile draft foundation for the proposed system. The new structural system is a skeleton system constructed with utility floors using members that support only vertical force (rigid connection at bottom and sliding bearings at the top), and is an earthquake-resistant system interconnected with a core tower which bears most of the lateral force during earthquakes, through springs and dampers.

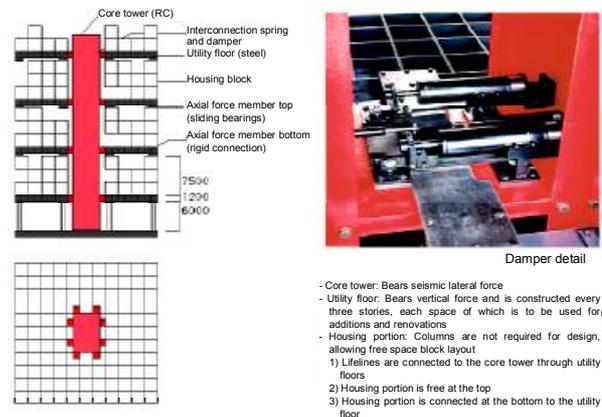


Fig. 2 Non-frame SI system

### 2.3 Proposal for a housing form and environmental control technology under hot/humid climate conditions

Porous building models that actively introduce the outdoor environment into the building interior were studied (Fig.3), and environmental control technologies were studied, after which the environmental engineering effectiveness of the high-density housing models under hot/humid climate conditions was examined. Specifically, (1) the effect of improvement in natural ventilation performance by

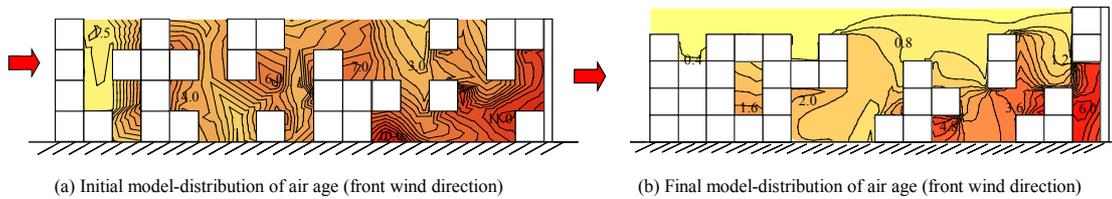


Fig. 3 Analysis of airflow rate for Hanoi experimental house by CFD

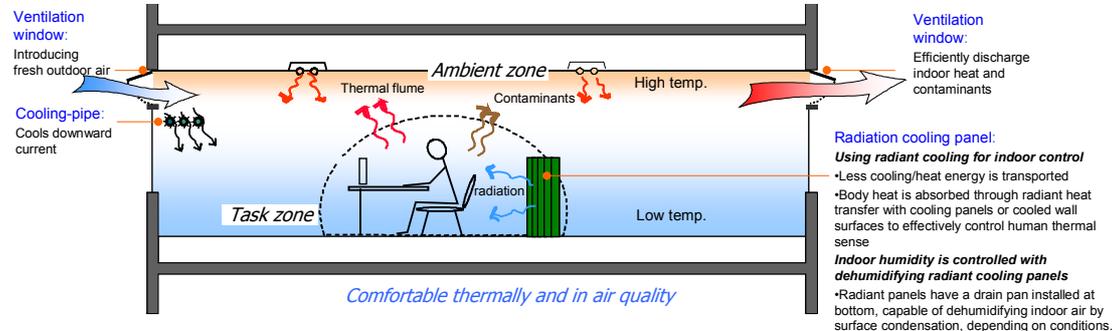


Fig. 4 Environmental control method under hot/humid climate conditions:

porosity on reducing the cooling load and LCCO2 was examined; (2) the effect of the system on reducing cooling load and LCCO2 was examined, proposing a natural ventilation-assisted radiation cooling system (see Fig. 4) as an environmental control technology to reduce environmental load; (3) the effect of introducing other environmental load-reductive technologies, such as solar heat and eco-materials on minimizing environmental load was examined.

#### 2.4 Tokyo and Hanoi models (Fig. 5 and 6)

This project proposes Tokyo and Hanoi models considering characteristics of Asian regions based on research results (1) to (3) noted above. Specifically, an experimental housing complex of more than 500 m<sup>2</sup> in total floor area was constructed in Hanoi, Vietnam to confirm the effectiveness of the high-density housing model developed as results of the project. Furthermore, the Tokyo model, taking into account the possibility of earthquakes and cooling/heating for peak hours during summer/winter, was developed (see Fig. 5). Tokyo and Hanoi model achieved a 30% reduction in LCCO2.



Fig.5 Tokyo model (concept model)



(a) A photo of Hanoi experimental housing (b) Porous space

Fig. 6 Hanoi experimental house

### 3. Concluding Remarks

This project proposes environmental load-reductive high-density housing models for hot/humid climate in Asia, where problems of extreme population density and congestion abound. The housing model proposed in this project significantly reduces environmental load and support healthy human living in large Asian cities. Through the working plans for Hanoi, Vietnam and Tokyo, we put housing models into practical use and confirmed their effectiveness. These models have demonstrated flexibility to respond to the rapidly changing social system, and are highly sustainable and economical.

Note: The project leader in this project was changed from Shuzo Murakami to Shinsuke Kato owing to the change of former leader's affiliation, however Shuzo Murakami looked after the project to the last minute.

#### Primary Publications

- (1) Y. Shiraishi, S. Kato, S. Murakami et al., Research of Environmental Load-reductive Housing Under Hot/Humid Climate Conditions Part 1, Architectural Institute of Japan Collected Papers on Planning No. 558, pp. 15-22, 2002.
- (2) T. Hirano, S. Kato, S. Murakami et al., Research of Environmental Load-reductive Housing Under Hot/Humid Climate Conditions Part 2, Architectural Institute of Japan Collected Papers on Planning No. 566, pp. 87-93, 2003.
- (3) D. Song, S. Kato, S. Murakami et. al, Study on Hybrid Cooling System coupled with Radiation Panel Cooling and Natural Cross Ventilation (part1) Transactions of The Society of Heating, Air-conditioning and Sanitary Engineers of Japan No.87, pp. 61-68,2002.
- (4) Suzuki, Ishii, Morita and Takanashi, Experimental Research of Failure Characteristics in Defective Column and Beam Weld Joints, JSSC Collected Papers on Structure Vol. 6, pp. 149-164, 1999.
- (5) K. Takanashi, Recent Achievements in Substructuring On-line Pseudodynamic Tests in IIS, Stability and Dynamic of Steel Structures, Elsevier, pp. 143-152, 1999.

### 3. Environmental Conservation in the Asian Region

#### (1) Research Promotion Committee Members

- Hiroya KAWANABE (Lake Biwa Museum)
  - Yukiko KADA (Kyoto Seika University)
  - Takeshi KUROKAWA (The Institute of Behavioral Sciences)
  - Masahiro KURODA (Keio University)
  - Satohiko SASAKI (Nihon University)
  - Toshiaki TADANO (Tokyo University of Agriculture)
  - Masanobu HASATANI (Nagoya University)
  - Tsuneyuki MORITA (National Institute for Environmental Studies)
- : Committee Chairperson

#### (2) List of Research Projects

No.	Research Project	Project Leader
1	<a href="#">The Comprehensive Development of Ecosystem-Reconstruction and Sustainable Biological Production in the Southwestern China</a>	Katsuhiko DEMURA (Hokkaido University)
2	<a href="#">A Study on Undesired Effects of Development on Local Societies and Strategies for their Mitigation</a>	Ryutaro OHTSUKA (The University of Tokyo)

# The Comprehensive Development of Ecosystem-Reconstruction and Sustainable Biological Production in the Southwestern China

## Project Leader:

**Katsuhiko Demura**

Professor, Graduate School of Agriculture,  
Hokkaido University



## 1. Objective:

This project was carried out in the karst mountainous area (Qibeilong) in southwest China. The purpose of the project was to discover co-existence principle of the natural ecosystem and human activity in Qibeilong, where forest degradation is severe, and the forest ecosystem has low self-recovery power. Furthermore, the study shows an Asian model based on a new co-existence principle that encourages both natural ecosystems and sustainable human activity, by restructuring damaged forest ecosystems and by raising the productivity of cultivated land.

## 2. Summary

### 2.1 An estimation of human carrying capacity by ecological footprint

“Carrying Capacity” (CC) is a notion of showing a permissible maximum which can be held in the global ecosystem. A technique which was introduced as an index verifying the relationship between this CC and human activity is the “Ecological Footprint” (EF) analysis. EF is an index representing various loads to an ecosystem, caused by human activities, which is shown as a unit of “an area of the land and the sea”.

The EF analysis was carried out in Nongshitun community in the Qibeilong area and the human carrying capacity was estimated. The land area used in Nongshitun community was 79.92 a/ person (Figure 1). The result except the relational undulation of materials from community to community, the land area needed by residents in Nongshitun community (no matter where the location is) was 71.16a/ person. The breakdown of the number as follows: the land area used for food production is 30.68a/ person, the forest land area needed for the assimilation of carbon dioxide exhausted from fuel needed in daily life is 40.28 a/ person. The maximum human carrying capacity in Nongshitun community is 64 people according to the EF analysis based on the land use shown above and the current land area in Nongshitun community. This maximum human carrying capacity exceeds the current population in Nongshitun, which is 94 people. The reasons why the current population is bigger than the maximum human carrying capacity are interpreted as follows: there is an excessive environmental load in the area and an over population caused by bringing things from the outside area. Furthermore, We compared three other communities with different ecosystems, and sought the

human carrying capacity by the land use restructuring in the green space.

