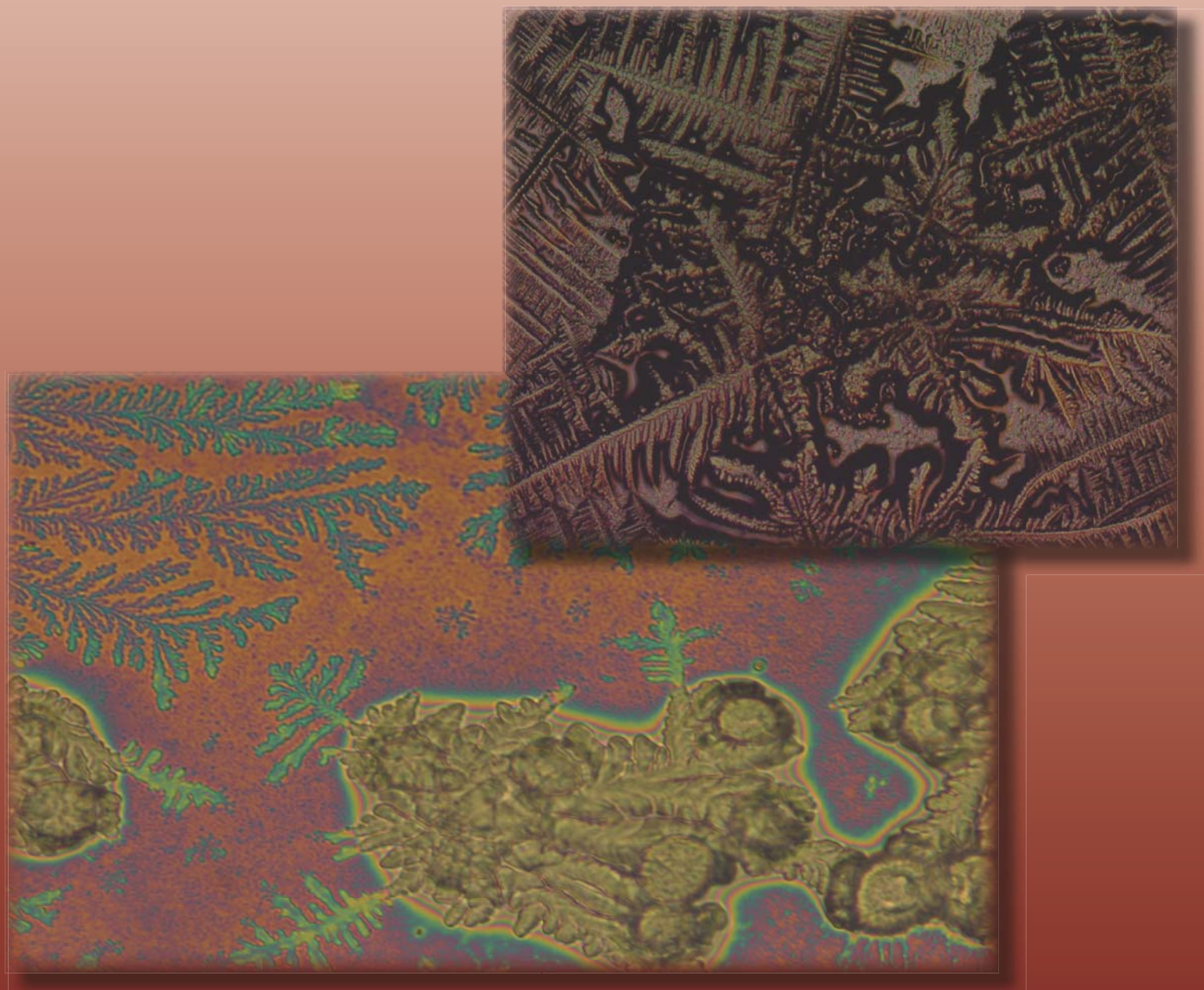
KIST Europe

Korea Institute of Science and Technology

Annual Report 2012



Illustrations (photos taken at KIST Europe):

Cover:
Cancer Cells in lysine buffer on silicon chip surface

Back:
Cancer Cells in lysine buffer on silicon chip surface
Chip device for free flow electrophoresis (FFE), microfabricated from silicon

Publisher:

KIST Europe
Korea Institute of Science and Technology

Korea Institute of Science and Technology (KIST)
Europe Forschungsgesellschaft mbH

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03/2013

Preface - Greetings of the Institute Director

Dear readers,

My name is Ho Seong Lee. I have been working as the 6th Director of the Korea Institute of Science and Technology Europe (KIST Europe) since September 2012.

In this position, I advocate a new vision and a managerial philosophy which can be explained with "Four Cs".

The first C stands for Communication. There is no doubt about the importance of good communication skills, but even more important is the will to communicate. Therefore the communication channels among the members as well as between me and the members shall always be open and thereby grant free communication and ban isolation.

The second C is for Cooperation; and strengthening the cooperative ties between Korea and Europe was the ultimate reason that KIST Europe was established in Saarbrücken in 1996. To succeed in this, it is necessary to establish cooperation among the members of KIST Europe and I'll commit full effort in creating an environment and atmosphere where cooperation between person-to-person, organization-to-organization, and nation-to-nation will be achieved.

The third C stands for our Clients who are affecting us directly as well as indirectly, who have impact on our identity, who deserve all our attention, for whose benefit we work and whose trust we gain.

The last C means Challenge. The research itself is like taking the road less traveled before. And people are often afraid when they are uncertain of what lies ahead or what will occur in the future. However, we have to overcome this kind of fear and challenge the uncertain future continuously.

On the following pages you can find our research results of the past year. And it's my pleasure to present this annual report, containing the most notable events of the year 2012, along with a selection of our scientific highlights such as an overview of our research output and the publications in international scientific journals.

It is not only about looking back to the year 2012; but it's about looking toward our future as well. We bravely take on challenges that will change the world.

Lastly I gratefully acknowledge the Korean government and KIST Korea for their continued sponsorship and their support. I am also thankful to the Saarland government and the Saarland University for their warm interest and support.

Prof. Dr. Ho Seong Lee
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Greetings of the Research Director

Dear readers,

I am very proud to present the research activities and achievements of KIST Europe during 2012 to you in this annual report.

The year was dominated by the loss of both group leaders, Dr. Chang-Hoon Nam and Dr. Jörg Ingo Baumbach, and by a redefinition of the overall research theme.

This process is ongoing and will need our attention for parts of 2013. The scientific direction is still along the lines of converging biological sciences and engineering, or more specifically, develop technology and biotechnology for applications including infectious disease, cancer and drug discovery. The bibliometric research output continues to improve, and is unlikely to suffer from the above mentioned changes.

On the front of infrastructure, we are happy to possess now a high-safety biological laboratory (class S3*, infectious disease, 50m^2), a new electron microscope, a scanning confocal microscope (Nipkow disk based) and several new chip manufacturing instruments (CVD for silane and thiol based surface chemistry, XeF_2 etching for silicon substrates).

We are all very happy with the laboratories here at KIST Europe, and the opportunity to work here. We try to do our best to promote collaborations between Korea and Europe, to establish independent high-quality research, and to get involved in local academic education.

I would like to acknowledge the generous funding by the Korean government, the goodwill by the Saarland University and many of its faculty members, as well as to the co-workers and everybody else involved in or supporting our research.

Prof. Dr. Andreas Manz
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Contents

KIST Europe - Introduction

The Korea Institute of Science and Technology Europe Forschungsgesellschaft (KIST Europe) was established in 1996 in Saarbrücken, Germany, as an overseas branch of the KIST in Seoul, Korea.

A decade ago, at a time when the globalization of science and technology was a new concept, the KIST Europe was the first and only Korean government commissioned R&D institute abroad with R&D capability in its own right.

Since then, the KIST Europe has endeavored to build global S&T networks with prominent EU research institutes in the field of basic and application oriented researches.

Its partners are research institutes and industrial companies in Europe and Korea. Together with its partners, the KIST Europe solves problems and develops technologies which can be utilized on both continents. Its vision is to be the core of scientific and technological cooperation between Korea and EU countries.

The KIST Europe has grown from the small seed sown 17 years ago, and is now preparing for another powerful leap forward after the construction of a 2nd research building and a high-safety biological laboratory.

Over the next 10 years, the KIST Europe plans to further accelerate cooperation between Korea and EU countries.

By the end of the next decade, KIST Europe aims to be one of the most respected and top-quality R&D institutes recognized by EU community.

Facts & News

November 19, 2011 - February 07,2012

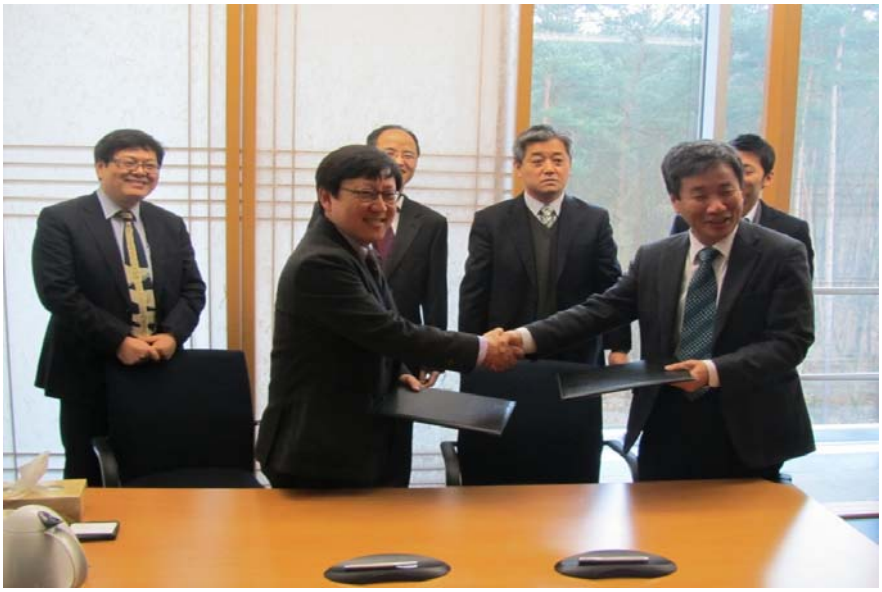
Teaching Lab Course

The second teaching lab course (bachelor, mechatronics) „Lab on Chip / Microfluidics“ took place at KIST Europe under responsibility of Prof. Dr. Andreas Manz. More than 80 students took an active part in the course.

March 2, 2012

MoU with Diatech Korea

KIST Europe‘s invention providing primer sets for diagnosing Ankylosing Spondylitis (AS) and the method for diagnosing AS using the same has been transferred to the Diatech Korea Co. Ltd..



March 6-10, 2012

CEBIT Hannover

In March 2012, KIST Europe displayed the latest results of breath analysis in a common booth together with the Max Planck Institute for Informatics (MPII) and the Saarland University. A cooperation was established in 2011 with the Computational Systems Biology Group at MPII. An ion mobility spectrometer was on display to demonstrate the possibilities of metabolic profiling within less than 10 minutes total analysis time. Volatile metabolites could be correlated to different diseases like infections, cancer, inflammation and others. The use of modern algorithms and bio-statistics supports database assisted interpretation of human breath profiles.

March 14, 2012

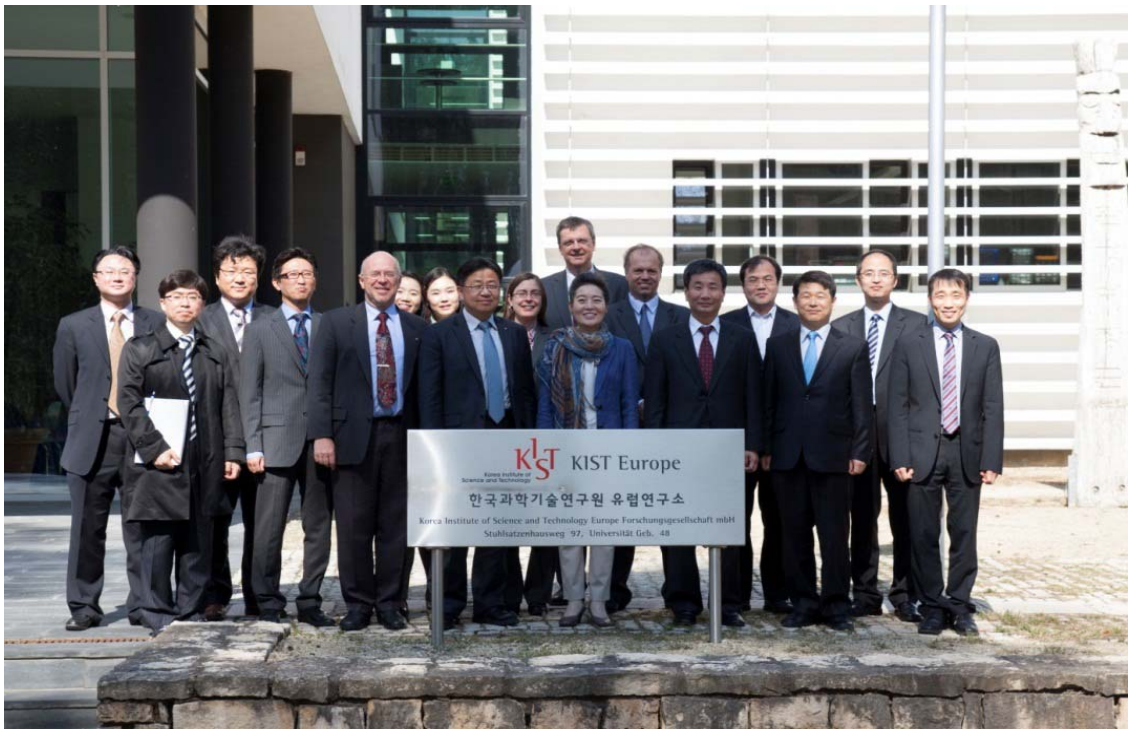
Workshop „Metabolites in Human Breath“

The 5th Workshop „Metabolites in Human Breath“ was held at the BioMedicalCenter in Dortmund. Here, the latest developments in the various clinical applications of ion mobility spectrometry for intensive and point of care applications were presented directly by medical doctors.

