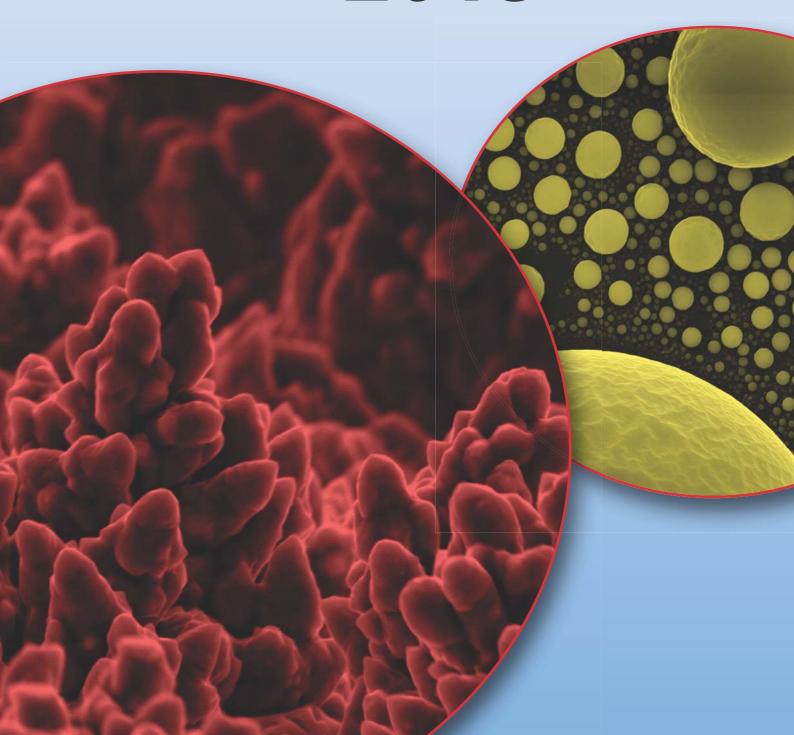


# Annual Report 2013



### **Illustrations (photos taken at KIST Europe):**

Cover:

left: Electrolytical hierarchally structured copper surface

right: Tin on carbon

Back:

Glass chip with 36 units grouped in 9 sets of four units HUVECs (Human Umbilical Vein Endothelia Cells) forming tube like structures in-vitro KIST employee working in the laboratory

### **Publisher:**

Mail:



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

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# Greetings of the Institute Director

Dear Readers,

It has already been a year since I was inaugurated as the director of KIST Europe. I am proud to say that everyone has given their best effort in 2013. Personally, what was most satisfying for me was being able to get to know our employees a little better through our 'Lunch with the Director' and 'Tea with the Director' programs.

Another highlight was being able to host the workshop to discover a joint research theme with our neighboring institute, INM (Institute for New Mate- rial), in April, 2013, which was especially meaningful. The momentum from this carried over leading to KIST Europe holding the Korea-EU workshop in October with the theme of "Future Materials and Safety". A total of 70 distin-guished participants from KIST, KRISS, KRICT, and KRIBB from the Korean side, and INM, Saarland University, and Max Planck Institute-FKF from the German side participated in the workshop to establish a joint research subject. Further to this, after the workshop, KRCF (Korea Research Council of Fundamental Science and Technology) provided financial support for a preliminary study to determine and plan the joint research subject. As a result, a joint research on two themes, 'Bio material' and 'Nano safety' with INM will kick off from January, 2014.

Also, in cooperation with KIRD (Korea Institute of Human Resources Development in Science & Technology), KIST Europe hosted an Executive Training Program for Korea-EU International Collaboration from October 8-19. Participants from 16 Korean organizations visited KIST Europe, JRC (Joint Research Center) in Brussels, Belgium and OECD in France and learned about EU's R&D programs and international cooperation efforts.

KIST Europe provided support to establish the Korea Research and Innovation Center-Europe in Brussels and will also participate in EU policy and technology trend research in the future by sending manpower.

The year 2013 was the start of KIST Europe branching out and expanding from Germany to other parts of Europe. I would like to express my sincere gratitude towards MSIP (Ministry of Science, ICT and Future Planning), KRCF and KIST Seoul for all of their support.