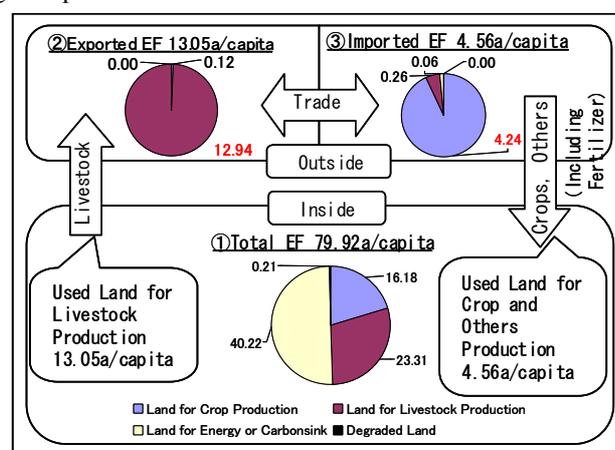


Figure1 EFA Analysis -Nongshitun-

### 2.2 Effects of land use on the vegetation, site condition and the recovery from degradation of a karst landscape in Southwest China.

To clarify the processes and causes of degradation of karst landscape in terms of human disturbances, we compared the land use patterns in four villages showing different levels of degradation. We adopted the vegetation cover on the slopes as the index of degradation. We addressed the following questions: 1) How the pattern of land use has changed during the decrease in vegetation cover? and what causes and what processes are involved in these changes?; 2) Is the degree of change in land use patterns correlated with the vegetation structure such as biomass distribution, species diversity, the dynamics of successional pathways of the vegetation and the site condition measured as soil characteristics. Finally, in order to find a rational strategy for the rehabilitation of this degraded regional ecosystem, we propose a blue print for land use and a feasible methodology for rehabilitation of the area.

Six types of land use were identified: forest, shrubland, grassland, maize field, fields of other crops and bamboo forest. These types included maize fields and grassland, both originating as a result of human activities. As human activities increase, the maize fields and the grassland become more extensive on the slopes. Forest was confined to only a few areas on the upper slope of the depressions. On the middle slope, forest changes to shrubland after tree-felling and subsequent grazing. At the bottom of the depressions, the



# A Study on Undesired Effects of Development on Local Societies and Strategies for their Mitigation

**Project Leader:**

**Ryutaro Ohtsuka**

Professor, Graduate School of Medicine,  
The University of Tokyo



## 1. Objective:

This project aimed to elucidate undesired effects of development on local societies and to seek strategies for their mitigation, based on anthropological fieldwork, with particular interest in:

- (1) Commercial logging of tropical forest in Solomon Islands (R. Ohtsuka et al.),
- (2) Rural development in Hainan Island, China (T. Shinohara et al.), and
- (3) Construction of oil storage stations in Okinawa Islands (T. Matsui et al.).

## 2. Summary

### 2.1 Commercial Logging of Tropical Forest in Solomon Islands

In Solomon Islands, a county of depending on commercial logging to the highest extent in the world, its effects on natural environment and community life markedly differed from place to place. Reforestation in association with clear-cut harvest, which has been widely recognized environmentally sound, was introduced to several areas by the government a few decades ago when the owing right of village land was transferred to the government. Despite increased cash income of the villagers, however, such trials have caused unexpected consequences: in particular, serious clash of logging operation with the **inhabitants' sense of environmental preservation**, excessive number of in-migrants as employees of logging companies, increase of **inter-household difference in economic level** and weakened social solidarity. It is thus difficult for the villagers to make their own development plans. On the other hand, selective harvest deteriorated natural environment but, due to short operation duration, has made it possible for the inhabitants to begin their own replanting in the abandoned sites. In conclusion, the efforts of the government, foreign companies and NGOs should direct to upgrading the villagers' capacity since their own plan of 6-8 years' rotational use of replanted fast-growing trees will provide the present-level log harvest by using less than 10% of the previously-cut forests.



Fig. 1. Eucalyptus groves reforested by a foreign company in Solomon Islands.

### 2.2 Rural Development in Hainan Island, China

In the vast peripheral zone of China where ethnic minority groups have inhabited, the government-led projects of arboricultural cash cropping of rubber and fruit-bearing trees and tourism development have been progressing. The former temporarily increased the inhabitants' cash income but has extinguished their environmentally sound agricultural system, including terraced paddy cultivation, together with managements of long-running irrigation and pasture for cattle used for tillage, which had long been maintained with the inhabitants' **sense of "commons"**. Its gradual collapse has enlarged inter-household difference in income, increased young people's out-migration and increased debt for purchasing machineries and agricultural chemicals, endangering not only **environmental justice** but also the future survival of local communities per se. In contrast, the villagers in a tourism development area have upgraded their living standard by application of their **traditional technologies** for growing edible grasses around paddy fields and small-scale cash cropping and fish culture. Needed for mitigation are pace-down of development and repeated **environmental impact assessment** of the original plans. It is considered that these findings are applicable to other Asian countries such as those in Indochina peninsula.



Fig. 2. A village of Hainan Island, China, under tourism development.



Fig. 3. Oil storage stations constructed in Yokatsu peninsula, Okinawa.

### 2.3 Construction of Oil Storage Stations in Okinawa

Large oil storage stations, which have been beneficial for urban dwellers in terms of their daily living and for the administrative bodies in terms of increase in tax revenues, were constructed in Yokatsu islands, one of the remote areas, by multinational enterprises with administrative approval by the Okinawa prefecture and the local town council. Despite increase in convenience of daily life owing to construction of a long bridge linking with the main island as the compensatory action of the companies and the government, the inhabitants have faced serious situations, particularly **clash of interests** among their various groups, such as the adjacent islands with different interests due to discriminative guarantee amounts and the occupational groups, which were gradually formed or activated through the development project. Serious damage to fishing and other local industries due to frequent oil leakage and excessive increase of young people's out-migration also occurred. Furthermore, as typically seen in transfer of the villagers' cemeteries from their traditional holy places, this development project has impaired their religious beliefs and sense of environmental preservation. These situations, together with the villagers' failure at the lawsuit against the enterprises and the government, have brought "soft resistance" feeling in their mind. The basic causes stemmed from the development planners' lacks of awareness on the unexpected consequences and interim assessment and/or re-adjustment of the original plans. More generally, the nationwide **consensus formation** for development at the sacrifice of the peripheral zone is indispensable even in developed countries like Japan.

### 3. Concluding Remarks

As the major factors damaging local societies, this project identified the development planners' paternalistic attitude and the lack of awareness on unexpected consequences and enlargement of inter-household economic difference as the major factors against environmental justice. It was also revealed that development projects have increased the inhabitants' cash income and convenience of living but do not guarantee their long-term quality of life (QOL) nor matches with their sense of environmental preservation. The most effective way for mitigation of such damages is to support capacity building of the local communities and to increase their participation in development projects. Finally, this project has suggested significant roles of establishment of equity relations between peripheral areas and central areas in each country or the Asian region as a whole in environmentally sound and long-term sustainable development.

#### Primary Publications:

- (1) Ohtsuka, R. [Ed.]: *The Impact of Development on Island Habitat 1: The Solomon Islands: Tropical Forests as the Last Frontier*. University of Tokyo Press (2004).
- (2) Shinohara, T. [Ed.]: *The Impact of Development on Island Habitat 2: The Death of Slash-and-Burn Agriculture on Hainan Island, China*. University of Tokyo Press (2004).
- (3) Matsui, K. [Ed.]: *Cultural Science for Development and Environment: Various Factors on Regional Social Changes in Okinawa Islands*. Youju-shoin (2002).
- (4) Ohtsuka, R.: Environmental preservation and community welfare under development projects: a comparative ethnoecological study in Asian and Oceania. In: Sasaki, T. (Ed.), *Nature and Human Communities*, Springer-Verlag (2004).

## 4. Electronic Social Systems

### (1) Research Promotion Committee Members

- Shigeo TSUJII (Chuo University)
- Tetsuhiko IKEGAMI (The University of Aizu)
- Kazunori ISHIGURO (The University of Tokyo)
- Takaya ENDO (NTT Advanced Technology Corporation)
- Hisatake KATO (Tottori University of Environmental Studies)
- Hiroshi SAITO (Senshu University)
- Kotaro SUZUMURA (Hitotsubashi University)
- Kei TAKEUCHI (Meiji Gakuin University)

○ : Committee Chairperson

### (2) List of Research Projects

No.	Research Project	Project Leader
1	<a href="#">Toward the Establishment of the New Legal System for the Emerging Electronic Commerce — The Strategy of US &amp; EU and the Japan's Role</a>	Minoru NAKAZATO (The University of Tokyo)
2	<a href="#">Legal Models of Information Market in the Near Future</a>	Zentaro KITAGAWA (International Institute for Advanced Studies)
3	<a href="#">Information Technology and the Market Economy</a>	Masahiro FUJIWARA (The University of Tokyo)
4	<a href="#">Foundations of Information Ethics</a>	Masahiko MIZUTANI (Kyoto University)

# Toward the Establishment of the New Legal System for the Emerging Electronic Commerce — The Strategy of US & EU and the Japan's Role

**Project Leader:**

**Minoru Nakazato** Professor, Graduate School of Law and Politics,  
The University of Tokyo



## 1. Objective:

Here is a very brief summary of our research plans we had in mind when we started the project.

- (1) Legal and political analysis of the international competitions in e-commerce among the states.
- (2) Legal analysis of e-commerce from various perspectives.
- (3) Policy proposals on e-commerce for the Japanese government.
- (4) Analysis of the implications of historical background in law in the formation of legal policy in e-commerce.

## 2. Summary

Here is a very brief summary of the conclusions of our research project. More detailed description of the outcome of our study is found in the following book: Minoru Nakazato and Kazunori Ishiguro (eds.), "E-society and Legal System" (Shinseisha, 2002, written in Japanese).

Please note that our study is theoretical and pragmatic at the same time. Thus we not only published papers, but also tried to make a contribution to the governmental policy formation.

- (1) International competition in e-commerce is severe. We should provide clear and efficient legal framework for the transactions.
- (2) We have accomplished detailed theoretical studies in each area of law, on which you could find in the above book of ours.
- (3) Each member tried to reflect the findings and conclusions of our study in the policy formation of the Japanese government through our comments in various advisory committees.
- (4) Various players are trying to take initiatives in the competitions in the area of e-commerce. It is surely important to establish a certain degree of unification, but it is also very important to assure the

independence of each player. For example, in the area of law, it is better to preserve the legal tradition at least to a certain degree.