March 28, 2012

Visit of the Korean Minister of Environment

Dr. Young Sook Yoo, Korean Minister of Environment, came to visit with the concern of research activities on environment of KIST Europe.



April 27, 2012

Advisory Board Meeting

The annual Advisory Board Meeting was held at KIST Europe.

Participants:
Dr. Moon (KIST), Mr. Flackus (Saarland Government), Mr. Lee (MEST), Mr. Jörgens (BMBF), Dr. Park (SAIT), Prof. Dr. Linneweber (University Saarland), Prof. Dr. Rombach (IESE) and Mr. Yang (Hyundai).

June 16, 2012

Open Day at University Saarland

In June, the University Saarland offered an Open Day in which KIST Europe participated and provided informations for all interested in research and Korean culture.

July 3, 2012

Visit of NSTC

Dr. Kichul Lim, Standing Commissioner of National Science & Technology Commission, visited KIST Europe.

July 24, 2012

Summer Workshop „Lab on Chip“

The theme of the summer Workshop 2012 at KIST was „Lab on Chip“. Well-known speakers from the universities of Washington, Berkeley and Toronto as well as from KIT Karlsruhe (Germany) held interesting lectures about microfluidics, micro-/nano-tools and their use in diagnostics and biology.

July 24, 2012

Biolab Opening

The new infectious disease research lab (Biolab) was opened at KIST Europe in a great opening ceremony.

High Safety Bio-Laboratory (Biosafety Level 3)

July 25, 2012

Round Table Meeting

Round table meeting for discussion on development of KIST Europe took place with participation of the VIPs in science and technology.

Participants (from left to right):
Dr. Dongil Jung (KEITI), Dr. Seock Joon Kim (KIMM), Dr. Yun Chul Chung (NRF), Dr. Ho Seong Lee (KIST Europe), Dr. Jae-Won Kwak (KOFST), Dr. Kil-Choo Moon (KIST), Dr. Youseung Kim (DGMIF), Dr. Myungja Kim (KOFWST), Dr. Sang-Dai Park (KOFST), Dr. Kwang Ho Kim (KIST), Dr. Mihye YI (KRICT), Dr. Kyung Ho Shin (KIST), Mr. Joon Haeng Heo (MEST), Dr. Tae-woon Kim (DGMIF)

July 25, 2012

MoU with KOFWST and KEITI

KIST Europe signed the MoU with Korea Federation of Women's Science and Technology Association (Dr. Myungja Kim) and Korea Environmental Industry & Technology Institute (Dr. Dongil Jung).

July 27, 2012

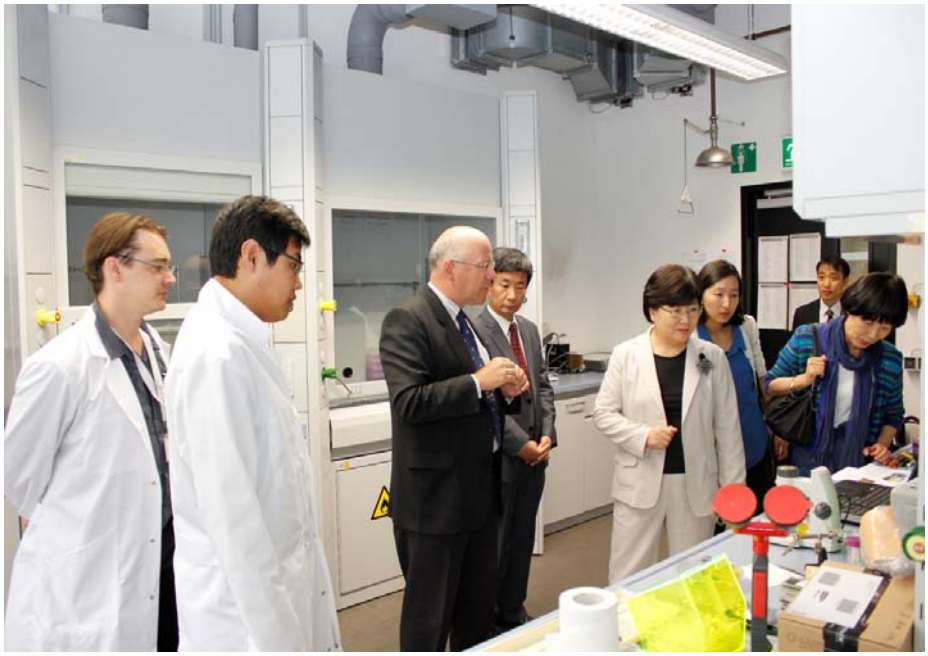
Participation in EKC

KIST Europe joined in the Europe Korea Conference in Berlin.

July 30, 2012

Visit of Ewha Woman's University

Dr. Sun Uk Kim, President of Ewha Woman's University, visited KIST Europe.



September 3, 2012

Inauguration of the new CEO

After 3 years, the former CEO Prof. Dr. Kwang Ho Kim discharged. His successor, Prof. Dr. Ho Seong Lee, was inaugurated at KIST Europe in Saarbrücken.

September 17, 2012

Welcome of the internship students from Korea

As in the past years, KIST Europe welcomed the internship students from Korea at the headquarter in Saarbrücken.

September 18, 2012

Visit of UST

Prof. Dr. Un Woo Lee, President of University of Science and Technology, visited KIST Europe for signing the MoU.

November 16, 2012

Visit of Yonsei University

Three professors of Yonsei University - Prof. Dr. Joong-Gill Choi, Prof. Dr. Seung Han Park, Prof. Dr. Jae-Chul Pyun - visited KIST Europe for discussion about researcher and student transfer.

November 23, 2012

Korean Council of Environmental Industry, Technology & Policy in Europe

The 1st Korean Council of Environment in Europe took place at KIST Europe.

November 24, 2012

Ländertag Korea

KIST Europe took part in the Ländertag Korea on the university campus.

December 2, 2012

Korea-Europe R&D Collaboration Workshop

Participants of the R&D Collaboration Workshop started their first program at KIST Europe.

December 13, 2012

Visit of KRCF and MEST

Prof. Dr. Keon Kim (Chairman of Korea Research Council of Fundamental Science and Technology) and Dr. Young Soon Kang (Director-General in MEST) visited KIST Europe.

Summary

MoUs

Diatech Korea	02.03.2012
Korean Institute of Energy Technology Evaluation and Planning (KETEP)	27.04.2012
Korea Environmental Industry & Technology Institute (KEITI) Korea Federation of Women's Science & Technology Association (KOFWST)	25.07.2012
University of Science and Technology (UST)	18.09.2012

Visits

Dr. Young Sook Yoo (Korean Minister of Environment)	02.03.2012
Dr. Kichul Lim (Standing Commissioner of National Science & Technology Commission; NSTC)	03.07.2012
Prof. Dr. Sun Uk Kim (President of Ewha Woman's Univ.)	30.07.2012
Prof. Dr. Un Woo Lee (President of University of Science & Technology; UST)	18.09.2012
Prof. Dr. Joong-Gill Choi, Prof. Dr. Seung Han Park, Prof. Dr. Jae-Chul Pyun (Yonsei Univ.)	16.11.2012
Prof. Dr. Keon Kim (Chairman of Korea Research Council of Fundamental Science and Technology), Dr. Young Soon Kang (Director-General in Ministry of Education, Science and Technology)	13.12.2012

Events

Workshop „Metabolites in Human Breath"	14.03.2012
Advisory Board Meeting of KIST Europe: Dr. Kil-Choo Moon / Mr. Changyune Lee / Dr. Jae Chan Park / Mr. Seungwook Yang Mr. Jochen Flackus / Prof. Dr. Volker Linneweber / Prof. Dr. Dieter Rombach / Prof. Dr. Günther Fuhr / Mr. Christian Jörgens	27.04.2012
Summer Workshop „Lab on Chip"	24.07.2012
Biolab Opening	24.07.2012
Round Table Meeting: Dr. Sang-Dai Park (KOFST) / Dr. Myungja Kim (KOFWST) / Dr. Youseung King (DGMIF) / Dr. Kil-Choo Moon (KIST) / Dr. Jae-Won Kwak (KOFST) / Dr. Yun chul Chung (NRF) / Dr. Seock Joon Kim (KIMM) / Dr. Mihye Yi (KRICT) / Dr. Dongil Jung (KEITI) / Dr. Kyung Ho Shin (KIST) and more	25.07.2012
Inauguration of the new CEO, KIST Europe	03.09.2012
Korean Council of Environmental Industry, Technology & Policy in Europe	23.11.2012
Korea-Europe R&D Collaboration Workshop	02.12.2012

Participations

Expo „CEBIT Hannover"	06.-10.03.2012
Open Day of University Saarland	16.06.2012
EU-Korea Conference 2012 in Berlin	26.-28.07.2012
Ländertag Korea	24.11.2012

Photosensitizer delivery by living immune cells

cell-mediated drug delivery, cytotoxic T lymphocytes, photosensitizing agents

The Cellular Immunotherapy team aims to develop next generation cancer therapies by combining immune cell-based therapeutic strategies with cell-mediated drug delivery. Recent in vitro data suggest that the natural cytotoxic activity of bispecific antibody-redirected T lymphocytes can be significantly enhanced if the cells are used as "Trojan horses" for a photosensitizing agent. Upon light-induced activation of the drug, photosensitizer loaded T cells were more effective against co-cultivated cancer cells than unloaded T lymphocytes.

Cell-mediated transport of therapeutics has emerged as a new drug delivery concept in the past decade. Certain cells types, such as immune and stem cells, actively trace and infiltrate or can be redirected to diseased sites which makes them ideal candidates for the development of novel drug targeting strategies. Furthermore, various defense cells possess an intrinsic cytotoxic or phagocytic activity that can additionally be exploited to fight diseases. So far, numerous preclinical studies have demonstrated the feasibility of the innovative concept, but these trials also revealed a major drawback of the novel strategy: Especially when malignant tumors were treated with chemotherapeutic agents-loaded living cells, a strong impairment of the carrier cell's functions and viability was observed.

To preserve the functionality of cellular drug carriers, we pursue a completely new strategy: By loading patient-derived T lymphocytes (T cells) with a stimulus-sensitive therapeutic agent we ensure that, when reinjected into patients, cells can trace the diseased tissue and exert their natural killing function until the drug effect is specifically activated. Diseased target cells are thus eliminated through the consecutive action of cytotoxic immune cells and the delivered, stimulated pharmaceutical.

To prove the feasibility of the novel concept, a straight-forward in vitro study was conducted involving ex vivo activated, bispecific antibody-redirected human donor T lymphocytes and a water-soluble formulation of a light-inducible photosensitizing agent (kindly provided by Jun. Prof. Dr. Marc Schneider, Saarland University, Germany). Flow-cytometer analyses showed that human T cells internalize the fluorescent photosensitizer (PS) very efficiently. T cell viability was not impaired if upon loading cells were kept in the dark. However, irradiation with a halogen light immediately caused T cell death indicating that the drug effect can be induced at a desired point in time. Fluorescence microscopy and FACS analyses revealed that the drug was transferred from PS-loaded T lymphocytes to co-cultivated cancer cells.

To evaluate the combined T cell-drug effect irradiated co-cultures of T lymphocytes and clinically relevant tumor cell lines were subsequently analyzed with respect to cancer cell survival. We found that bsAb-retargeted, PS-loaded T cells kill tumor cells more efficiently than redirected, unloaded T lymphocytes (figure 1). Interestingly, retargeted, PS-containing T cells were also superior to a mixture of unloaded T lymphocytes, bsAb and separately applied PS strongly suggesting that combining T cell-mediated photosensitizer delivery, immunotherapy and phototherapy could become a powerful option for the treatment of cancer.

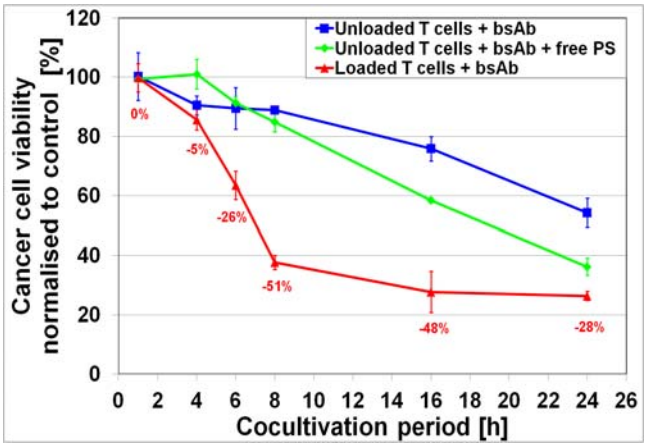


Figure 1: WST-1 assay based determination of cancer cell survival in co-cultures of lung carcinoma cells and bispecific antibody-retargeted, unloaded T cells (blue curve), redirected, photosensitizer (PS) loaded T lymphocytes (red curve) and retargeted, unloaded T cells in combination with separately applied PS (green curve). To activate the photosensitizing, agent co-cultures were illuminated for 15 min with a halogen light two hours before the measurement.

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Further information: a.blaudszun@kist-europe.de

Publications

Stöhr, T.; Blaudszun, A.-R.; Steinfeld, U.; Wenz, G.: Synthesis of glycosylated peptides by NCA polymerization for recognition of human T-cells. *Polym. Chem. 2 (2011) 2239-2248*

Droplet Generation on W/PEG/W system

droplet, aqueous two phases, PEG, microfluidics

For bio-medical applications by using droplets in microfluidic platforms, practically an aqueous based two or more phases system can provide wide range of methods. Therefore, a droplet generation with W(water)/PEG aqueous two phase has been theoretically studied. Based on the results, PEG conjugated droplets and its nano-scaffold generation can be tested in fully aqueous condition.