Prof. Dr. Ho Seong Lee

Institute Director mail: hslee@kist-europe.de

### **Preface**

# Greetings of the Research Director



**Prof. Dr. Andreas Manz**Research Director
mail: Manz@kist-europe.de

Dear readers,

I am very proud to present the research activities and achievements of KIST Europe during 2013 in this annual report.

This year, the research at KIST Europe had some changes in personnel: First, Prof. Leon Abelmann was hired as a new group leader in Nano-Engineering. He is from the MESA+ at the University of Twente (The Netherlands) with a background in magnetics and nanoscale engineering. He started his work at KIST Europe in July 2013, and will mainly work on magnetotactic bacteria and magnetic imaging. He is also active in novel forms of education.

The Convergence Bioscience Group had Dr. Myung Hee Jung as an ad-interim leader from March till August 2013, until Dr. Sanghun Kim took over the group in September. This was a very important step to secure continuation in Nanomedicine. As a new team leader, Dr. Young Jun Kim was in charge since September 2013. His activities will mainly cover bacteriophages, bioMEMS, diagnostics and drug discovery.

A new group was established under Dr. Sanghun Kim on Environment and Energy. During the year, additional laboratory space was equipped and many new researchers / students joined. The main activity will be in ecotoxicity of novel nanomaterials in the environment.

These recently started activities will continue to draw our attention in the coming year. The bibliometric research output keeps improving, and new collaborations within Korea and Europe will be in our focus for the near future. Our excellent infrastructure is placed in 1,500 m² of lab space, and the workforce in our research labs stands at about 50 students and coworkers. We are all very satisfied with the research opportunity we have here at the Saarbrücken site, and I would like to acknowledge, in the name of all, the generous funding by the Korean government, the goodwill of our host, the Saarland University, and the continued support by our collaboration partners in Korea and Europe.

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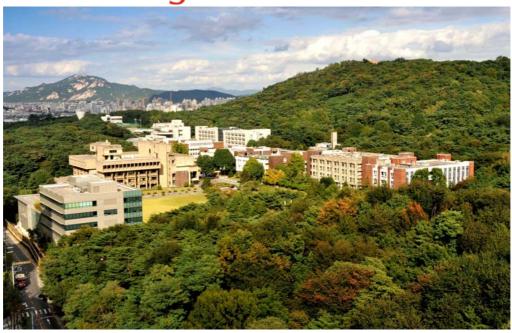


# **KIST Headquarter**

### Introduction

# Korea Institute of Science and Technology (KIST), Seoul (Korea)





KIST was established in 1966 with the primary goal of developing creative, original technologies which would build Korea's science and technology capacity by transferring research results and achievements to industry.

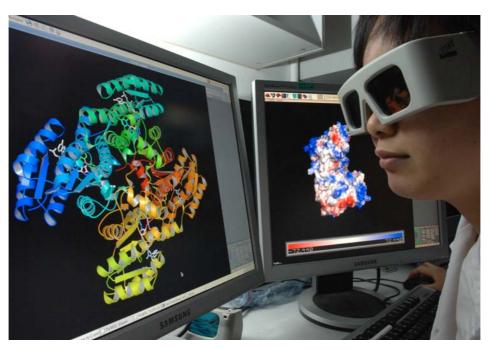
By concentrating on urgently needed technological development during the early stages of Korea's industrialization, KIST contributed to the modernization of industry and helped push ahead a period of rapid, remarkable economic growth for Korea. In addition, over the years KIST has produced a pool of premier S&T talent and spun-off numerous specialized research institutes. These accomplishments have guaranteed its continued role as the country's leading S&T institute. Putting its years of accumulated R&D expertise and versatility to work, KIST now is looking to expand its role and become one of the world's leading R&D institutes by taking on largescale, long-term, interdisciplinary fusion and system-oriented R&D projects that are too challenging for universities or the industrial sector to carry out.

KIST envisions itself solving national problems as well as making significant contributions to a broader range of global issues affecting the world in general.