On the other hand, we could not finish everything we had intended to pursue. We surely have made a certain degree of success in the clearing process of the chaotic situations, but there are still many topics that we could not discuss in details. Please note that there is not an ultimate comprehensive legal framework on e-commerce anywhere in the world. We try to continue our efforts.

## 3. Concluding Remarks

The development of e-commerce will have a great impact on every area of law. In the past, strong arguments were made to show that the traditional legal system would not work at all in cyberspace. In our project, however, we have tried to prove that such argument does not have strong grounds. We instead believe we could modify the traditional legal system and extend it for the future developments.

In the course of our discussions, we have come to notice the important roles played by various social norms in the field of e-commerce. We will continue to make a research on the role of "soft law" in our COE project which started in 2003 following this e-commerce project.

## Primary Publications

Please find the list of major English publications at : [http://www.jsps.go.jp/j-rftf/saishu/h14/f05\\_e.html](http://www.jsps.go.jp/j-rftf/saishu/h14/f05_e.html).

Here is a list of several books written in Japanese that you could find relatively easily:

- (1) Minoru Nakazato and Kazunori Ishiguro (eds.), "Denshi-shakai to Hou-system" (Shinseisha, 2002, 358pp. ISBN4-88384-045-X).

- (2) Mitsuru Iwamura (ed.), “IT-kakumei wo Yomitoku”  
(Gijutsu-Hyoronsha, 2001, 301pp. ISBN4-7741-1320-4).
- (3) Hidetaka Aizawa (ed.),“Denshi-money to Tokkyohou”(Koubundo,1999,324pp.,ISBN4-335-35200-X).
- (4) Kazunori Ishiguro,“Denshi-shakai no Hou to Keizai”(Iwanami,2003,233pp.ISBN4-0002-7041-9).
- (5) Mitsuru Iwamura,“Cyber Economy”  
(Toyokeizaishimposha,2000,316pp. ISBN4-4923-9329-3).
- (6) Minoru Nakazato,“Tax Shelters”  
(Yuhikaku,2002,319pp.ISBN4-641-12911-8).

## Legal Models of Information Market in the Near Future

**Project Leader:**

**Zentaro Kitagawa** Vice Director, International Institute of Advanced Studies



### 1. Objective:

The theory of “law model of the near future” aims at solving problems which do not exist within the framework of modern law, or those important issues which cannot be appropriately handled within this same framework (Zentaro Kitagawa “Law Model of the Near Future” IIAS Selected Book No.5, P51-54, 1999).

The Objective of this research is to construct an intellectual property transaction market model concerning the rights management and dissemination of intellectual property, typified by copyright. It also pursues the “Copymartization” of the product of individual studies. To be more concrete, we carried out basic research as well as applied research of Copymart. As a result, we constructed the IIAS model called the “Academic Information System,” which would integrate the processes and outcomes of these researches. This IIAS model is applicable to further academic information systems at universities and research institutions. The research result report of this project was itself compiled by the application of this IIAS model.

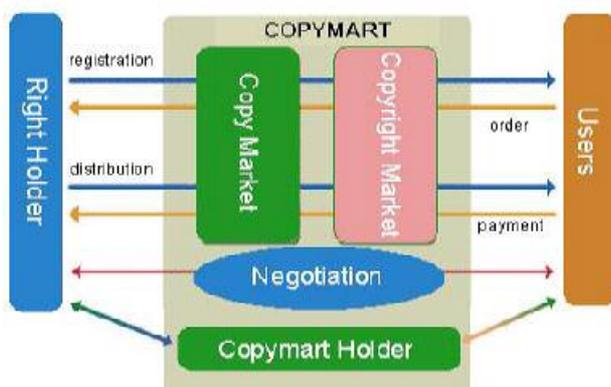


Fig. 1 Conceptual Figure of Copymart

### 2. Summary

(1) The research on a law model of the near future aims at the discovery and identification of critical issues on

information market, as well as the examination of a system for resolving these issues. The issues include a wide variety of topics such as a near-future model of copyright and intellectual property on the Internet, a law model of science and technology (genetic testing, ES cells, etc.), a future model of Asian law, and a future model of information law. We achieved a variety of positive research results from study meetings and symposiums. Such various results demonstrate the importance and diversity of the theories on a law model of the near future. These results have been published under the IIAS Academic Publications.

(2) Copymart is a law model of the near future with the purpose of resolving copyright issues on the Internet. This research project aimed at the construction of this law model. In this sense, copyright, electronic transactions, and information studies in (1) are connected with the basic aspects of law in (2). The construction of Copymart will require fundamental research. In the research on “information value” with regard to a Copymart price, the so-called “proposed equation for Copymart price” was devised in reference with the IIAS Academic Publications. In the research on “Copymart Code,” we devised a Copymart code that could provide meta-data of the “IIAS Academic Information System” that integrates research process with research result. As for the research on “Copymart System,” both an online and a book versions of the IIAS Academic Publications are in operation. “Copymart Technology” is the fruit of analysis on related technologies, and a part of the results has been Copymartized. The research on “Copymart Minimum” examined the criterion for Copymart. This study clarified the versatility of Copymart, and thereby the applied research section of Copymart developed dramatically. In the field of the “IIAS Model of Industry-University Cooperation,” we published Kitagawa, “The IIAS Model of Industry-University Cooperation” (IIAS Report, 2003). This IIAS Model of Industry-University Cooperation has already been

employed in some industry-university cooperation projects. The “Chemical Substance Copymart” achieved the Copymartization of more than 120 research papers regarding polycyclic aromatic hydrocarbons and built a system of providing each substance; it thereby pioneered in developing an expanded Copymart that would integrate products with copyrights. In the “Electronic Publishing Copymart,” we, in cooperation with the Japan Book Publishers Association, sorted out the problems regarding electronic publishing, and examined a business model of publishing Copymart. The “Image and Movie Copymart” attempted the development of a business model of Copymart that deals with audiovisual contents.

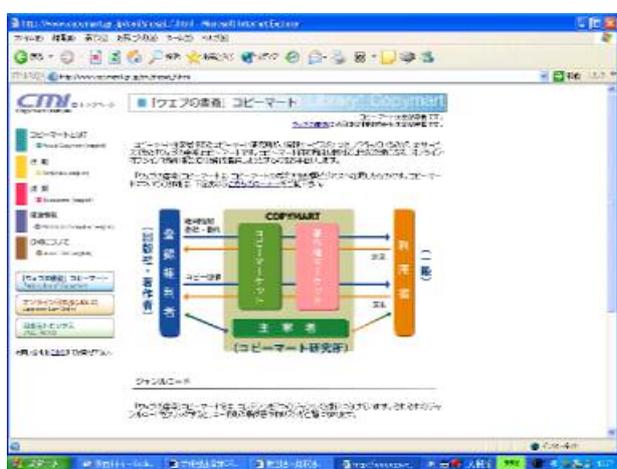


Fig 2. “Web no Shosai” Copymart  
([http://www.copymart.jp/cmi/shosai\\_f.html](http://www.copymart.jp/cmi/shosai_f.html))

The IAS Academic Publications built as a Copymart published over 20 titles of IAS Senso (IAS Selected Books) and IAS reports in book format. In addition, these titles are being provided on the “Web no Shosai” Copymart (Cf. Figure 2).

### 3. Concluding Remarks

As a result of our analysis on various issues with regard to a law model of the near future in an information market, we discovered that each of the issues could constitute a large-scale research topic. As for one such issue, Copymart, a copyright transaction market model, furthermore the IAS Model of Academic Information System was constructed.

The Copymart Model has been influencing copyright management policies and business abroad even before the inception of the project. For instance, Mr. Bruce Lehman, former U.S. Commissioner of Patents and Trademarks, admitted his shock when he found that the Copymart concept was identical with the idea behind the “Information Superhighway”

policy that had been proposed by the Clinton administration. Mr. Lehman says that their policy was influenced in no small measure. Another example is Mr. Ralph Oman, former U.S. Register of Copyrights, who almost overestimates Copymart as follows: “In the book “The New Organum” (1960), Francis Bacon elucidated Nature and established utilitarianism to rule and utilize Nature. Copymart, however, may succeed in what Bacon failed to achieve.” (Kitagawa (supervising ed.), Copymart Study Meeting (ed.), “Copymart: A Copyright Transaction Market on the Internet” (Shinsei-sha, 2003) pp. 196-).

What is important in the future prospects is the versatility of Copymart that is not exhausted by its function as a copyright management system. From this research have grown a variety of types of Copymart applied in various fields such as personal genetic information, technology transfer, and chemical substances. A lively exchange has been underway among experts in and outside Japan, not only among legal scholars but also between researchers in the fields such as natural science and medical science. The versatility of Copymart that was proved by applied research on Copymart has clarified that Copymart will not remain merely an information transaction market of copyright and intellectual property of whatever digital information; Copymart can also serve as a legal infrastructure of an unexplored information market that will integrate information-related products and systems.

### Primary Publications

- (1) Zentaro Kitagawa (ed.) “*Copymart: The Product and Its Prospect*” IAS Forum, 2003.
- (2) Zentaro Kitagawa “*IAS Model of Industry-University Cooperation*” IAS Study, 2003.
- (3) Zentaro Kitagawa “*Copymart: Legal Infrastructure in the Information Society*” Yuhikaku, 2003.
- (4) Zentaro Kitagawa (supervising ed.), Copymart Research Group (ed.) “*Copymart: Copyright Market on the Internet*” Shinsei-sha, 2003.
- (5) Zentaro Kitagawa “*Law Model of the Near Future: A Thought on the Present from the Perspective of the Near Future*” IAS Selected book No.5, 1999.
- (6) Zentaro Kitagawa (ed.) “*Copyright and Business in the Information Society*” IAS Selected Book No.11, 2001.

# Information Technology and the Market Economy

**Project Leader:**

**Masahiro Fujiwara**

Professor, Graduate School of Economics,  
The University of Tokyo



## 1. Objective:

Increasing use of information technology affects functioning of the market economy. By investigating it theoretically and empirically, we tried (a) to predict how the economic system will change in the 21<sup>st</sup> century, and (b) to analyze/ evaluate the desirable policy/institutional changes as well as possible organizational/behavioral strategies to cope with it. We organized seven research projects in the following three research areas.