A W/PEG emulsion in which aqueous droplets consist of two phases with a PEG droplet can provide several advantages on microfluidic applications.

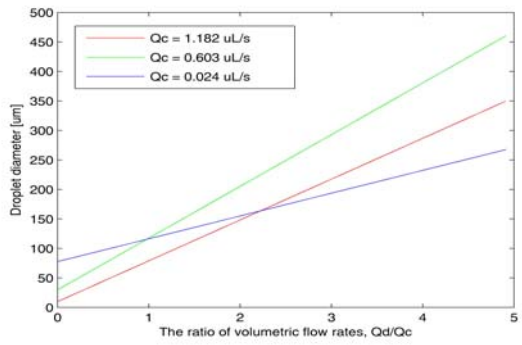

Figure 1: Dependence of the droplet diameter on the ratio of volumetric flow rates (Q_d/Q_c) when the flow rates of continuous phase (Q_c) are fixed value ($0.024*10^{-9}m^3/s$, and $1.182*10^{-9}m^3/s$ when h=40um)

Previous studies of droplet breakup at microfluidic T-junctions have focused on the relationship between Ca (capillary number) and the emerging droplet. According to previous studies, at the critical capillary number-about 0.1 to 0.01, the surface tension forces break the liquid thread to droplets, minimizing the interfacial area and surface energy. Especially, when the width of continuous phase inlet is larger than that of discontinuous phase inlet, the droplets which has smaller diameter than the channel width will be generated when 0.01 < Ca < 0.5. And in this breakup regime, the size of droplet generated depends predominantly on Ca and volumetric flow rates of continuous phase, not only on volumetric flow rates of discontinuous phase. According to these criteria, the flow rate of continuous phase, the PEG phase, should be 0.012 - 0.591, 0.024 - 1.182, and 0.036 - 1.773 [10-9 m3/s] when the height of channel is 20, 40, and 60 um. Based on these results, the size of the merging droplet can be determined. In this study, the extended scaling model for droplet breakup has been utilized with the assumption that there are three main forces which effect on the process of droplet formation. And also simulated the droplet formation using the lattice Boltzmann method has been presented.

T-junction devices were used for this study as one of the most common strategies in droplet formation. Two liquid phases emerge into the channel with the respective volumetric flow rates (continuous Qc and discontinuous Qd), under the each inlet pressure.

There are three main forces acting on the discontinuous liquid's surface, which govern the droplet formation process: capillary force, viscous shear force and squeezing pressure force. When these three forces sum to zero, the droplet will be generated.

$$(1 - b/wc)3 = b/wc * Ca$$

where the b is the estimated size of the droplet and wc is the width of continuous phase inlet.

On the other hand, to get the final length of droplet at its detachment, the additional length of the droplet from the onset of thinning to detachment should be considered. If we assume the time needed for the neck to be thin to zero, then the final size of droplet generated will be given by

$$L = wc (b/wc + (Qd/Qc)*(Wd/Wc)*(b/Wc)-1)$$

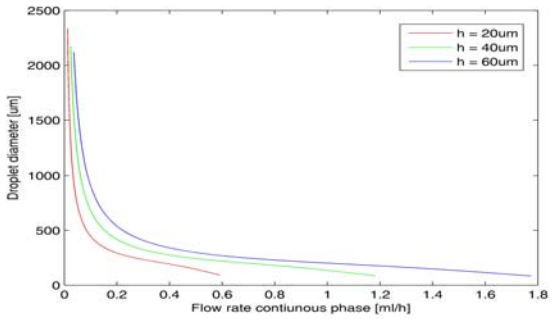

Figure 2: Dependence of the droplet diameter (h=20, 40 and 60 um) on the flow rate of continuous phase (Q_c) when the ratio of flow rates (Q_d/Q_c) are fixed value (0.5, 0.85 and 1.2)

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Bacteriophages as templates for manufacturing supramolecular structures

bacteriophage, fd-SN, bundle, counterion condensation, AFM, nanobiotechnology

Bacteriophages are viruses that can infect bacteria. fd-p8SSGDD phage (SS-phage) was genetically engineered by replacing 2 N-terminal aa of the p8 coat protein of fd-phage. AGE and zeta potential measurements showed that SS-phages carry at least 1/4 less net negative surface charge than fd. Morphology of phages was studied at different counterion concentrations (1, 10 and 100 mM) by AFM, SEM and immunofluorescence analyses. Bundles induced by $CoCl_2$ and $CaCl_2$ were either metallized by chemical reduction or biomineralized for apatite-like material formation. Energy dispersive X-ray spectroscopy confirmed the presence of Co, P and Ca peaks in mineralized samples. Such bottom-up manufactured phage scaffolds could be applied in bioengineering studies.

Phage bundles obtained with 100 mM $CoCl_2$ were treated with different concentrations of reducing agent (0.15 mM, 1.5 mM and 15 mM $NaBH_4$). Presence of Co on phage filaments was confirmed by SEM and EDX analyses. Reduction of Co_2+ ions with $NaBH_4$ led to the formation of cobalt and cobalt oxide clusters on phage bundles (Fig 1). As $NaBH_4$ concentration was increased, larger metal aggregates were detected (Fig 1). Chemical composition of reduced phage bundles were confirmed by EDX measurements. Although Co peaks were detected for SS-phage bundles treated with 0.15 mM $NaBH_4$, no

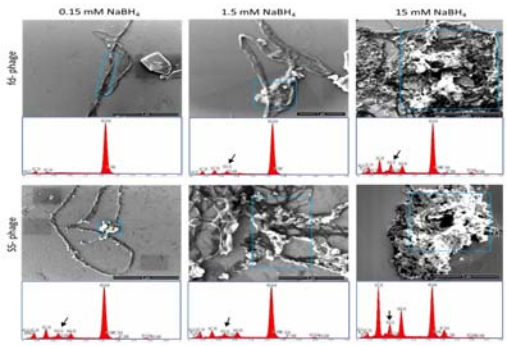

Figure 1: SEM images of fd- and SS-phage bundles treated with 100 mM $CoCl_2$ after treatment with various concentrations of $NaBH_4$ solution (0.15 mM, 1.5 mM and 15 mM). Pictures were taken at 25 kV. The corresponding EDX spectra are shown below each picture. Co peaks are indicated with the arrows. EDX analyses were conducted inside the blue boxes.

peak was observed for fd-phages. Corresponding SEM images support the EDX data. Whilst SS-phages treated with 0.15 mM $NaBH_4$ were observed to be decorated with metal clusters, fd bundle was plain without any particles. For 1.5 mM and 15 mM cases, thicker and denser aggregates were detected for SS-bundles. This implies that SS-phages carry more nucleation points for the growth of Co particles after reduction. By changing the reducing agent concentration, we could obtain metallized bundles of different thickness and morphologies.

To investigate the effect of $CaCl_2$ treatment on apatite-like layer formation, $CaCl_2$ (10 mM and 100 mM) induced phage bundles were incubated in 5 x SBF solution. Fig 2 shows the fd- and SS-phage bundles after SBF treatment. After 1 day of incubation at 37°C, phage bundles were coated with

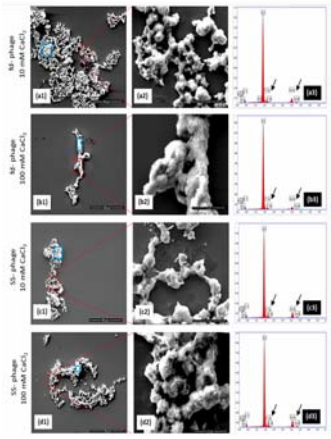

Figure 2: SEM images of fd- and SS-phage bundles treated with 10 mM (a,c) and 100 mM $CaCl_2$ (b, d) and incubated in 5xSBF soluation at 37 °C for 1 day with various magnifications. Pictures were taken at 5 kV. The corresponding EDX spectra are shown on the right. Ca und P peaks are marked with the arrows. EDX analyses were conducted inside the blue boxes.

a thick mineral layer (Fig 2). SEM images (Fig 2 a2-d2) revealed that the size of apatite-like material was increased with increasing $CaCl_2$ concentration. Longer incubation times in SBF solution resulted in growth of larger apatite-like material (data not shown). Bound Ca_2+ ions act most probably as exposed nucleation sites for mineral growth. Thus as the $CaCl_2$ concentration was increased from 10 mM to 100 mM, number of nucleation sites increased gradually forming thicker layers. EDX spectra confirmed the presence of Ca and P on apatite-like mineral layers (Figure 2 a3-d3). Ca/P ratios for 10 mM and 100 mM $CaCl_2$ induced fd-phages after 1 day of SBF treatment were calculated to be 1.41 & 1.58 and 1.42 & 1.49 for SS-phages respectively (Figure 2 a3-d3). Ca/P ratios of phage derived apatite-like mineral layers are slightly lower than 1.71 which is the value for the human bone.

Further information: n.korkmaz@kist-europe.de

Publications

Korkmaz, N.; Kim, Y.J.; Nam, C.H.: Bacteriophages as templates for manufacturing supramolecular structures. *Macromolecular Bio-science. 20.11.2012, doi:10.1002/mabi.201200290*

Single point characterization of segmented flow and surface properties

segmented flow, laser induced fluorescence, stroboscopic imaging

We propose the use of single point measurements of droplet fluorescence, in conjunction with stroboscopic imaging, as an alternative technique for segmented flow characterization. We demonstrate that this method can yield information on analyte concentration, droplet width and velocity, and flow rate ratio of the phases. Finally, it is also possible to characterize the nature of the channel surface with regards to its hydrophobicity or hydrophilicity.

The segmentation of flow in microfluidic channels is a common method of achieving compartmentalization of analytes or reactions in microfluidic devices. Briefly, two immiscible streams are mixed at a T-junction, resulting in the break-up of one phase into droplets dispersed in the second or carrier phase. The carrier phase is chosen to preferentially wet the walls of the channels of the device, so that the dispersed droplets never come into contact with their surface. The droplets can then be used as individual nano to picoliter reaction vessels to perform a variety of chemical and biochemical operations. Droplet analysis is typically done by fluorescent imaging using a microscope. This method, however, can severely limit the rate at which data can be collected, unless expensive ultra-high speed cameras are used.

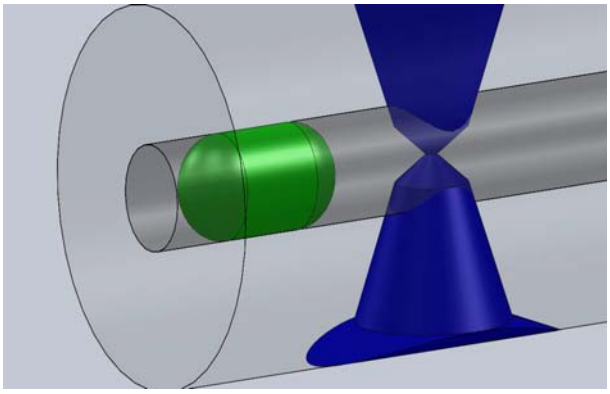

Figure 1: CAD representation drawn in Solid Works software of blue laser beam interaction with fluorescein droplet inside

An alternative method is the use of laser induced fluorescence coupled to detection with a photomultiplier tube. For droplet characterization, the inner surface of a fused silica capillary with an inner diameter of 50 µm was rendered hydrophobic with fluoroalkylsilane (FAS) by chemical vapour deposition. Representation of laser interaction with the droplet of fluorescein is shown in Fig. 1. Two detection windows were burned on the polyimide coating, about 20 cm apart. Segmented flow was generated by mixing an aqueous fluorescein solution with hexadecane inside a T-junction, the outlet of which was connected to the coated capillary. The first detection window was placed in the viewing field of an inexpensive GE5 AIGO microscope (see Fig. 2) the second one into a custom-built laser induced fluorescence (LIF) detection system with photomultiplier tube (PMT). Once segmented flow was observed, fluorescence measurements from the PMT were recorded by an oscilloscope, while droplet images were taken simultaneously with the microscope. Since the droplets are moving past the detector at a high velocity, imaging cannot be done continuously. Rather, images were taken using an LED flashing at a rate of 1 to 2 Hz. This results in

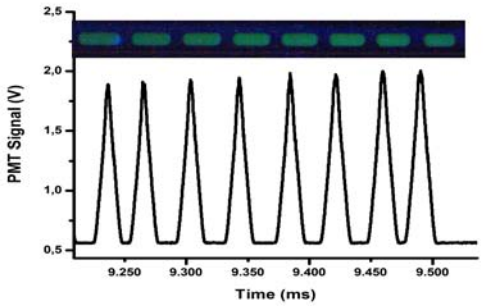

Figure 2: (top) Optical fluorescent image of a segmented flow formed by fluorescein and oil. The capillary had hydrophobic coating. (bottom) Corresponding optical amplitude detected by PMT

representative images that can be used to measure droplet length which, along with the time-domain data from the oscilloscope, can be used to calculate a linear velocity for the drops.

Similar measurements at different flow rates shows the relationship between the oscilloscope signal and droplet width and flow rate ratios. Measurements were also performed on a hydrophilic surface (uncoated fused-silica capillary) and a partially hydrophobic surface (not fully coated fused silica capillary). The shapes of the curves of the moving droplets will be compared to a SolidWorks model for different flow rates, allowing the characterization of each surface (i.e. coating efficiency).

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Free flow electrophoretic microdevice for cancer detection

microfluidics, free flow electrophoresis, cancer, biomarkers, proteins

Free flow electrophoresis (FFE) is a separation method which can be used for purification of charged molecules from complex mixture. The fabricated chip-based device is low-cost and easy-to-adapt for applications such as high yield protein isolation. The system will be used to assist the identification of biomarkers from complex human samples such as urine. It will be used as early detection method for cancer enabling quick results and timely treatment.