- (1) Changes of Performance of the Economic System  
(Kiyohiko Nishimura, Noriyuki Yanagawa, others)
- (2) Changes of Consumer and Corporate Behavior  
(Hotaka Katahira, Junjiro Shin-taku, Takahiro Fujimoto, others)
- (3) Economic Analysis of Public System and Policies  
(Katsuhito Iwai, Yoshitsugu Kanemoto, Masahiro Okuno-Fujiwara, others)

## 2. Summary

### 2.1 Changes of Performance of the Economic System

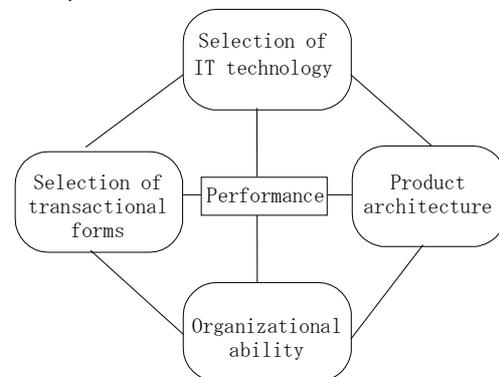
Effects of information technology do not show up in the GDP statistics unless software expenditures are counted as business investments and price indexes that account for quality changes are adopted. We constructed a detailed database, taking account of these points, and showed that the technological innovations in the 90's had negative influences on the Japanese productivity which depended on traditional production method.

We also conducted a case study of Japanese Game Contents industry. Firms in the industry adopted diverse corporate strategies, which depended upon their past experiences. The Industry has flexible division of roles between hardware and software firms, which enabled it to cope with a rapid change in its environment.

### 2.2 Changes of Consumer and Corporate Behavior

Through the analyses of intercorporate information network and 3 dimensional CAD, we demonstrated that whether or not introduction of information technology results in improvement of corporate/industrial competitiveness depends upon fitness between product characteristic and/or transaction forms and information technology, and organizational capability in utilizing the technology.

IT and Organizational Ability, Transactional Forms, Architecture



We also analyzed a change that is occurring in the US automobile distribution by the emergence of online buying service (OBS) firms. The third party OBS, which has no financial tie with manufacturers or dealers, makes it possible for customers to obtain information quickly about a variety of cars with low cost, to make one-stop-shopping without visiting a large number of dealers, maintain neutrality and fairness of information. Disintermediation is not necessarily the outcome of the introduction of the IT technology.

### 2.3 Economic Analysis of Public System and Policies

We examined the telecommunications policy and intellectual property policy from the view of the core commons, the view to separate ownership of infrastructure (spectrum band or literary work) and its use (wireless LAN or fair use of copyright), and allow open access to the latter as commons. We also made public proposal to auction off leasing right of spectrum band.

We demonstrated the needs for reform of business architectures, such as electric bidding system, in the area of e-government. Emphasis was made for the adoption of the Value Analysis that selects policy tools with high benefit/cost ratio. We also examined changes in the collective decision process. The use of IT will enhance, enlarge and deepen the citizens' participation.

### 3. Concluding Remarks

It is important for many firms to develop a superb organizational capacity for manufacturing. For that purpose, it is urgently needed for firms with excellent organizational capacity to further improve their abilities, and transmit their capacities to other firms and industries.

In order to properly understand the diffusion of information technology, an appropriate data reduction is required. Furthermore, reexamination of policies and institutions is recommended after carefully studying the intrinsic changes of economic structures that the propagation of information technology will bring about. It includes the concept of Core Commons in the telecommunications and the intellectual property industries, and the Value Analysis for policy evaluation to create e-government.

#### Primary Publications

- (1) Masahiro Okuno-Fujiwara, Akimichi Takemura, Junjiro Shintaku, eds., *Electronic Society and Market Economy*, Shinsei-sha, pp.1-355, 2002.
- (2) Masahiro Okuno-Fujiwara, "Changes in the Role of Government in the Electric Society"; Makoto Abe, "A Media Planning System for the Broadband Era"; Koichiro Hayashi and Nobuo Ikeda, "Property versus Commons in the Communications Network"; Kentaro Nobeoka, Yoko Takeda and Yaichi Aoshima, "The Influence of 3D-CAD Usage on Product

Development Organization"; Hajime Oniki, "Efficient Utilization of Radiowave Resources---Electronic Society and Market Economy"; Koichi Shimokawa, "The Future Prospect of Internet Automobile Distribution: Revolution and Its Problems"; Hotaka Katahira, "How Has the Internet Changed the Behavior of Firms and Consumers?"; Jiro Kokuryo, "Revenue Models for Information Goods"; Miho Nojima, Junjiro Shintaku, Yoko Takeda and Jiro Kokuryo, "Risk Reduction Mechanism at Internet Shops: A Questionnaire Survey on E-commerce Users"; Noriyuki Yanagawa, "Innovation: Learning from Video Game Industry"; Yoshitsugu Kanemoto, "Information Technology and the Spatical Structure of Cities"; Masahide Horida, "Information and Communication Infrastructure for Political Decision-Making"; Kazuhito Ikee, "Information Technology, Economic Transactions and Organizations"; Kiyohiko G. Nishimura, Kazunori Minetaki, Makoto Shirai and Futoshi Kurokawa, "Information Technology (IT) Innovation: What Do Data Tell?"; Akimichi Takemura, "Value of Personal Information in Electronic Statistical Information and Electronic Money"; Takahiro Fujimoto, "Manufacturing Industries and Role of Information Technology in the 21<sup>st</sup> Century: The Key is Organizational Capability"; in various issues of *Computer Today*, 2001-2003.

- (3) Junjiro Shintaku, Tatsuo Tanaka and Yanagawa Noriyuki eds., *Economic Analysis on Game Industry*, Toyokeizaishinpo-sha, pp.1-380, 2003.

# Foundations of Information Ethics

**Project Leader:**

**Masahiko Mizutani**

Associate Professor, Graduate School of Letters,  
Kyoto University



## 1. Objective:

Information Ethics is one of the newest fields in the field of applied ethics, in the sense that applied ethics deals with and tries to resolve the conflicts between advances in technology and people's life in the modern society. The remarkable development of computer and network technology has given rise to so unprecedented and so diversified a set of ethical and moral problems that attempts at solving these problems undoubtedly will take philosophically focused and technologically well-informed intellectual efforts. The problems we are facing include the ethical status of "unauthorized" uses of computers and networks, the cultural and economic imbalance between groups with different historical, regional backgrounds in the globalized information infrastructure, the new dimensions of human rights like privacy and copyright in the digitized world, and the moral obligations and duties as professionals in information technology. The researchers working on the project are well aware of the urgency and imminence of the tasks and decided to collaborate.

## 2. Summary

The project has aimed at the following targets

- (1) To delineate the intellectual domain of information ethics which is both philosophically consistent and technologically up to date
- (2) To demarcate the domain of information ethics within a wider context of applied ethics, including biomedical ethics and environmental ethics
- (3) To enlighten both ourselves and the intellectual community with the importance of "case studies," which analyze the conflict of interests involved in actual cases and suggest a possibly wide range of measures to take, in the hope that we will generalize over the cases into a generic understanding of the future society
- (4) To propose a specific frame of reference against which relevant problems are analyzed, discussed and resolved in a way that groups with apparently conflicting interest can agree, or at least mutually understand each other

- (5) To create domestic and international interdisciplinary for a in which people of the common interest discuss the relevant issues in light of the cutting the edge technology
- (6) To define the possible method and content of future pedagogical enterprise, both at home and in school, that makes much of the importance of information ethics

## 3. Concluding Remarks

It has been pointed out that the rapid dissemination of information technology in Japan will pose various problems while it provides the people with a great deal of convenience. Under the circumstances, we need not only to build the infrastructures and consolidate the legal system, but to cast a critical eye on them in order to achieve the intended objective of 'good life of our citizens.' It is also necessary for us to ensure in practice that the people as the users of information technology come to acquire information moral. At one of the bases for our project at Hiroshima University, for instance, we conducted research on information-moral education of children underpinning the prosperous information society in the future, critically examined the situations hitherto surrounding such education in Japan, and elucidated what was necessary for our future educational program. These attempts, we strongly believe, were of great significance as researches on the safe and prosperous social foundations. Furthermore, in response to various requests from society, we conducted studies on issues concerning, for example, medical information and privacy in a manner such that we could meet the real needs; we also collected the basic literature on fundamental and important ethical issues, writing papers based on them.

This way we have contributed to the formation of intellectual assets in academic fields.

## 4. Primary Publications

- (1) Masahiko Mizutani, Helen Nissenbaum (eds.), Proceedings of the First International Workshop for Foundations of Information Ethics, Kyoto University, 1999.

- (2) Koichiro Itai, Atsushi Asai, "Clinical Ethics Discussion: Should a physician be allowed to prescribe psychotropic drugs for a delusional patient without explicit explanation regarding diagnosis and treatments?", *Eubios: Journal of Asian and International Bioethics (EJAIB)*12-1, 2002.
- (3) Masahiko Mizutani, James Dorsey, James H. Moor, "The Internet and Japanese conception of privacy", *Ethics and Information Technology*, 6-1,2004.