Diagnosis of cancer in an early development stage increases drastically the chances of successful treatment. Many detection systems used today are bulky, expensive and require biopsy tissues as samples. Four years ago p53 tumor protein was discovered in urine. Its presence could indicate an early stage of cancer. This detection technique could be used for mass population screening as it is non-invasive and urine is collected during regular annual checkups.

A suitable technique for the purification of proteins from samples containing a complex mixture of various compounds is free flow electrophoresis (FFE). The FFE device consists of a separation chamber with

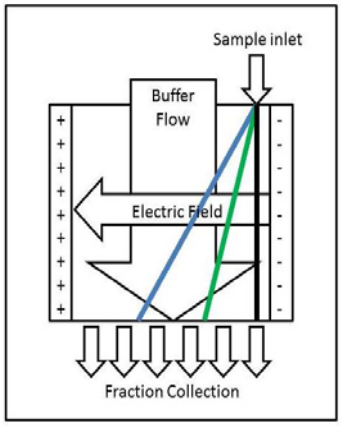

Figure 1: Schematic representation of urine sample injected into an FFE device consists of three differently charged compounds. Black color represents neutral compound, green color shows low charge density compounds and blue color indicates compounds with high charge density.

applied electric field perpendicular to the buffer and sample flow direction (see Fig. 1). Buffer and samples are connected to the FFE chip and pumped through by syringe pumps. The electric field causes charged molecules to deflect their motion through the chamber based on their electrophoretic mobility and electric field intensity.

Here, we demonstrate a chip-based unit for continuous FFE. The microfluidic device was photolithographically patterned and either etched by hydrofluoric acid (glass substrate) or by XeF_2 vapor (silicon substrate) as shown in Fig. 2. Once the

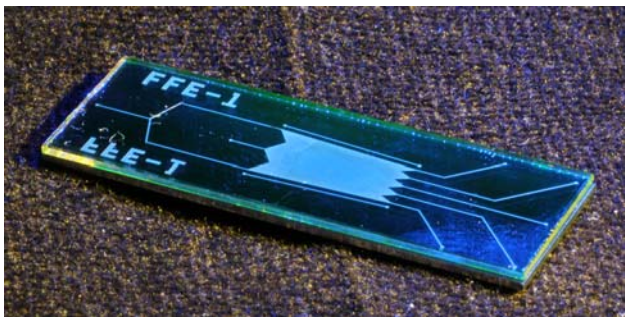

Figure 2: Fabricated FFE microdevice by photolithography from silicon capped with glass. Its dimension are 34mm x 12mm. The typical channel width and depth are 210 µm and 70 µm, respectively. For better visualization, the microdevice is filled with fluorescein.

channels and FFE chamber were etched into the substrate the devices was capped by a cover glass. Fluids interconnections were provided by capillaries connected to the chamber via holes drilled into the cover glass. After optimization of separation conditions we expect to isolate p53 in one of the outlets as pure substance for further analysis by mass spectrometry or immuno-detection.

The device testing will be first performed with artificial urine spiked with p53 protein as a sample for optimization of separation conditions as flow velocity and applied electric field. The real clinical sample of urine contains substances which could complicate further analysis. Concentration of urea in urine is up to 7M. It also contains uric acid, degradation products of Hemoglobins (Bilirubins), calcium sulfate, calcium oxalate, kreatinins, hormones, and many others. The typical concentration of proteins is only in the range from 2 to 8 mg per 100 ml of urine. The FFE chip provides fast, easy-to-use and reproducible device for the enrichment of the cancer biomarker protein.

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Droplet Pyrosequencing - Rapid DNA sequencing on magnetic particles

ssDNA (single-stranded) sequencing, microdroplet-based system

Pyrosequencing is a DNA sequencing method where the nucleotide presence in the ssDNA is determined by amplitude of emitted bioluminescence. Here, we present a rapid and simple microdroplet-based system. The DNA is bound to functionalized magnetic particles. The particles together with DNA are moved between different stations with nucleotides using magnetic force. This method can be further expanded for sample preparation for PCR.

Pyrosequencing is a method where nucleotides are sequentially added (anneal) to the complementary strand of ssDNA. The reaction requires presence of number of enzymes such as polymerase, luciferase, sulfurylase and apyrase. In event of nucleotide annealing bioluminescence is produced and subsequently recorded. In conventional platforms, presence of apyrase is essential as after every annealing this enzyme destroys free nucleotides. It allows adding more and more nucleotides and thus detecting the DNA sequence. This dilutes the pyrosequencing mixture which eventually stops the reaction. Also these commercial systems are costly and bulky.

We have demonstrated a solid-phase DNA pyrosequencing. The ssDNA is bound to magnetic particles and together moved between nucleotide-containing droplets. Instead of apyrase enzyme we use a washing station to remove unreacted nucleotides. This way the reaction mixture does not suffer dilution.

The reaction is performed at the hydrophobic surface of glass cover slide. This glass is mounted on a translation stage with a permanent magnet fixed beneath the slip. Five droplets are spotted in a cross pattern onto the cover slip (see Fig. 1).

Figure 1: Photograph of the setup, showing the central washing station and the four nucleotide droplets around it. The magnetic particles can be seen in the washing station with the magnet underneath the glass slide. The glass slide was moved via a translation stage while the magnet was fixed in position.

Four droplets with volume of 25 µL contain the nucleotides (A, C, G, T) together with the pyrosequencing enzymes. The large central droplet with volume of 100 µL is the washing station. All the droplets are covered with layer of mineral oil to prevent water evaporation and changing the concentration of solutions. Biotinylated ssDNA from *Bacillus subtilis* is bound to streptavidin-functionalized magnetic particles with the size of 2.8 µm. First the particles are introduced into the washing station WS. From there, they are sequentially moved into droplets with nucleotide in this sequence: WS -> A -> WS -> T -> WS -> G -> WS -> C -> WS (see Fig. 2). This sequence is repeated number of times until the DNA sequence is determined based on recorded the bioluminescence signal using photomultiplier tube (PMT) and oscilloscope.

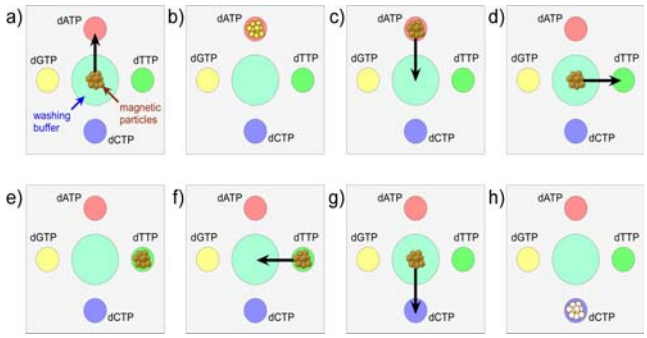

Figure 2: Droplet-based pyro-sequencing. (a) Magnetic particles featuring ssDNA are pulled into the first nucleotide droplet (dATP), where (b) dATP is incorporated and light is given out. The particles are then (c) washed, and (d) pulled into the dTTP droplet, where (e) no light is produced, hence dTTP is not incorporated. The procedure continues thus (f-h) and the cycle is repeated until the DNA has been sequenced.

This method uses efficient washing step between reactions with nucleotides elliminating apyrase. It results in longer read-lengths of the ssDNA.

We have demonstrated a droplet-based device capable of performing pyrosequencing. It is a simple and inexpensive tool for rapid and efficient sequencing. We foresee integration of this device into a system capable of providing an all-in-one sample preparation, PCR as well as pyrosequencing. It could be made into fully automated system.

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Ultra-high voltage separation using van de Graff generator

van de Graff generator, microfluidics, capillary electrophoresis

We are developing a capillary electrophoresis (CE) analytical system (in collaboration with ETH Zurich) with 200 times higher efficiency and 14 times higher resolution compare to the conventional CE systems. The experimental setup consists of three parts: a microfluidic sample- injection system, a laser-induced fluorescence (LIF) detection system and the van de Graaff generator providing voltage of up to 6 MV.

Capillary electrophoresis (CE) is a powerful tool for separation and detection of complex compounds. The efficiency and resolution of CE depends on applied voltage. Commercially available CE systems can typically work with voltage up to 30 kV. Higher voltage power supplies are not readily available. We are working on integration of van de Graaff generator from ETH, which provides up to 6 MV, with our CE system. If successful we will achieve 200 times the efficiency and 14 times resolution compare to the conventional CE which will result in highest CE resolution ever reported.

We have fabricated a microfluidic device for sample injection system (see Fig. 1 left). The chip was

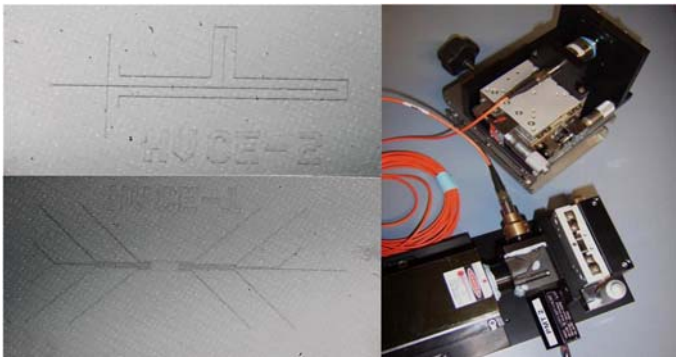

Figure 1: Injection chips for the ultra-high voltage capillary electrophoresis (left). Laser induced fluorescence detection (LIF) system (right). The LIF system is split into two parts connected by optical fiber.

fabricated using conventional photolithography process using Pyrex 4770 glass as substrate. The sample is detected by laser induced fluorescence (LIF) system (see Fig. 1 right).

First we have tested our sample injection system with ready-made microfluidic chip. This chip consists of two channels intersecting each other. One is for sample injection, the other one is for sample separation. The desired sample pinching was observed at intersections of two channels (see Fig. 2) and have injected sample into the separation channel. Both injection and detection systems have been integrated and they will be tested with fused silica capillary tube with inner diameter of 5µm. This small diameter will assure high electrical impedance along the capillary leading to minimized Joule heat dissipation.
High voltage testing in ETH Zurich will be challenging. as there is no access to the high voltage source once

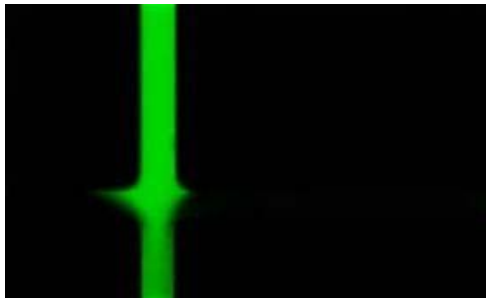

Figure 2: Microscope image of the sample focusing in microfluidic chip

it is operating. The injection system will be placed outside of the van de Graaff generator as well as part of the LIF such as laser source and photomultiplier tube. The rest will be mounted inside the body of the generator. There is about 10 meter distance between measurement point (inside) and instrumentation (outside). These two points will be connected by capillary and fiber optics.

In conclusion, together with ETH Zurich, we are developing capillary electrophoresis (CE) analytical system with highest resolution. It will provide significantly increased yield for the efficiency and resolution in the field of analytical chemistry. This will help KIST-Europe to play significant role in CE and will pave the way for the novel types of chemical analysis.

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Breath analysis of ventilated rats with induced sepsis using MCC/IMS

breath analysis, IMS, sepsis

Sepsis is a condition in which the body is fighting a severe infection that has spread via the blood stream and is one of the main causes of death in intensive care units. For this study, we developed an on-line system to monitor breath during sepsis. MCC/IMS makes it possible to measure volatile organic compounds from exhaled breath. We established a ventilated rat model for sepsis studies and investigated volatile organic compounds in the breath of healthy rats and with induced sepsis.

Abbreviation:
MCC/IMS: Ion mobility spectrometry with multi-capillary column

In case of sepsis the whole body is in an inflammatory state (called a systemic inflammatory response syndrome or SIRS) caused by severe infections, such as commonly bacteria, but also fungi, viruses, or parasites in the blood, urinary tract, lungs, skin, or other tissues. Sepsis can lead to multiple organ dysfunction syndrome and finally death.

For continuously rat breath measurements, we established an on-line system between the IMS device and the ventilated rat. In this system, the ventilator is connected to the synthetic air bottle used for operating the MCC/IMS to avoid entering room air inside the device. Therefore, the rat's mechanical ventilation was carried out with synthetic air during all the experiments. Second, the sampling tube from the MCC/IMS was directly connected to the exhaled line of the ventilator. For this study we induced a polymicrobial sepsis in 6 rats, whereas 6 healthy rats were used as a control group.

The IMS coupled with a multi-capillary column-ion mobility spectrometer(MCC/IMS) was used for breath analysis. The multi-capillary column (MCC, OV-5, Sibertech Ltd, Novosibirsk, Russia) was applied to preseparate the complex of analytes at 40 ºC constant temperature by gas chromatography due to specific polarity. 10ml of exhaled breath were collected in the sample loop of the MCC/IMS before entering to the MCC column. The ventilator was measured in closed circuit and without any rat, in order to measure the background and to avoid any conflict with the signals coming from the rat. Detected peaks from the MCC/IMS were characterized using the software Visualnow (B&S Analytik, Dortmund Germany). The MCC/IMS analysis of the rat's breath from this experiment was compared with ventilator before the breath analysis to exclude background signals. The set of peaks found in the measurements analyzed together in a cluster procedure.

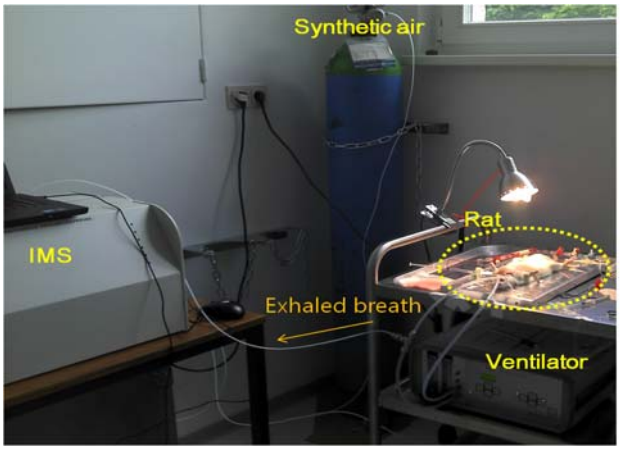

Figure 1: On-line experimental setup for rat breath measurements

We found 35 peaks in the ventilator and 38 peaks from the exhaled breath of the rat. Specially, some peaks show time dependent differences sepsis rats and control rats. The intensity of these peaks is decreasing in septic rats, whereas in control rats it remain almost at the same level. Our results mayhave the potential to help identifying biomarkers for sepsis in breath.

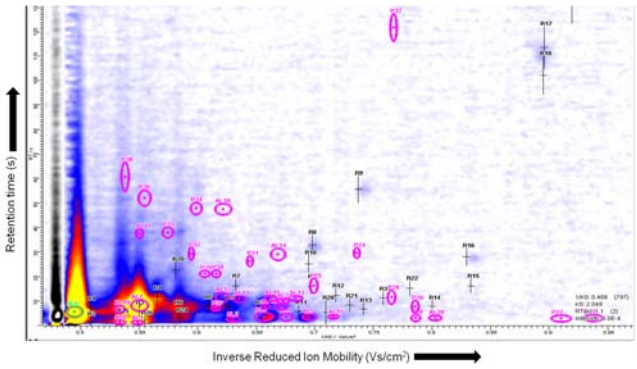

Figure 2: IMS Chromatogram of all detected peaks in ventilated rat (pink circle: the peaks in only breath of the rat; black cross: the peaks in only the ventilator).

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Publications

Alexander Wolf, Astrid Kleber, Jörg Ingo Baumbach, Kathrin Rupp, Sasidhar Maddula, Patrick Favrod, Mi Jang, Tobias Fink, Thomas Volk, Sascha Kreuer: Multicapillary column - Ion mobility spectrometer (MCC-IMS) measurementes in ventilated rats: Establishment of a new animal model, 2013 - *in progress for submission*

Alignment between MCC/IMS and GC/MS measurements

ion mobility spectrometry, gas chromatography, alignment

To identify the molecules in a complex breath sample a reference data base is necessary. In order to get a first set of references different substances were measured with the multicapillary column ion mobility spectrometry (MCC/IMS) and gas chromatography mass spectrometry (GC/MS) in parallel. The retention times of the IMS and GC measurements were aligned and a mathematical function was obtained. Using this function a prediction of the retention times from GC/MSD to MCC/IMS and vice versa is possible.

To prove the accuracy of the alignment between these two techniques the retention time of the MCC/IMS measurement was predicted for three test compounds knowing their GC retention time. After verifying the prediction, the mathematical function was integrated into the IMS software VisualNow 3.4 to compare the spectra directly in the software, which makes the analysis of the peaks much more efficient.

The aim of this study is the automated alignment of MCC/IMS and GC/MS data as well as the possibility to increase the MCC/IMS database. An alignment function and an alignment of both chromatograms with the software VisualNow 3.4 is shown. The aligned chromatograms serve to identify unknown compounds in the MCC/IMS by knowing the GC retention time. In order to get a first set of analytes measured with MCC/IMS and GC/MS 13 references and three test compounds which are related to diagnostic potential in medical and biological studies were selected. Finally, peak alignment has been demonstrated for a mixture of seven different compounds.

In the Fig.1 the GC retention times are located on the x-axis and the MCC retention times are located on the y-axis.

With the alignment the following exponential function turned out:

$$RT(MCC)calc = A1*exp(x/t1)+A2*exp(x/t2)+y0 \quad (1)$$

This mathematical function was used to visualize the match of both chromatograms in the software VisualNow 3.4. One example for the visualization is shown in Fig. 2.

The deviation of the compounds differs from 0.22 s to 10.04 s whereas in the study of Jünger et al. a deviation from 0 s to 36.32 s was shown. In contrast to Jünger (1) et al. our alignment was done in the software and not by hand, which is time-saving. Another existing paper related to this topic is the work of Bunkowski (2), which correlated the MCC/IMS and the GC chromatograms not by a measured mathematical function but by alignment with a software. The drawback of this method is an inaccurate fitting of the GC chromatogram in several retention time sections. In summary, our automated alignment function based solution for comparison of MCC/IMS and GC chromatograms improves existing methods concerning speed of operation and fitting accuracy.

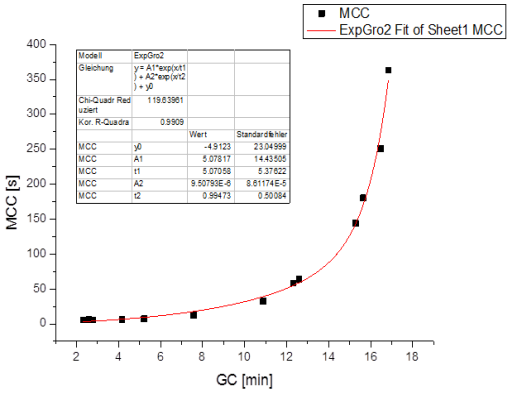

Figure 1: Alignmentfunction of MCC and GC retention times

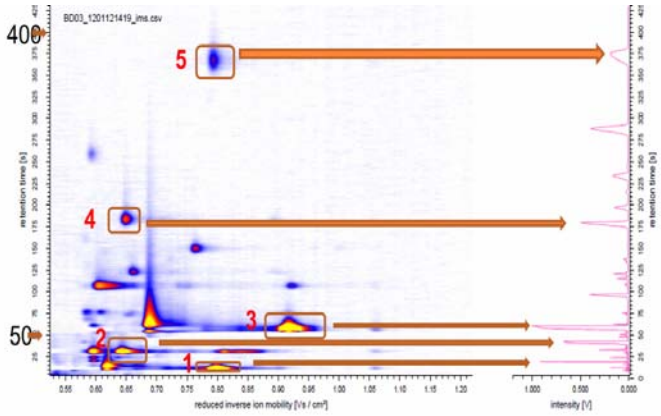

Figure 2: Alignment of a mixture of different compounds

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Publications

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Software for analysis of parallel MCC/IMS and GC/MS measurements

ion mobility spectrometry, gas chromatography, layer, automation

The identification of molecules in a complex sample as breath necessitates a reference database. Existing ion mobility spectrometry (IMS) databases are still growing and an explicit identification is not always possible. Hence, gas chromatography-mass selective detector (GC/MSD) analysis are used in parallel to identify detected analytes by comparison of the chromatograms. We developed a software tool to speed up and automate the comparison by generating an analyte layer for the IMS software VisualNow using the corresponding GC-MSD chromatogram.

Using multiple capillary column (MCC)/IMS databases for the identification of indistinct peaks can be challenging due to several reasons. One problem is the partial superimposition of the peak localization. In such cases analysis of the mass spectrum can help to identify the peaks. On the other hand manual analysis of the GC/MS data coupled to MCC/IMS measurements is tedious and requires many different manual steps and parallel running software tools.
The whole process can be automated using our new software tool named MIMA (MS-IMS Mapper). Analysis can be organized in the following two main steps (fig. 1). In the first step, the batch functionality of a mass spectrometry software is utilized to automatically process a set of chromatograms. Then a list of all detected peaks and the corresponding best match in the NIST mass spectral library are exported.
In the second step, MIMA is parsing the peak list files and extracts the identified analyte names and their chemical abstract service number (fig. 1).

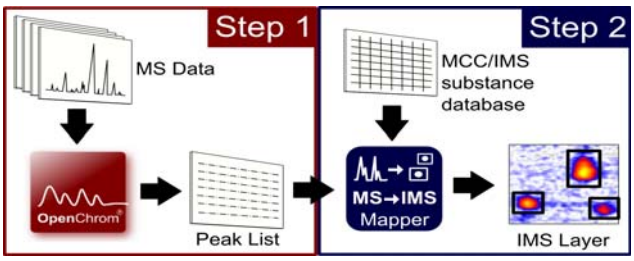

Figure 1: Schematic step by step process

This so called CAS-number is matched to the entries within the MCC/IMS substance database. The matching entries are used to create a new file, named IMS layer. This layer mainly contains a list of analytes and the corresponding coordinates within the IMS-chromatogram. Finally, this layer can be superposed on the IMS chromatograms, which corresponds to the processed mass spectrometric chromatograms.

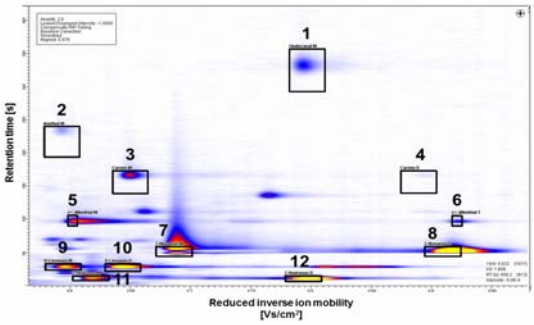

Figure 2: MCC/IMS spectrum including the automatic generated layer

The reliability of the software was tested by parallel GC/MSD MCC/IMS measurements of a reference substance mixture with 7 components and a subsequent automated analysis. Using MIMA an IMS layer was obtained from GC/MS measurements and superposed on the corresponding IMS-chromatogram (fig. 2).

In total 12 signals in the IMS chromatogram could be identified by the layer, which arise from the reference analytes and their dimers and trimers.
Thus, the functionality of our software solution was demonstrated successfully by identification of all reference compounds in the automatic generated layer superposed on the IMS chromatogram. However, an assignment to be solved is the limited number of analytes in the IMS databases, which are used in comparison with the NIST to generate the layer. The fact that missing analytes cannot be displayed despite their presence in the NIST necessitates the enlargement of IMS databases.
In comparison to other methods our software solution provides a tool, which improves peak identification in MCC/IMS chromatograms by automation of parallel GC/MSD data analysis. Our new method providing suggestions for an indistinct MCC/IMS signal within minutes.

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Publications

(1) Eisinger, K.; Maurer, F.: Hauschild, A.-C.; Baumbach, J.I.: Identification of analytes in MCC/IMS chromatograms by aligning accompanying GC/MS measurement data with a new software tool. *Submission to IJIMS - in progress*

Development of a PLS-based integrated addition model for mixture toxicity

mixture toxicity, partial least squares, integrated addition model

As a novel approach to predict the toxicity of chemical mixtures using knowledge on their mixture components, a partial least squares-based integrated model (PLS-IAM) was successfully developed in this study. The PLS-IAM combined the concentration addition model with the independent action model and was based on the partial least squares regression technique. Through the four validation test datasets, this study showed that the PLS-IAM overall outperformed the existing reference models.

The predictive capacity of the conventional models, concentration addition (CA) and independent addition (IA) models can be strictly restricted unless accurate MoAs on all mixture components are readily available. Knowledge on such MoAs of chemicals still remains lacking. Therefore, these facts create need for developing integrated addition models (IAM) and combining CA and IA concepts, at least for calculating additive toxicity of non-interactive mixtures regardless of whether mixture components produce

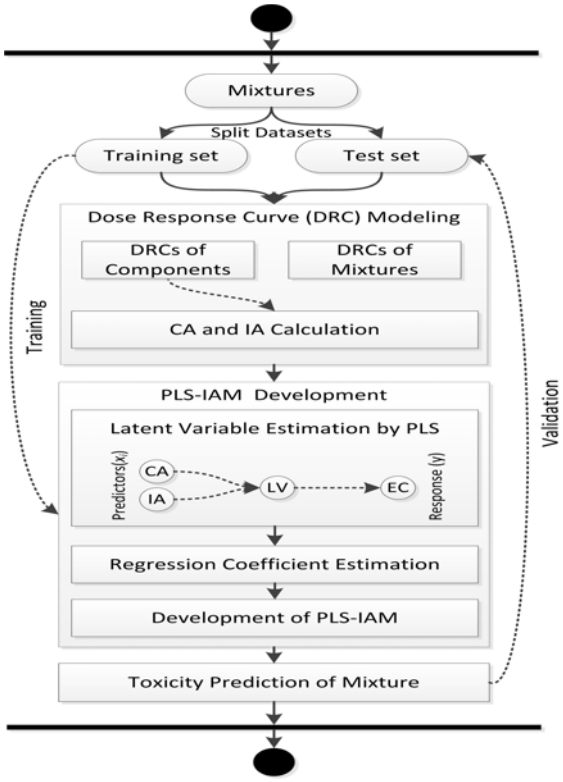

Figure 1: The PLS-IAM approach for determining the toxicity of mixtures.

similar, dissimilar, or both similar and dissimilar MoAs. An IAM, called the 'integrated concentration addition with independent action based on a multiple linear re-gression (ICIM) model', was recently developed by Qin et al. based on a multi-linear regression (MLR) technique to combine the CA and IA models. However, in case of a linear relationship between any pair of predictor variables (i.e. presenting a multicollinearity problem), prediction results through the OLS regression used in the ICIM model cannot be strictly guaranteed to work statistically well despite its ability to calculate good prediction values.