**Projects Proposed by Advisory**  
**Committee to University-Industry**  
**Cooperative Research Committees**

## 1. Research and Development of New High Damping Materials Based on Atomistic Design

### (1) List of Research Project

Research Project	Project Leader
Research and Development of New High Damping Materials Based on Atomistic Design	Shin TAKEUCHI (Tokyo University of Science)

### (2) University-Industry Cooperative Research Committee

Name of Committee :

133<sup>rd</sup> Committee on Microstructures and Functions of Materials

Committee Chairperson :

Naohiro IGATA (The University of Tokyo)

## 2. Development of Analytical Instruments for 3D Atomic Structure Determination of Surface and Interface by Photoelectron Spectro-Holography

### (1) List of Research Project

Research Project	Project Leader
Development of Analytical Instruments for 3D Atomic Structure Determination of Surface and Interface by Photoelectron Spectro-Holography	Yoshimasa NIHEI (Tokyo University of Science)

### (2) University-Industry Cooperative Research Committee

Name of Committee :

141<sup>st</sup> Committee on Microbeam Analysis

Committee Chairperson :

Michio HIBINO (Aichi Institute of Technology)

### 3 . Research and Development of Ultra-fast and Ultra-high Contrast Ratio Full Color Liquid Crystal Display

( 1 ) List of Research Project

Research Project	Project Leader
<a href="#">Research and Development of Ultra-fast and Ultra-high Contrast Ratio Full Color Liquid Crystal Display</a>	Shunsuke KOBAYASHI (Tokyo University of Science ,Yamaguchi)

( 2 ) University-Industry Cooperative Research Committee

Name of Committee :

142<sup>nd</sup> Committee on Organic Materials Used in Information Science and Industry

Committee Chairperson :

Naoyuki KOIDE (Tokyo University of Science ,Yamaguchi)

### 4 . Research on Ultra Low-power System LSI Technology

( 1 ) List of Research Project

Research Project	Project Leader
<a href="#">Research on Ultra Low-power System LSI Technology</a>	Takayasu SAKURAI (The University of Tokyo)

( 2 ) University-Industry Cooperative Research Committee

Name of Committee :

165<sup>th</sup> Committee on Ultimately Integrated Devices and Systems

Committee Chairperson :

Masataka HIROSE (National Institute of Advanced Industrial Science and Technology)

## 5. Development of Ultimate Precision

### Bio-Nanomanipulator

(1) List of Research Project

Research Project	Project Leader
Development of Ultimate Precision Bio-Nanomanipulator	Atsushi IKAI (Tokyo Institute of Technology)

(2) University-Industry Cooperative Research Committee

Name of Committee :

167<sup>th</sup> Committee on Nano-Probe Technology

Committee Chairperson :

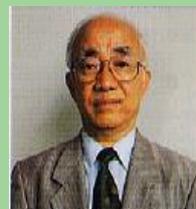
Seizo MORITA (Osaka University)

# Research and Development of New High Damping Materials Based on Atomistic Design

## Project Leader:

**Shin Takeuchi**

Professor, Faculty of Industrial Science and Technology,  
Tokyo University of Science



## 1. Objective:

In the modern society, it is a crucial problem to achieve to control vibrations of materials in solving noise problems, precision positioning-control, fatigue fracture problem, etc., in various fields such as electronic and information machineries, transport industries, nano-technologies, etc. In the present project, in order to cope with such demands from the society and industries, we aimed at:

- (1) development of high-strength (or high-specific strength) and high-damping materials,
- (2) development of high-temperature high-damping materials under high stresses,
- (3) development of characterization methods of high-damping, and
- (4) application of developed high-damping materials to practical purposes.

## 2. Summary

We describe below summary of the results for each purpose (1) to (4) mentioned above.

### 2.1 High-Strength or High-Specific Strength and High-Damping Materials

As high-strength, high-damping materials, we succeeded in developing ① Ti-Ni-Cu and Ti-Ni-Cu-H intermetallic compounds having high-damping capacity of 40%, ② Co-added Fe-Cr-Mn stainless steel with higher than 5% of damping capacity, ③ hydrogenated bulk metallic glasses having amplitude-independent damping capacity of about 10%, all of which have the strength 1GPa or higher. In Fig. 1, we plot the relation between the damping capacity and the strength of the material for the above three materials (plotted by double-circle) together with conventional materials. As high-specific strength and high-damping materials, we developed ④ high-damping Mg-Cu-Mn alloys by powder metallurgy and ⑤ aluminum alloys in which high-damping is achieved by a controlled deformation and annealing process.

### 2.2 High-Damping Materials at High-Temperature (under High Stress)

We have developed ⑥ new ceramics including  $\text{YNbO}_4$  with superplastic character, which show a

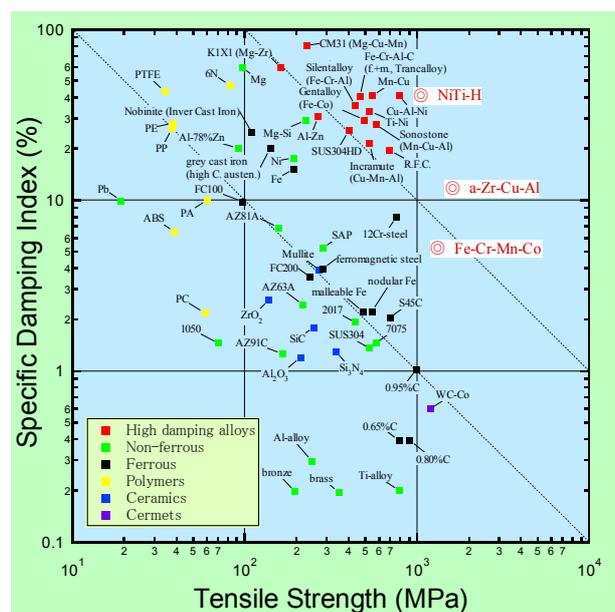


Fig. 1 The relation between the damping capacity and the tensile strength for various materials.

⊙ shows developed material in this project.

high-damping up to 800°C, ⑦ high-damping C/C composite materials by an oxidation procedure, ⑧ high-damping Ni-based cermet which exhibits higher damping than Rene superalloy. We have also discovered new high-temperature, high-damping properties for magnetite and  $\text{LaCrO}_3$  ceramic materials.

### 2.3 Characterization Method

⑨ We have developed a damping measurement apparatus under stress at high-temperatures for the first time in the world (Fig. 2). ⑩ We have established the method of high-damping capacity measurement with an ultrasonic-echo method.

### 2.4 Application of High-Damping Materials to Industries

In the latter half of the project period, the largest effect has been devoted to apply developed materials to practical purposes. With collaboration of 28 companies, trial manufacturing and the test of the manufactured samples have been made for a variety of 37 items for the developed materials. Among them,

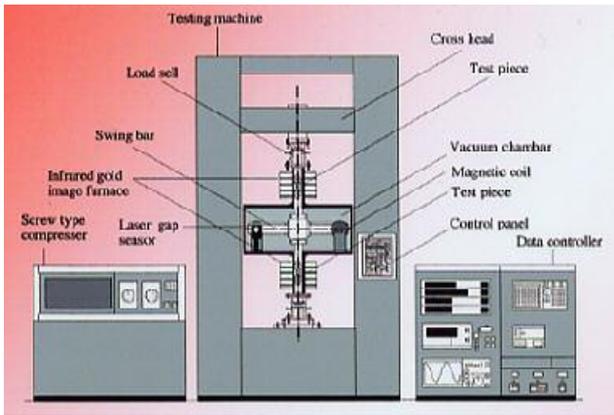


Fig. 2 A new apparatus for damping measurement at high-temperature and under high-stress.

18 items have been found to be successful and 13 items have been found to be effective with some problems remaining. For other items, definite conclusion has not been obtained yet.

The materials used for the tests were Fe-Cr-Mn stainless steel, M2025 (Mn-based alloy), MCM (Mg-based alloy), TiNi-based alloy, cast iron, composite material, etc. Application was expanded to a variety of fields, including audio-machineries, precision machineries, electronic machineries, measurement machineries, transport industries, energy-related industries, etc. Two examples are given below. Figure 3 shows the comparison of the impact number dependence of the binding force of bolt & nut between the conventional steel and the developed high-damping steel, showing the latter does not get loose even under a weak binding. An example of vibration tests of the material for driving arm of magnetic hard disc driver is shown in Fig. 4. The photograph shows the vibration test machine. Below are shown the vibration spectra for NiTi alloy and the developed TiNi-H alloy. Almost all the resonance peaks have disappeared in the latter.

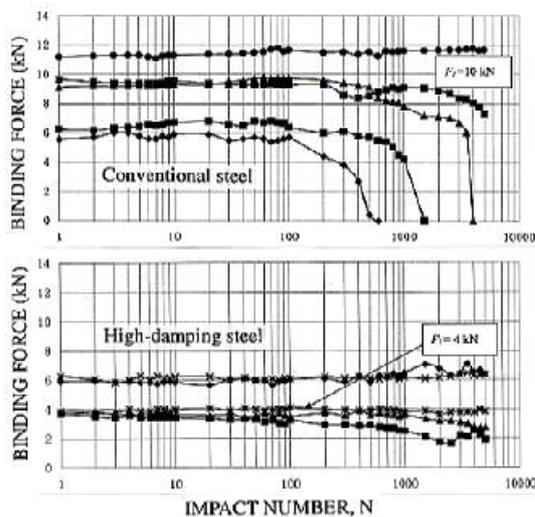


Fig. 3 Impact number dependence of the binding force of bolt & nut for the conventional steel and the developed high damping steel.

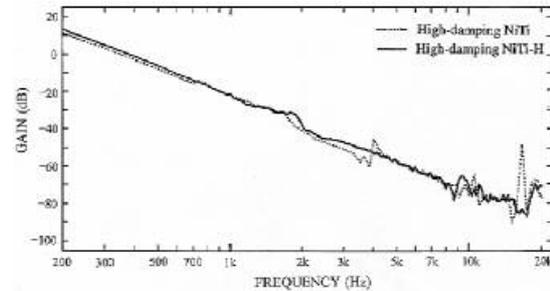
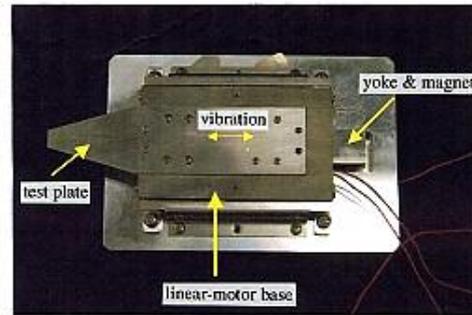


Fig. 4 Vibration testing machine of magnetic hard disc driver arm (above) and the test results for TiNi and TiNi-H high-damping materials.

### 3. Concluding Remarks

In this project, for the purpose of solving vibration problems from the materials side, a variety of high-damping materials have been developed, and the developed materials have been shown to be useful in a variety fields such as precision machinery, electronic machinery, audio-machinery, etc.