The objectives of this study were to develop and evaluate a PLS-based integrated addition model (PLS-IAM) not only to overcome the multicollinearity problem, but also to combine the independent variables into an IAM model using the latent variable that accounts for most of the variation in the response. The predictive performance of the PLS-IAM was evaluated by four validation datasets. Each dataset was composed of training data for developing the PLS-IAM and test data for validating the developed PLS-IAM. Dataset 1 was developed from the present study for the mixture toxicity of ten pesticides *on Vibrio fischeri*. As additional datasets, Datasets 2, 3, and 4, derived from previously published studies, were used for further validation of the PLS-IAM. Those three additional datasets were divided into three types of data as follows: Type 1, a mixture with similarly acting components [Dataset 2: eight chloroacetanilides on *Scenedesmus vacuolatus*]; Type 2, a mixture with dissimilarly acting components [Dataset 3: 16 organics on *Scenedesmus vacuolatus*]; and Type 3, a mixture with similarly and dissimilarly acting components [Dataset 4: five herbicides and four metals on *Vibrio qinghaiensis*].

On the basis of the adjusted R^2_{test} and Akaike's Information Criterion scores, the prediction results of PLS-IAM on the target mixtures were evaluated with comparing to those of CA, IA, and ICIM models. For the four mixtures, the PLS-IAM overall outperformed the other reference models, the CA, IA, and ICIM models. An IAM, called the 'integrated concentration addition with independent action based on a multiple linear re-gression (ICIM) model', was recently developed by Q the other reference models, the CA, IA, and ICIM models.

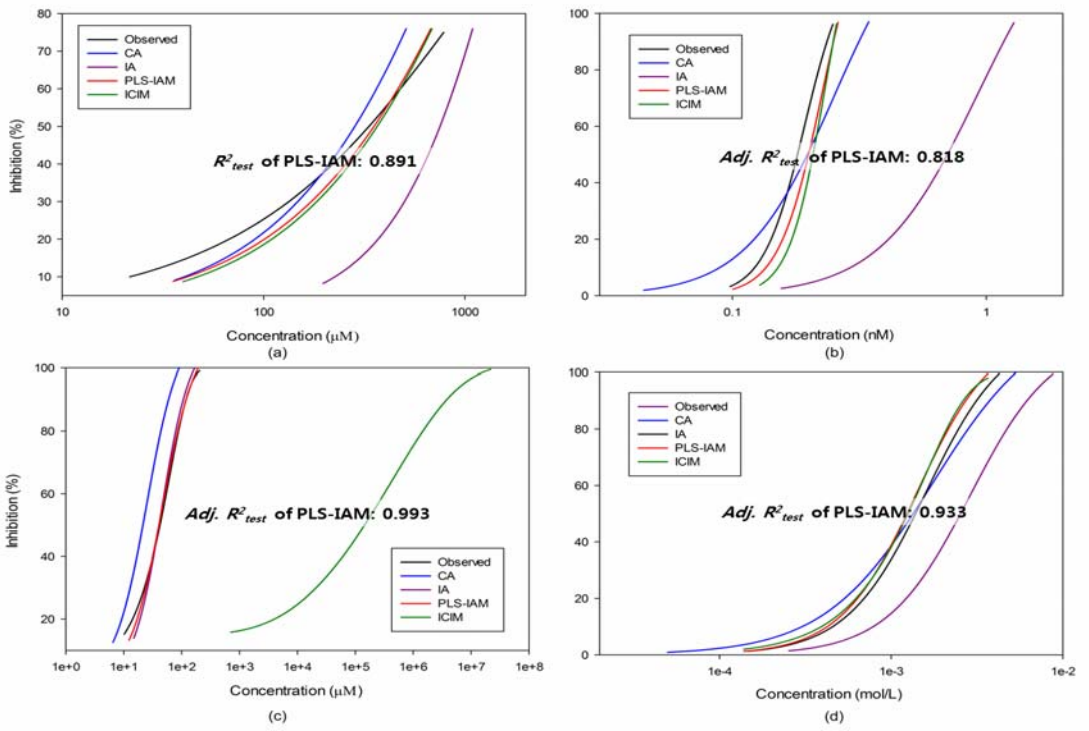

Figure 2: Overview on the comparison of CA, IA, ICIM and PLS-IAM predictions against observed toxicity for validation datasets (EC50 ratio mixtures): (a) Dataset 1, different modes of action groups (10 pesticides); (b) Dataset 2, similarly acting organics (8 chloroacetanilides); (c) Dataset 3, dissimilarly acting organics (16 compounds); (d) Dataset 4, different organic and metal compounds (5 herbicides, 4 heavy metals). The EC50 ratio mixture is an equitoxic mixture based ratio at 50% effect concentration of each compound.

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Publications

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(2) Jongwoon Kim, Sanghun Kim, Gabriele E. Schaumann: Reliable Predictive Computational Toxicology for Mixture Toxicity: Toward the Development of Innovative Integrated Models for Environmental Risk Assessment. *Environmental Science and Biotechnology, 2012, doi: 10.1007/s11157-012-9286-7*

Alternative Path to the EU Mixture Risk Assessment Scheme

mixture risk assessment, case study, computational simulation

Two methods, Key Critical Component (KCC) and Composite Reciprocal (CR), are presented in the EU draft technical guidance documents. The KCC considers only one key critical component as the whole mixture of equal danger, whereas the CR indirectly considers the contribution of each component to mixture toxicity. In this study, the two methods were firstly evaluated via a case study and a computational simulation. In addition, as a tentative alternative to the two methods, a tiered approach combining 'Enhanced KCC' and 'CR' methods was proposed.

The EU presents the draft technical guidance documents (TGDs) for deriving the Predicted No Effect Concentration (PNEC) and Derived No Effect Level (DNEL) in mixture risk assessment. These values are used as sources of information for risk characterization as part of an environmental and human risk assessment scheme. The main approaches in the EU draft TGDs for estimating the PNECs and DNELs of mixtures are Key Critical Component (KCC), and Composite Reciprocal (CR) PNEC and DNEL methods.

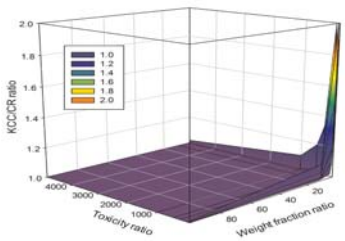

Figure 1: Result of the 3D computational simulation of the KCC/CR ratio variation, according to the toxicity ratio and weight fraction ratio between 2 components in a mixture.

The objective of this study was to evaluate the KCC and CR methods used to determine the PNEC and DNEL of a mixture through a case study and a computational simulation. The case study on coating products (having different compounds) and computational simulation were performed while considering influencing factors and focusing on the causes of the discrepancy in estimations between the two methods. This study discusses not only the limitations of the two methods in terms of concept, implementation, and performance, but also what can be considered for the use of the two methods for regulatory purposes. The case study and simulation were conducted for experimentally evaluating major factors (e.g., toxicity values, weight fractions, number of components, and assessment factors) that influence the discrepancy in estimates between the KCC and CR methods. The number of components that had similar toxicity values, assessment factors, and weight fractions were confirmed as the key factors that triggered the difference between the results of the KCC and CR methods. The assessment factor can be a less dominant factor than the others, assuming that valid toxicity datasets are used for the PNEC or DNEL values. This observation leads us to conclude that the number of mixture components with similarly weighted PNECs and DNELs in the same exposure pathway first requires checking before the application of either KCC or CR methods.

From a precautionary principle point of view, the following tentative solution can be suggested to effectively apply the KCC and CR methods: we

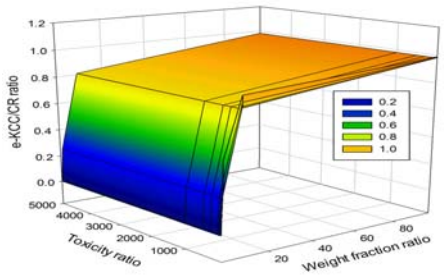

Figure 2: Result of the 3D computational simulation of the e-KCC/CR ratio variation, according to the toxicity ratio and weight fraction ratio between 2 components in a mixture.

propose a two-tier approach combining the enhanced KCC (e-KCC) and CR methods as an alternative to using either the KCC or CR methods. Through this approach, the e-KCC method might be used to maintain the advantage of the original KCC method and reduce concern about the non-additive toxicity concept of the KCC method. The PNEC and DNEL values calculated by the e-KCC method were less than those produced from the CR method. Therefore, the CR method can be considered as the second tier only when the risk characterization ratio (e.g. exposure levels to DNELs or PNECs) derived from the e-KCC method exceeds 1.

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Toxicity of Surface Modified Nanoparticles in Aqueous Solution

metallic nanoparticles, surface modifications, MARA

Four types of nanoparticles coated with different techniques, where nanoparticles bind through physical and chemical mechanisms and have hydrophilic and amphiphilic characteristics, were investigated. Gold nanoparticles with citrate, silver nanoparticles with citrate, polyethylene glycol (PEG) and bovine serum albumin (BSA) coatings were used. Their physicochemical properties were measured and bioassays were performed using a microbial assay for risk assessment (MARA) screening system containing 11 genetically diverse microorganisms.

Metallic nanoparticles are widely used in the medical application because of their optical properties and easy surface modification. However, their application has been limited because of their unstable conditions in the aqueous solution. Surface modification is a popular technology adopted to overcome the weakness of nanoparticles by increasing biocompatibility, stability and reactivity, which also increases the concerns on the adverse effect. However, their toxicity researches have not been widely investigated and mainly focusing on the cytotoxicity. Considering the increased use of surface modified metallic nanoparticles (SMMNPs), it is inevitable to study the adverse effects on not only the human health but also on the environment. Therefore, a study to elucidate the potential environmental fate and ecotoxicity of SMMNPs according to their physiochemical characteristics was performed in this work. In addition, bioassay on SMMNPs was also performed as a screening test for ecotoxicity in the aqueous solution.

SMMNPs with the size 10-20nm were selected as target materials. Their physical properties as the shape, size, and aggregation were measured by UV-vis spectrometer and Scanning Electron Microscope (SEM). UV-vis spectrum shows that citrate-coated gold nanoparticles (AuNPs) have the peak absorbance at 524nm, which is the general peak absorbance observed with AuNPs with the size of around 20nm. Contrarily, UV-vis spectrum shows that silver nanoparticles (AgNPs) have the peak absorbance around 403-406nm. With AuNPs, the red shift was observed in absorption spectra as a consequence of aggregation during the sample preparation including dilution process.

For a toxicity test, MARA toxicity test kit, where each bacterium seeded on to 96 well plates (Fig. 1(A)), was used, and growth rate of each bacterium and yeast was measured with a scanner with a MARA software provided and an ELISA reader (Fig. 1(B)). All SMMNPs

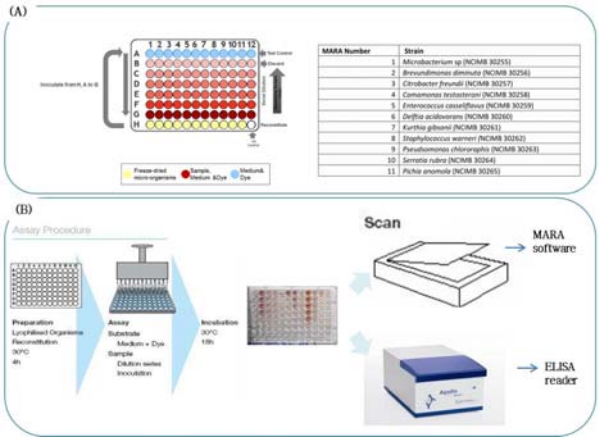

Figure 1: (A) MARA toxicity test kit and the organisms in the MARA system. (B) The assay procedure of the MARA system with a scanner and an ELISA reader: Test samples are injected into each well with serial dilution followed by color measurements after 18h incubation under 30 °C. The depth of color and pellet size is measured by a scanner and the color change is analyzed using the software provided. The absorbance is measured by ELISA reader.

did not show any toxicity with them in the concentration ranges from 0.25 to 0.01mM and from 0.157 to 0.005mM for AuNPs and AgNPs, respectively (Fig. 2(A)). The growth rate of citrate-coated AuNPs was also investigated by an ELISA reader (Fig. 2(B)), and visible decreases in growth rate of No. 7 and 8 bacteria and No. 11 yeast were observed. Among them, there was significant difference for No. 8 bacteria (Pseudsomonas chlororaphis).

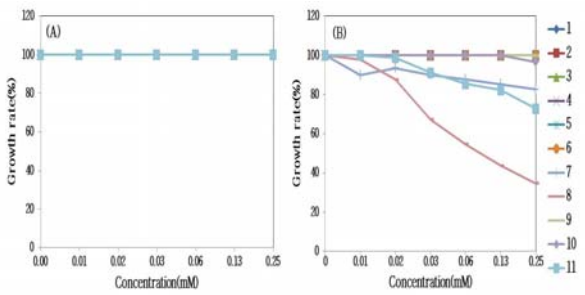

Figure 2: Percent (%) growth rate of citrate-coated AuNPs by (A) a scanner with a MARA software and (B) an ELISA reader.

Consequently, it is known that the MARA system with bacteria and yeast has some limitations to use. The MARA system with a scanner was less sensitive than an ELISA reader. Other systems for ecotoxicity of nanoparticles and species such as daphnia magna with better sensitivity on nanoparticles have to be considered in future works.

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Analysis of the network in the clean development mechanism

clean development mechanism, collaborative technology innovation, social network analysis

This research analyzes dynamics of the global partnership networks of Clean Development Mechanism (CDM) by using social network analysis framework. The results of this research show that the density of CDM networks decreases by time. In addition, leading groups in CDM partnership networks tend to shift into host countries. Finally, a host country with more collaborative projects rather than unilateral ones better utilizes global knowledge resources. This research was done in collaboration with Chinese University of Hong Kong.

The CDM is a global collaborative action proposed at the Kyoto Protocol in response to climate change issues. The CDM contributes to cost-efficient reduction of greenhouse gas emissions in industrialized countries and promotes sustainable development in developing countries.