### Primary Publications

- (1) H. Okada, H. Sahashi, N. Igata and K. Miyahara: "Effect of  $\epsilon$  martensite and nitrogen on the damping capacity of high strength Fe-Cr-Mn alloys," *J. Alloys & Comp.*, **355** [1-2] (2003) 17-21.
- (2) I. Yoshida, D. Monma, K. Iino, K. Otsuka, M. Asai and H. Tsuzuki: "Damping properties of  $Ti_{50}Ni_{50-x}Cu_x$  alloys utilizing martensitic transformation," *J. Alloys & Comp.*, **355** [1-2] (2003) 79-84.
- (3) K. Nishiyama, T. Abe, T. Sakaguchi and N. Momozawa: "Damping properties of  $YNbO_4-Nb_2O_5-Y_2O_3$  ceramics," *J. Alloys & Comp.*, **355** [1-2] (2003) 103-107.
- (4) K. Nishiyama, M. Keino, Y. Furuyama, T. Sakaguchi and K. Takagi: "Internal friction of boride cermets at elevated temperatures," *J. Alloys & Comp.*, **355** [1-2] (2003) 97-102.
- (5) H. Mizubayashi, S. Murayama and H. Tanimoto: "Feasibility of High-Strength and High-Damping Materials by Means of Hydrogen Internal Friction in Amorphous Alloys," *J. Alloys & Comp.*, **330-332** (2002) 389-392.
- (6) F. Yin, S. Takamori, Y. Ohsawa, A. Sato and K. Kawahara: "Effects of static strain on the damping capacity of high damping alloys," *Mater. Trans.*, **43** [3] (2002) 466-469.

# Development of Analytical Instruments for 3D Atomic Structure Determination of Surface and Interface by Photoelectron Spectro-Holography

## Project Leader:

Yoshimasa Nihei

Professor, Faculty of Science and Technology,  
Tokyo University of Science



## 1. Objective:

Recent improvements to more minute, light-weight, high-density and high-functional products have been accomplished by the advancements of devices and components. However, the progresses in the analytical method on these devices are less promoted than the manufacturing technologies. In particular, there are a few analytical methods to research insides and interfaces of thin-film materials, which these devices are composed of, without destructions. We proposed a novel “Photoelectron Spectro-Holography” method to investigate 3-D atomic structures of these surfaces and interfaces. Researches and developments have been performed as follows:

- (1) Development and Instrumentation of a novel analytical method based on photoelectron diffraction and holography,
- (2) Practice of material analysis using this method and apparatus of “photoelectron spectro-holography”.

In particular, we will achieve not only the development of the method but also the instrumentation. This means that we should present the standnd alone apparatus for the developing analytical method to end users. This novel method and instrument enables us to give the world standard and to indicate the leading edge of analysis on the devices. The summary of our results are as follows.

## 2. Summary

### 2.1 Development of “Photoelectron Spectro-Holography” method and instrument

The combination of fundamental analytical methods and apparatuses we have developed originally, realized the novel analytical method and instrument for 3-D atomic structure determination of surfaces and interfaces, i.e. “Photoelectron Spectro-Holography”. Details are as follows.

#### (1) Multi energy high intensity X-ray source

Although synchrotron radiation (SR) is well known as an advanced and extremely powerful light source, most of SR facilities are much larger than several tens meter. We newly developed a small (~1m) but high

intensity X-ray source with the same intensity to SR X-ray source for using in laboratories. This apparatus can provide 3 high intensity X-ray sources with different energies. Figure 1 shows X-ray photoelectron diffraction (XPED) patterns (holograms) of a thin film composed of light elements by using two sources. Using different X-rays, we can easily obtain structural information from thin films and substrates individually.

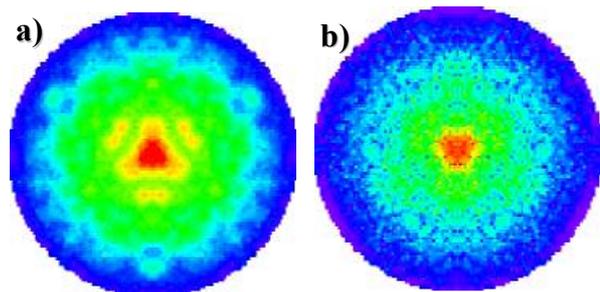


Fig. 1 XPED patterns (holograms) from a thin film composed of light elements excited by (a) conventional Al-K and (b) lower energy Cr-L sources. Different X-rays can separate structural information on films from that on substrates.

#### (2) High throughput, high energy and high angular resolution electron analyzer

The direction and energy of photoelectrons, which are emitted from a surface excited by X-ray, brings much information on the structure and state of the surface. If we can detect more precise XPED pattern with a higher throughput, more precise structure of surfaces and interfaces can be elucidated. The novel

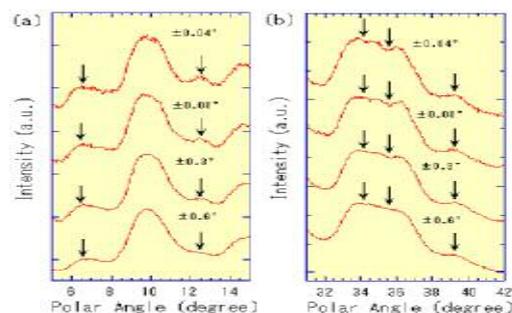


Fig. 2 In high angular resolution of XPED patterns, fine features appear more clearly with improving the angular resolution.

electron analyzer system realizes these kinds of analysis. We also developed the detector for high speed and high energy photoelectron measurements. Highly precise measurements using this apparatus can reveal fine features in XPED patterns.

### (3) Differential photoelectron holography

In holographic method, the interface between direct wave (reference wave) and scattered wave by the object (object wave) can be used to reproduce the image of the object. However, photoelectron holography is more difficult than general holography using lights because strong forward scattering peaks of photoelectrons in holograms disturb the image reproduction. Thus, summation of several holograms has been also performed but the reproduced images are still distorted. We found the difference between only two holograms can reproduce the atomic images more clearly and precisely without distortions. Using this method and improved one, we can easily obtain fine and precise 3-D atomic images.

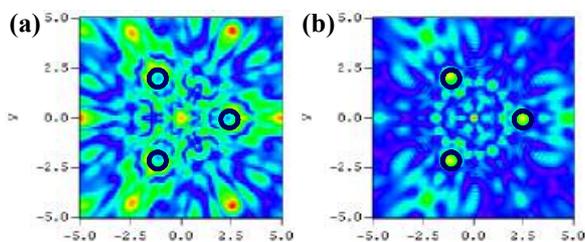


Fig.3 Reproduced atomic images by (a) two-wave photoelectron holography and (b) differential photoelectron holography. Open circles represent the positions of atoms.

## 2.2 Application to practical material analysis

Using the "Photoelectron Spectro-Holography" method and instrument, we carried out the structural analysis on several thin films.

There are some materials of which mechanisms are not perfectly understood although we generally used those. To progress advanced research and development of such materials, we need to fully understand the mechanism. Zr-O/W(100) surface is such kind of material. This material is widely used as sources of electron microscopes etc., where adsorbing Zr-O on the surface of conventional W filaments realizes a high brightness electron source. Because it is difficult to analyze at the working temperature of 1400 °C, the mechanism is not elucidated perfectly. Thus, we carried out structural analysis on this surface at the

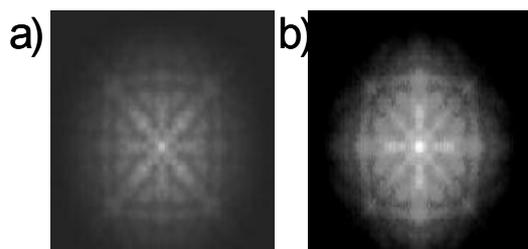


Fig.4. Holograms from a W substrate of the material for a high brightness electron source measured at (a) room temperature and (b) near working temperature (1330 °C). Fine features in holograms show high precise measurements are carried out at high temperature.

working temperature in order to elucidate the mechanism. These measurements promote not only to reveal the features of practical materials but also to present the guidance to new materials which we need in future. We also developed the methods to obtain the precise cross-sections of devices using focused ion beams and reactive gasses.

## 3. Concluding Remarks

- (1) We developed a novel multi-energy high intensity X-ray source in laboratories, with the same photon intensity as that of the 2nd generation SR.
- (2) High angular resolution up to  $\pm 0.04^\circ$ , which is 100 times as high as those of conventional high angular resolution measurements, has been realized by developing the high throughputs, high energy and high angular resolution electron analyzer system with the novel diffraction plane aperture input-lens. We also accomplished high speed and high efficient detector of photoelectron.
- (3) Differential photoelectron holography reproduces 3-D atomic images more precisely and clearly without distortions, where we need only two holograms. We also improved this method to obtain more precise atomic images.
- (4) The combination of these methods and apparatuses realized the "Photoelectron Spectro-Holography" method and instrument. This system realizes high performance analysis, such as high precision, high angular resolution, high speed up to 1 ms and high energy measurements.
- (5) Using this instrument, we investigated several thin layer materials, such as the material for high brightness electron sources at near their working temperature (1400 °C). In order to establish the 3-D analysis on practical devices, we are also developing the chemical assisted high-speed and low-damage microbeam etching system.
- (6) Through our research and developments, we applied for and got rights of 5 patents. We also promoted and contributed the education and training for young researchers and technician in both universities and companies.

## Primary Publications

- (1) S. Shiraki, H. Ishii and Y. Nihei: "Design of a high angle-resolving electron energy analyzer," *J. Electron Spectrosc. Relat. Phenom*, **113**, 199-205 (2001).
- (2) S. Omori, Y. Nihei, E. Rotenberg, J. D. Denlinger, S. D. Kevan, B. P. Tonner, M. A. Van Hove and C. S. Fadley: "Differential Photoelectron Holography: A New Approach for Three-Dimensional Atomic Imaging," *Phys. Rev. Lett.* **88**, 55504-1-4 (2002).
- (3) Y. Nihei: "Determination of 3D atomic structure of surfaces and interfaces by photoelectron holography," *Surf. Interface Anal.*, **35**, 45-50 (2003).
- (4) H. Ishii, S. Shiraki, K. Tamura, W.-G. Chu, M. Owari, R. Shimizu and Y. Nihei: "Development of the Novel Instrument for X-ray Diffraction and Holography," *Surf. Rev. Lett.*, **10**, 505-510 (2003).