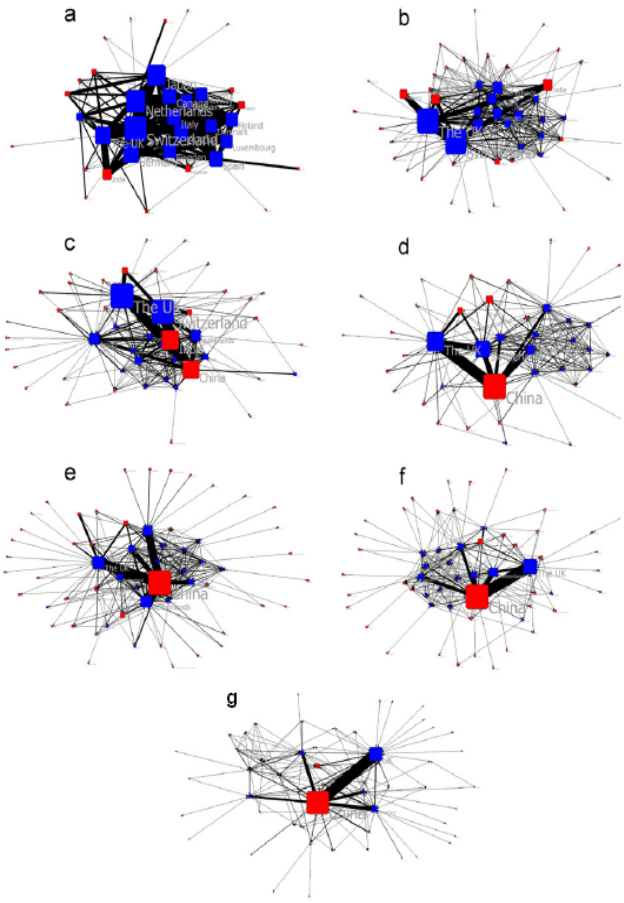

Figure 1: The dynamics of collaboration networks in the CDM partnership from 2005 to 2011. (a) 2005, (b) 2006, (c) 2007, (d) 2008, (e) 2009, (f) 2010 and (g) 2011

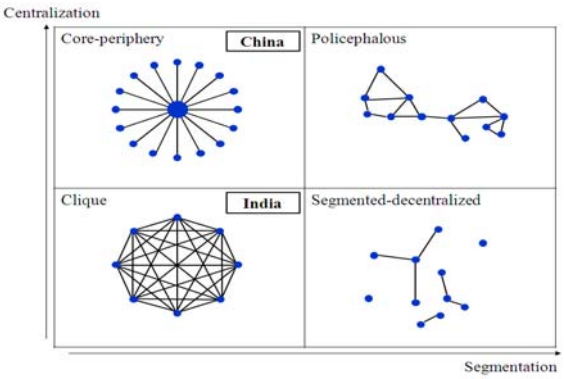

Figure 2: Network structure type (DIANI, 2003 cited in Ernstson et al., 2008)

Its fundamental framework is based on partnerships between industrialized and developing countries. This study employs social network analysis to investigate the dynamics of the partnership networks observed in 3,816 CDM projects registered in the database of the United Nations Framework Convention on Climate Change over the period of 2005 to 2011. Our three main findings can be summarized as follows.

First, the CDM partnership network is a small world; however, its density tends to decrease as the number of participants for a CDM project decreases. Second, the partnership networks' leading groups tend to shift from partner countries into host countries. Third, a host country that pursues more partnership-based projects takes better control of resources and knowledge-flow in the ego-network formed around that country, and can thus better utilize global resources for its CDM projects.

Compared with idealized network models (Diani, 2003 cited in Ernstson et al., 2008) in Figure 2, CDM networks have developed in the direction of the core-periphery model, with high centralization and low segmentation. CDM networks have observed the recent emergence of a few central countries, so the policephalous model can also be used to explain the current state of global CDM networks.

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Challenges and drivers for waste ink recycling business

sustainable waste management, waste ink recycling, business model, European printing industry

This research investigates main challenges and drivers for adopting innovative technologies for waste ink recycling in EU printing industry. For this purpose, we conduct an empirical survey of European printing companies and their up- and downstream partners in collaboration with Bureau van Dijk. This research is carried out under SuWAS (Sustainable Waste Management Strategy for Green Printing Industry Business) project funded by German Ministry of Education and Research and organized by EU ECO-INNOVERA programme.

By far, printing industry in EU has generated a large quantity of hazardous waste ink and most conventional technologies for the waste ink treatment is mainly based on incineration which produces enormous environmental pollutants such as CO2, NOx, and PAHs. With strengthening of regulatory pressures to recycle, recover, and reuse the waste, sustainable waste management in the printing industry has to be sought. For the purpose, advanced technologies for recycling of waste ink have been already developed. Nonetheless, the printing industry still confronts with difficulties in implementing the recycling technology due to absence of effective business model. Furthermore, main challenges and drivers for a successful adaptation of the recycling technology in the printing industry have to be identified as prerequisite essential for establishing the business model.

Therefore we are analyzing market structure and drawbacks of current waste ink management based on literature and opinion survey. Based on this, an empirical investigation into the European printing industry is being carried out for addressing up- and downstream stakeholders, not only existing waste ink treatment system but also potential systems which can be affected by implementation of the recycling process. As a consequence, this research is scrutinizing the challenges and drivers as key factors for business strategies of waste ink recycling that can benefit various stakeholders in the printing industry, which contribute to designing the business model for the waste ink recycling in the printing industry.

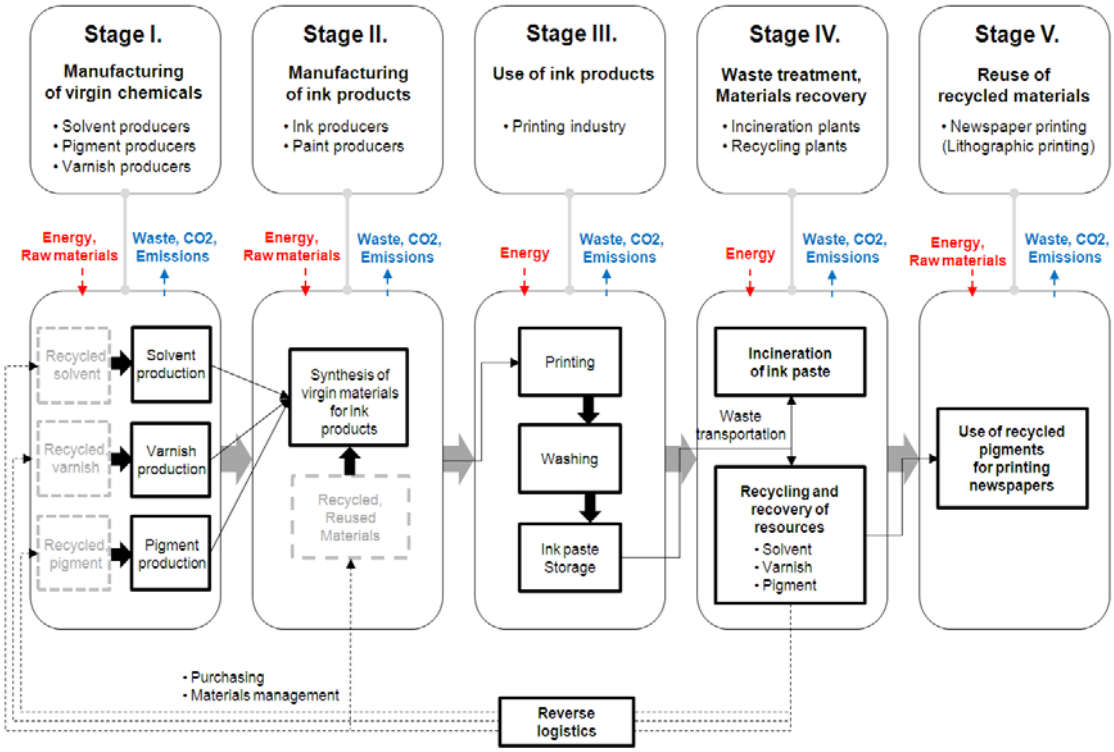

Figure 1: Life cycle of the printing ink

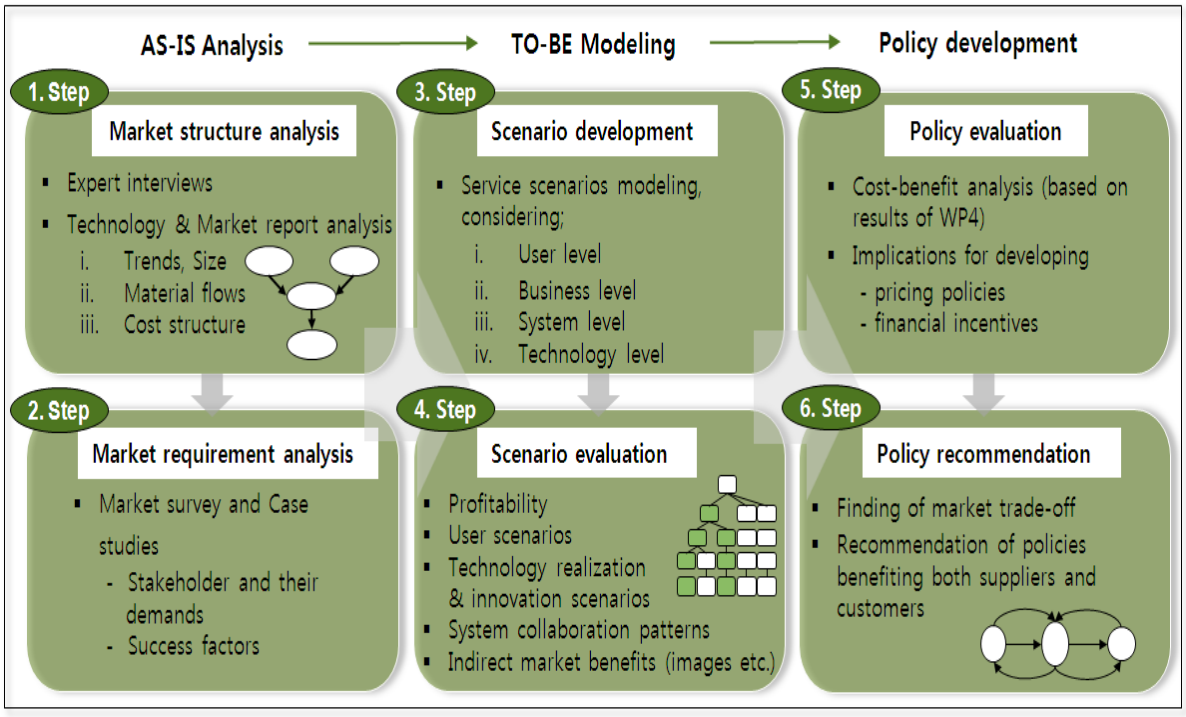

Figure 2: Work plan for development of sustainable business model and supporting policy

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Controlled Nucleation and Growth of Protein Crystals by Solvent Freeze-Out

zone I crystallization, controlled nucleation, metastable zone, KPVLs (key process variable levels)

The best quality of the protein crystals was produced with an initial protein concentration of 4 mg/mL. Consequently, the initial necessity for the optimized crystallization strategy was accomplished well at pH 4.4 and 5% (wt) NaCl with an initial protein concentration of 4 mg/mL under the temperature profile for the freeze out crystallization system. The numbers of tetragonal HEWL crystals were increased by the ice mass since the temperature with cooling rate applied to the system determines the ice growth rate, and therefore nucleation and growth rates of the protein crystals. Hence, nucleation of the given protein system can be controlled by adjusting moderately the ice growth rate and the temperature of the crystallization system.

Solvent freeze-out technology was developed as a new concept in the field of protein crystallization. This technology allows separation of the nucleation and growth steps, but requires understanding of the thermodynamics of the complex mixture of protein, solvent, salt and buffer at temperatures for which little accurate data currently exists. The phase diagram of the given protein system was systematically investigated and confirmed for the identification of optimal crystallization conditions for Zone I which is the best region of protein crystallization by employing a preliminary screening. As an initial necessity for protein crystallization the delicate balance between repulsive and attractive forces in the given protein system was found at pH 4.4, and 5% (wt) of NaCl.

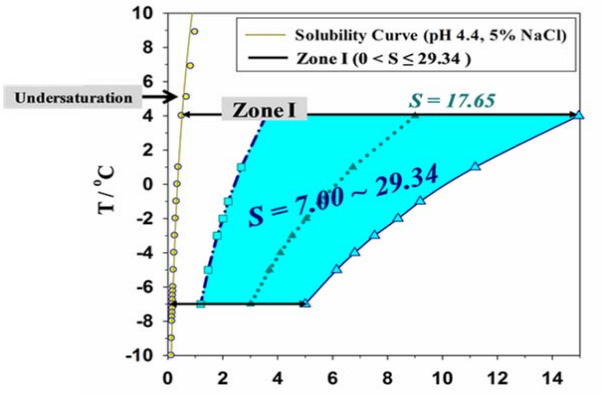

Figure 1: Phase diagram for HEWL with 5% (wt) NaCl at pH=4.4 in NaAc buffer (: solubility line at pH 4.4, : S=7, : S=17.65, S=29.34)

The precise value of the supersaturation level of Zone I was estimated to be 9.56 ≤ S ≤ 29.34 after a statistical analysis of the initial screening by a linbro test. Then the supersaturation levels for the metastable zone were identified to be 7.0 ≤ S < 17.1 from the statistical analysis of all experimental results from both the linbro test and the individual crystal growth measurements. Protein crystallization in Zone I by the freeze out technology was carried out and evaluated. The key process variable levels (KPVLs) were operated within the boundary of the phase diagram which is confirmed by the preliminary screening. At 5% (wt) NaCl concentration with pH 4.4 quite a good quality of tetragonal HEWL crystals were produced due to proper tuning of a net surface charge of HEWL even with a very low value of the initial protein concentration.