## Research and Development of Ultra-fast and Ultra-high Contrast Ratio Full Color Liquid Crystal Display

### Project Leader:

**Shunsuke Kobayashi**

Director, Liquid Crystal Institute,  
Tokyo University of Science Yamaguchi



### 1. Objective:

This research has been conducted with the aim of realizing a full color liquid crystal display exhibiting ultra-fast response and ultra-high contrast ratio using ferroelectric liquid crystals; and the contents of the research are:

- (1) To fabricate zig-zag defect free ferroelectric liquid crystal displays (FLCDs) based on the theoretical research, material science and cell fabrication technique.
- (2) To realize FLCDs exhibiting continuous V-shaped and H-V-shaped switching using side chain type mesogenic polymer-stabilization that are called PS-V-mode FLCD and PS-H-V-mode FLCD.
- (3) To realize good switchability of our PS-V-mode and PS-H-V-mode FLCD with TFTs and good reliability and image sticking free for a long-term operation.
- (4) Finally to demonstrate field sequential full color LCDs taking advantage of the fast switching of our PS-V-mode FLCD and PS-H-V-mode FLCD.

### 2. Summary

#### 2.1 Realization of high contrast ratio of 700:1 in terms of fabrication of the defect-free FLCD.

FLCD was invented by Clark and Lagerwall in 1980 and it attracted researcher's interest for its fast response and bistability; however it has passed 17 years until

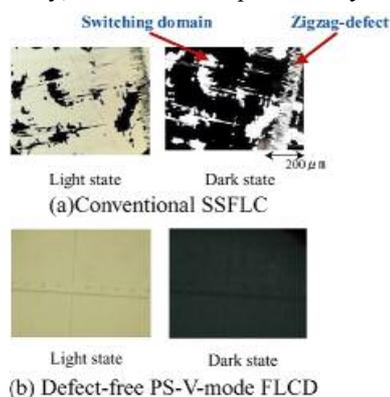


Figure 1(a) Textures of zig-zag defects and switching domains in a conventional FLCD cell; (b) textures of defect-free PS-V-mode FLCD

when our research group invented a method for fabricating zig-zag defect-free FLCD. This was done through theoretical research material science research and cell fabrication technique; actually we discovered that surface topography of the alignment layers gives an influence on the generation of zig-zag defects and we have shown that polyimide RN-1199 (Nissan Chem Ind.) is useful for this purpose. Besides rubbing technique, we investigated photoalignment and ion beam alignment and we showed that they are also useful. Figure 1 (a) shows defects and a domain appearing in a conventional FLCD and Figure 1(b) shows a texture of defect-free FLCD cell.

#### 2.2 Invention and realization of PS-V-mode FLCD and PS-H-V-mode FLCD with continuous grayscale operation

Conventional FLCDs are featured by their fast switching speed and bistability, but it is impossible to operate them in gray scale operation. To solve this difficult problem, we devised a method of polymer stabilization using mesogenic side chain polymers, where we apply an AC field to a cell during a photocure to fabricate an FLCD with V-shaped switching and apply a DC field to fabricate FLCD with half V-shaped switching; they are called PS-V-mode FLCD and PS-H-V-mode FLCD. For this purpose, we used photocurable monomers synthesized by Dainipon Ink and Chem Ind.

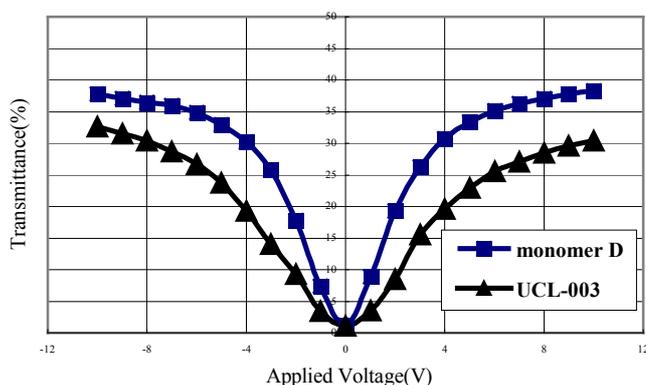


Figure 2. V-T curve of a PS-V-mode FLCD

### 2.3 Realization of good Switchability of PS-V-mode FLC and PS-H-V-mode FLC with TFTs

FLCDs developed in this research project are featured by good switchability with TFTs and high reliability and a image sticking free for a long term operation. While, other V-mode FLCDs reported by several groups are lack in the TFT switchability and in the reliability for a long term operation. Besides, our PS-V-mode FLC and PS-H-V-mode FLC, we also investigated an intrinsic H-V-mode FLC without adopting polymer stabilization, and it is shown that its reliability for a temperature cycling test was good but its reliability for a long term operation is insufficient.

### 2.4 Demonstration of field sequential full color LCDs

We succeeded in demonstrating field sequential full color LCDs taking advantages of our PS-V-mode FLC and PS-H-V-mode FLC such as their fast response characteristics and wide viewing angle with the specification of VGA and XVGA. Figure 3 shows an example of FS-FC LCD.

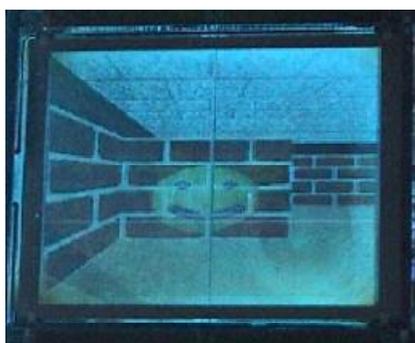


Figure 3. An example of field sequential full color LCD using PS-V-mode FLC with the specification of VGA.

## 3. Concluding Remarks

We have succeeded in fabricating zig-zag defect free FLCDs and also PS-V-mode FLC and PS-H-V-mode FLC that exhibit high contrast ratio of 700:1, fast switching with the time constant of  $300\mu\text{s} \sim 500\mu\text{s}$ , continuous grayscale operation, good switchability with TFTs, good reliability and image sticking free for a long term operation. We also succeeded in demonstrating field sequential full color LCDs with the specifications of VGA and XVGA. Residual problem is to have a good FLC material having switching angle of  $\pm 30^\circ$  or more to realize a high luminous level in a PS-V-mode FLC.

## Primary Publications

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# Research on Ultra Low-power System LSI Technology

## Project Leader:

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The University of Tokyo



## 1. Objective:

In recent years, various electric devices are abundant in our daily life and the shift has clearly started to a highly advanced information society. What is needed in this environment is an advanced technology with which anyone can handle various types of information, such as video and audio data, anywhere and anytime. Large scale integration (LSI) is a component used as the base for realizing such an information infrastructure, and is a very important technology determining the performance of all information devices and equipments. In this context, an LSI is not only high speed and highly functional but also it should be low power so that it can be used by a battery in mobile environments.

It is necessary to integrate memory and logic on one chip in order to meet the needs for small size and low cost. The status quo, however, has not reached the status where we can have solid knowledge and technical base on which we can realize the demanded high-speed yet ultra low-power LSI's. The purpose of this project is to establish a design guideline for realizing future ultra-low power and high-speed new system-on-a-chip. It can only be achieved by multi-disciplinary collaboration such as among industry-academia and among device, circuit, software and systems researchers. The collaboration is also needed among international entities and systematic understanding for realizing the low-power system-on-a-chip is a must. In order to cut down the power consumption by more than two orders of

magnitude, it is necessary to cultivate a new continent of design space of 0.5V operation voltage.

## 2. Summary

### 2.1 A 0.5V voltage hopping processor

The most effective method in cutting down the power consumption of LSI is lowering power supply voltage. However, the speed of circuit will be degraded significantly in the low VDD region. In this project, a voltage hopping system where the operation voltage of an LSI are changed between high supply voltage and low supply voltage adaptively to the workload was proposed. Power consumption was shown to be reduced to 1/3 with this mechanism. Since existing processor can not be operated at sufficiently low supply voltage, a high-speed processor which can be operated at 0.5V supply were design and manufactured to check the feasibility of the proposed voltage-hopping scheme.

It has been demonstrated that the voltage hopping processor not only shows high-speed and low dynamic power operation but it is also shown that the processor can cut down the leakage power significantly through the DIBL (Drain Induced Barrier Lowering) effect. Therefore, it turns out that the voltage-hopping scheme will be promising in the near future. For further future, we have to adaptively change the threshold voltage itself through a back-gate bias and for that purpose, VTH-hopping scheme has been also proposed and shown to be effective by using a test chip.

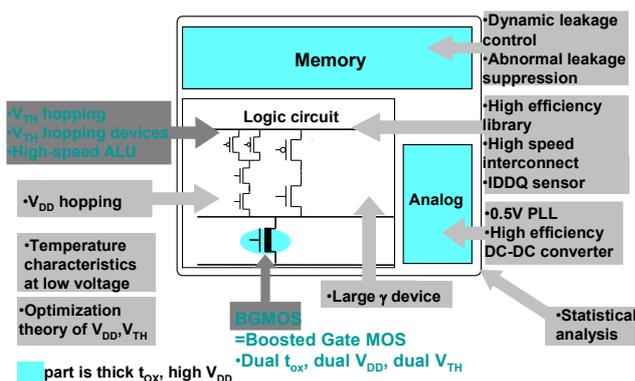


Fig.1 Proposed technology and theory

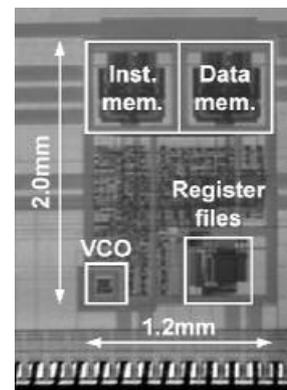


Fig.2 0.5V high performance processor

## 2.2 Low leakage memory

Power increase by leakage current is becoming a serious problem as device is scaled down and supply voltage is also scaled down. Thus coping with the leakage power is a subject of ultimate importance, especially for memory component of system-on-a-chip. In memory, most of the memory cells are dormant and leakage is more important than the logic part.