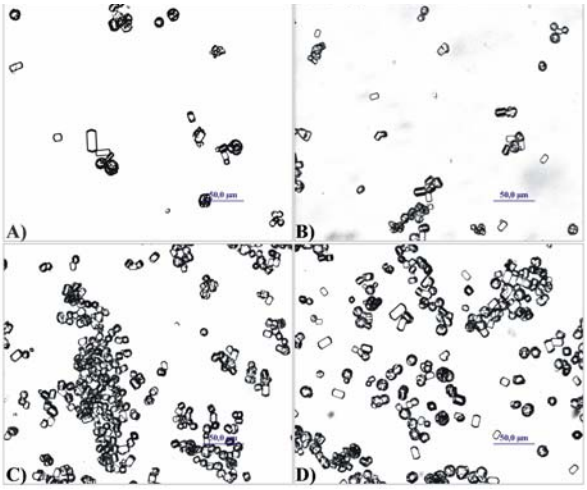

Figure 2: Tetragonal HEWL crystals for different ice masses (IMs) at pH 4.4 with 5 wt % NaCl concentration: (A) almost no ice, (B) IM = 4.24g, (C) IM = 26.48g, (D) IM = 33.72g)

The numbers of tetragonal HEWL crystals were increased by the increasing of the ice mass since the cooling rate applied to the system determines the ice growth rate, therefore nucleation and growth rates of the protein crystals. Hence, nucleation of the given protein system can be controlled by adjusting moderately the ice growth rate.

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Nano Letters	2010	Neuzil, P. et al.	30
Thorax	2009	Westhoff, M. et al.	29
Biosens Bioelectron	2009	Jose, J.et al.	18
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Lab Chip	2011	Rodriguez-Villareal, A.I. et al.	13
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Mol. Cell Biol	2011	Reiter, A. et al.	5.5
Polymer Chem	2011	Stöhr, T. et al.	5.3
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* author with other co-authors incl. actual KIST employees

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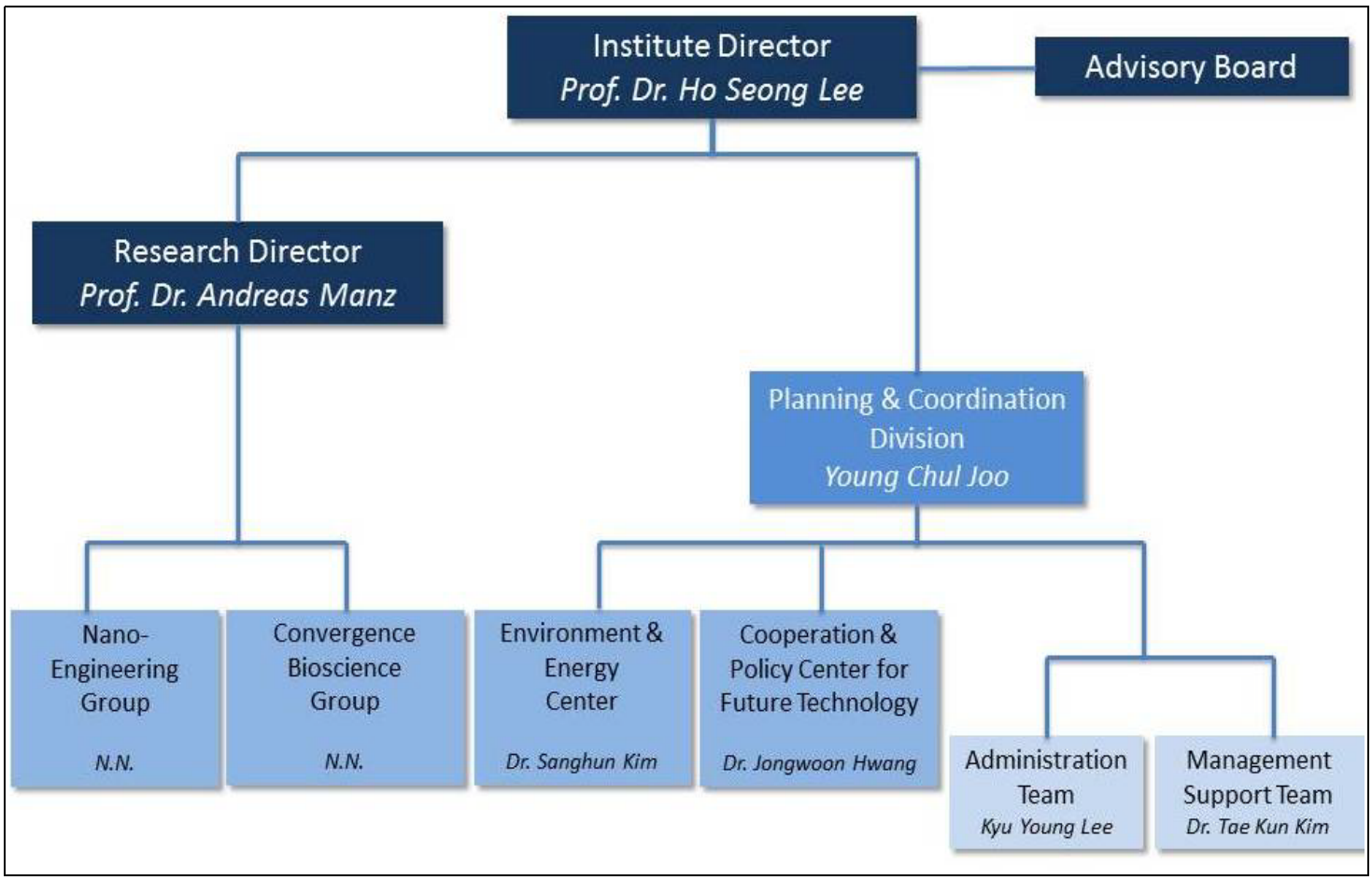

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from Frankfurt Airport:

By train directly from Frankfurt Airport Station to Saarbrücken Main Station by fast train (ICE/IC) or local train (RE/RB)
For more information, see:
www.bahn.de/i/view/GBR/en/index.shtml

By Taxi: please contact us for reservation (200-500 Euro per transport)

from Paris:

By train from Paris Gare de L'Est by fast train ICE/TGV

By Car

Information for the navigation system:
66123 Saarbrücken, Stuhlsatzenhausweg 97

From east (Frankfurt/Mannheim/Karlsruhe):
„A6 Mannheim-Paris“ motorway up to the exit „St. Ingbert West“. Follow road signs to „Universität“.

From north (Köln/Koblenz/Trier):
Motorway „A1“ until junction „Autobahnkreuz Saarbrücken“, then motorway „A8“ until junction „Autobahnkreuz Neunkirchen“. Then proceed towards Saarbrücken on motorway „A6“ (see „from east“).

From France:
Motorway „Paris-Mannheim“ until exit „St. Ingbert West“. Follow road signs to „Universität“.

From Luxemburg:
Motorway „A620“ until Saarbrücken, exit „Wilhelm-Heinrich-Brücke“. From there, follow road signs to „Universität“.

By Airplane (Airport Saarbrücken-Ensheim)

Airport Saarbrücken has direct connections with the following cities: Berlin THF, Dresden, Düsseldorf, Hamburg, Leipzig, Luxemburg and Munich. Taxi to KIST Europe takes app. 20 minutes.

By Train (Central Station of Saarbrücken)

More information:
www.bahn.de
(German Rail)

- from Frankfurt Airport via Mannheim by fast IC/ICE
- from Paris Gare de l'Est by fast ICE/TGV
- from north-west from Cologne via Koblenz/ Trier by Regional Express
- from north-east via Mainz by Regional Express

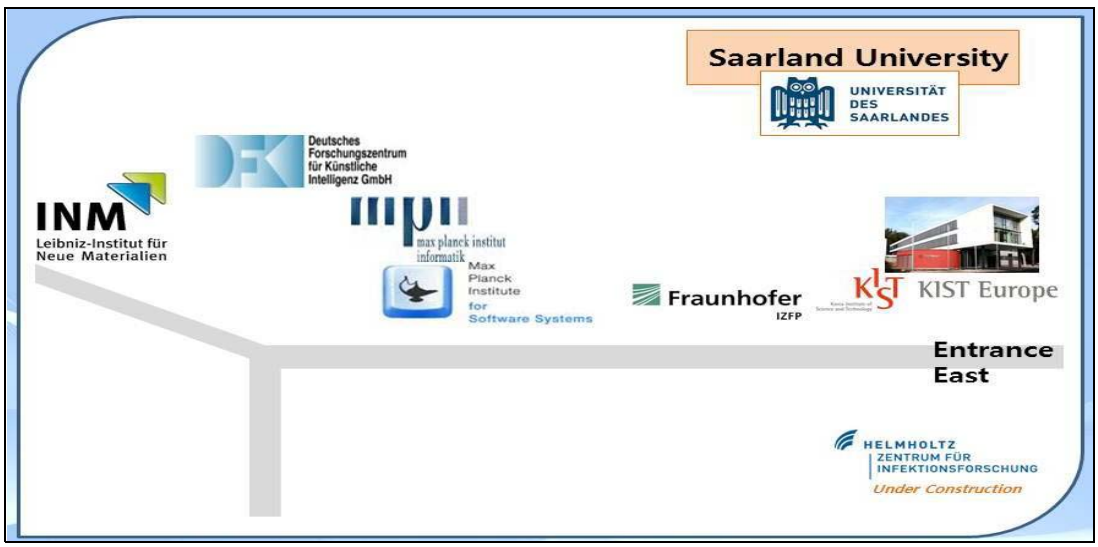
By Bus

More information:
www.saarbahn.de/de/fahrplan
(Saarbrücker Bus Timetable)

Busstop: „Universität Busterminal“

- from Saarbrücken Central Station: bus No. 102, 112 or 124 (every 30min.)
- from City Centre: bus No. 101, 102 109 or 111 (every 30min.)

On the campus of Saarland University



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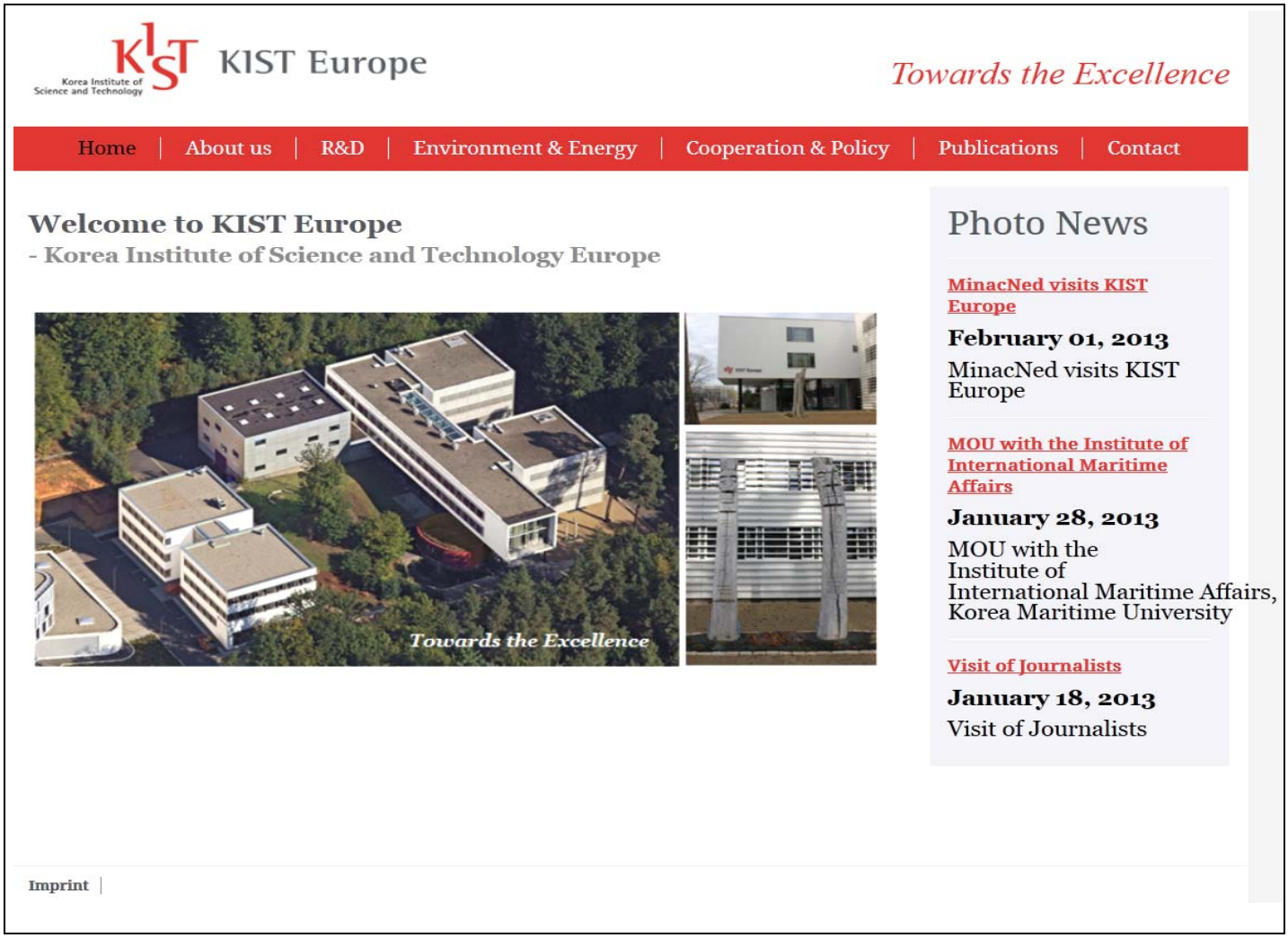

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