Two different approaches for reducing the leakage in memory blocks are proposed and verified. One is a dual-VDD system where a memory part is designed by high supply voltage while logic part is designed by low supply voltage. The other is a row-by-row control system where VDD and VSS of accessed memory cells are dynamically changed. It was shown that both effectively reduce the leakage by two to three orders of magnitude.

## 2.3 Collaboration with device and circuit

In order to reduce power consumption in a stand-by mode, we proposed a new device and circuit scheme named called Boosted Gate MOS (BG MOS) that drastically suppresses the standby leakage current for sub-0.1 $\mu$ m generation while maintaining circuit speed. By applying boosted gate voltage to a power-gating switch which is made with higher threshold voltage and thicker oxide, extremely low stand-by power is achieved. This is an good example of the outcome of collaboration with device and circuit researchers.

## 2.4 Intra- and inter-chip model of variation

LSI process is an integration of hundreds of individual process steps, each of which has certain process variation. This inevitably influences device characteristics. We found that distribution of the variability spreads in scaled down devices for high speed and low power.

ISSCC	6
SSDM	5
ISLPED	6
CICC	4
VLSI Symp.	5
ISQED	2
ICCAD	2
DAC	2
ASPAC	7
ISCAS	4
ISPD	3
SASIMI	3
others	26
TOTAL	75

JSSC	4
JJAP	8
TVLSI	1
TCAD	3
TED	2
IEICE Trans.	12
others	9
TOTAL	39

Fig.3 Publication at international conferences and in journals

In an LSI, added to the process variation, temperature and supply voltage vary. This would worsen the device variation, and enough design margins are needed. We have developed a technique to separate these intra-chip and inter-chip variation, and both variations have been modeled to be used in the design with variation.

## 3. Concluding Remarks

The results of this project would contribute to the semiconductor industry which is now in the leading position in manufacturing industries and which provides components other industries are based upon. Through the achievement of this project on ultra-low-power system LSI's, we believe more people in the world can realize more secure and more convenient life ever.

This was the first project where circuit and device researchers are teamed up together, sharing the same goal of the low-power LSI's. The success of this project will open up a new horizon for future ultra-low-power LSI systems.

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## Development of Ultimate Precision Bio-Nanomanipulator

### Project Leader:

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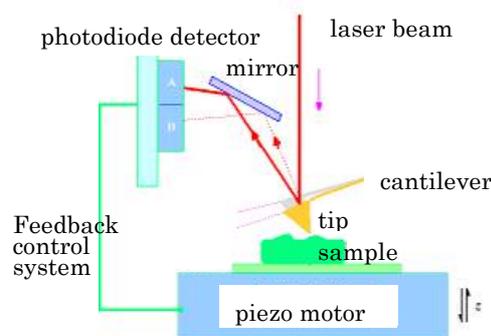
### 1. Objective:

The aim of this project is to develop methods to measure mechanical properties, such as hardness and softness of proteins, DNA, chromosomes, cell membranes and cells that constitute biological structures and to cultivate processes of artificial manipulation of bio-macromolecules and bio-structures. Establishment of reliable methods of such manipulation is of essential importance as the basic technology in the gene therapy and regenerative medicine of future and also in replacing drug tests involving a large number of animals with those using cultured cells.

### 2. Summary

#### 2.1 Measurement of Mechanical Strength of Protein Molecules at the Single Molecular Level

For the development of nano-technology of tomorrow, technological advancement in handling and assembling at the level of single atoms and molecules is vitally needed. In bio-nanotechnology where functions of bio-macromolecules will be extensively used, artificial proteins, DNA, polysaccharides, lipid membranes are anticipated. For this purpose, information on the mechanical properties of bio-macromolecules will be vitally needed. This is understandable from an analogy with the construction processes of macroscopic machineries.



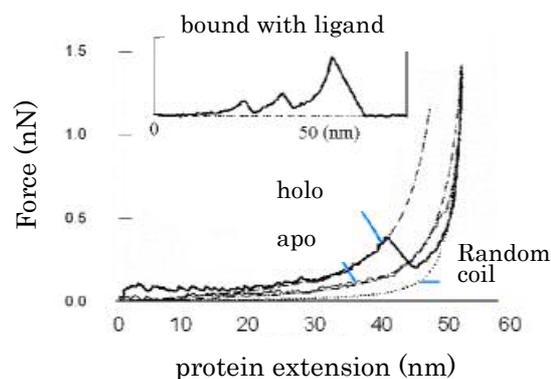
**Figure 1.** Basic construction of AFM. Laser beam reflected from the back of the cantilever detects the latter's deflection due to tip-sample interaction

For the basic material of construction, not only thermodynamic properties such as melting and freezing temperatures, specific heat, but also mechanical properties such as elastic modulus associated with bending and stretching of the material are needed. This project aims at supplying molecular basis of such studies.

First, we stretched polypeptides with helical conformation, calmodulin, carbonic anhydrase, and OspA from their two termini by the atomic force microscope (AFM, Figure 1 illustrates basic working principle of AFM) after covalently immobilizing each of them between the tip and the substrate of the AFM. Figure 1 shows the basic construction of AFM.

#### 2.2 Mechanics of Calmodulin Stretching

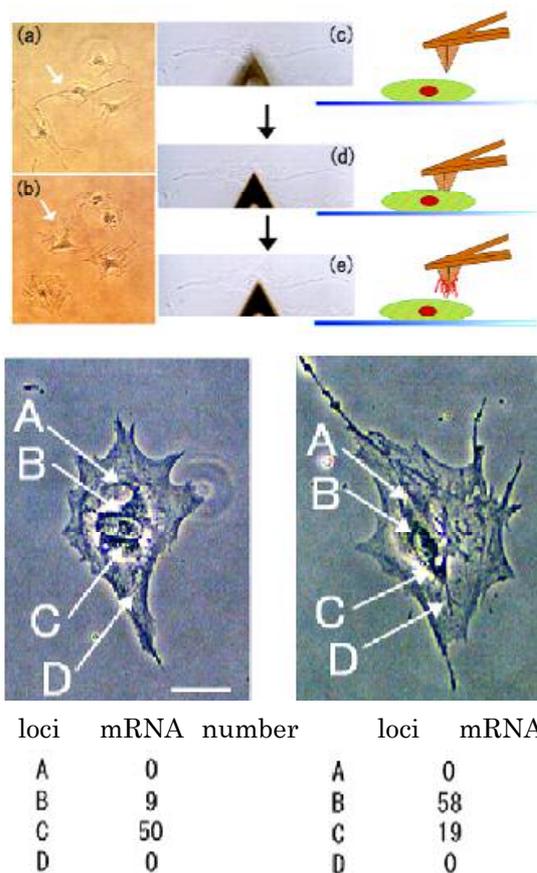
Calmodulin is a protein with MW=16,000 and 148 amino acid residues and has an extensively developed helical conformation. It is a good example to study mechanical properties of helical proteins. Calmodulin was sandwiched between the tip and substrate of AFM and stretched from its N- and C-termini. The result of stretching is given in Figure 2 as the relation between tensile force (ordinate) and the extension of the molecule (abscissa). There is little difference in helical content between  $\text{Ca}^{2+}$  bound form (holo) and unbound form (apo), and therefore, the marked difference in their mechanical properties shown in Fig.2 was considered to be due to the effect of  $\text{Ca}^{2+}$ . The force peak observed in the force curve of  $\text{Ca}^{2+}$  bound form is probably due to the release of  $\text{Ca}^{2+}$  ion.



**Figure 2.** Result of mechanical stretching of holo and apo calmodulin.

### 2.3 Extraction, identification and quantitation of mRNA from a single live cell.

When an AFM tip is inserted into a live cell and pulled out, many molecules are recovered from the inside of the cell by simple adsorption to the tip. For mRNA molecules among them, RT-PCR method can be used to artificially multiply chosen fragments. By this method, mRNA for actin was detected with reproducibility of 96%. Fig.3 shows the schematics of the method (top) and the result of quantitative recovery of actin mRNA from four different loci of two cells.

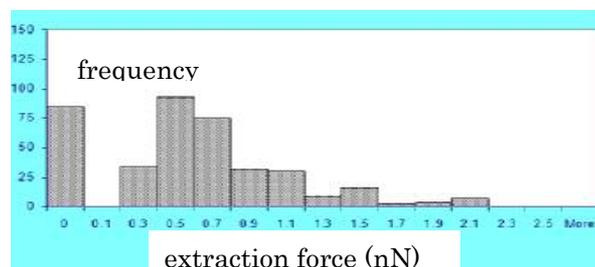


**Figure 3. Result of extraction of  $\beta$ -actin mRNA from cytoplasm of living cells. (Top) Schematics of the extraction procedure. (a),(b) cells under optical microscope, (c), (d), (e) approach of AFM tip to a living cell, with cartoons showing harvest of mRNA (red) (Bottom) Result of quantitative RT-PCR amplification of extracted mRNA.**

### 2.4 Extraction of intrinsic membrane proteins

On the biological membrane, extrinsic proteins associated to the membrane primarily through electrostatic interaction, and intrinsic membrane proteins encompassing the membrane reaching the inside of the cell. Among these membrane proteins, we are interested in so-called intrinsic membrane proteins some of which function as receptors and ion channels. We developed a method to collect such intrinsic membrane proteins on an AFM tip. First, an AFM tip is modified with covalent cross-linkers and it was brought in contact with the surface of a live cell. The cross-linker then forms covalent bonds with amino groups on the membrane proteins. After formation of

the bonds, the tip was separated from the cell surface and during this process intrinsic membrane proteins were extracted from the cell surface because the non-covalent interaction anchoring the intrinsic proteins to the membrane is weaker than the covalent bonds.



**Figure 4. Histogram of force required to extract intrinsic membrane proteins from living cells.**

### 3. Concluding Remarks

The method described in this report will be developed as important technology for the artificial manipulation of live cells and cell biology in general. For a wide use of the AFM based method in cell biology and medicine, it is necessary to develop a devoted instrument for cell manipulation which is easy to use and less costly compared with a general purpose AFM instrument of today.

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