# **KIST Headquarter**

# Introduction



### Research Areas

- Brain science: neuroscience, functional connectomics, neuro-medicine, biomicrosystem
- Biomedical research: bionics, biomaterials, theragnosis
- Multi-disciplinary convergence of matter: materials architecturing
- Opto-electronic convergence systems, computational science
- Green city technology: water resource cycle, environment, health and welfare
- Urban energy system, energy convergence, integrated risk
- Future convergence research: spin convergence, interface control
- High temperature energy materials, electronic materials, molecular recognition
- Chemical kinomics, nano quantum information
- National agenda research: fuel cell, clean energy, sensor system, photo-electronic hybrids, human-centered interaction & robotics, imaging media

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### Introduction

### KIST Europe GmbH, Saarbrücken (Germany)



On February 10th, 1996 KIST Europe was founded as an overseas branch in Saarbrücken (Germany) after an agreement in respect to this was reached between the Ministers of Research of Korea and Germany on the occasion of a state visit of the Korean president one year earlier. The foundation act took place on almost the same day as the 30th birthday of the KIST Seoul.

During the first years, 14 employees worked in leased office and laboratory space at the University of Saarbrücken, until it was decided to establish an own institute building on the University Campus.



Within the time from the ground-breaking ceremony on April 18, 1998 until its completion in the year 2000, the first KIST Europe Institute building was created on the grounds of the University of Saarland and in close proximity to prestigous research institutes - Leibniz Institute for New Materials (INM), the German Research Center for Artificial Intelligence (DFKI), the Max-Planck Institute for Computer Science (MPI) and the Fraunhofer Institute for Computer Science (IZFP) - providing office spaces for 60 employees such as laboratories and a technikum.

8 years later, in May 2008, the groundbreaking ceremony was held on the occasion of the establishment of a second university building, the Korea-EU Cooperation building, for providing space for the Industrial Cooperation Center, onsite laboratories of KIST and other Korean research organizations such as some more office -

and laboratory space for the growing number of employees. The construction works of the second institute building began in September 2008 and were completed in March 2010. In July 2012, the potential of use of the new building was maximized by the installation of a high standard biolab.

Under the direction of the 6th Institute's Director, Prof. Dr. Ho Seong Lee, and the Head of Research, Prof. Dr. Andreas Manz, KIST Europe today employs about 60 employees in the fields of research and administration, coming from different cultures and countries, such as numerous internship students and guest researchers from Korea and other parts of the world. The total amount of approximately 75 persons is spread over the two buildings with a total of more than 9.000 m<sup>2</sup>.

The areas of KIST Europe's research activities are wide spread and range from Microfluidics over Cellular Immunotherapy to Green Technology. At the time being, 11 projects are in process, some of which are partially or fully funded by third party funding. One of them is a Korean-German Joint Research on Algea Treatment with focus on testing the toxicity of cyanobacteria and on finding techniques for toxicity removal.



### Introduction





Besides KIST Europe, renowned partners are involved in the project: the Technical University Berlin (German Partner) and KIST, KRIBB, KICT, SNU,GIST, SKUU and others (Korean partners).

As a result of the research activities, KIST Europe currently has 18 national and international patents registered and 67 patent applications pending.

KIST Europe collaborates closely with numerous institutes and enterprises inland and abroad. Thus, since the completion of the second building, the Korean Institutes KETEP, KAERI and KICT such as the German Institute Ursapharm have successfully installed Branch-Labs in the Korea-EU Cooperation building. Furthermore, numerous MoU's (Memorandum of Understanding) have been signed between KIST Europe and for example the Korea Maritime University, KOFWST and KEITI, Yonsei University and University of Science and Technology and many more.

The participation in and the co-hosting of several international collaboration programs (EU FP7 KORRIDOR, KESTCAP, HORIZON 2020, EUREKA etc.), the organization of workshops (Bubble Tech to Bio App Lab on a Chip 2011, Germany-Korea Nano Bio Material Expert Workshop 2013 etc.) such as the cooperation with the German Federal Government in several projects (AiF Project, BM-Wi) round off the cooperation profile of KIST Europe.

Another field KIST Europe is taking careful account of is the improvement of the international link between Korea and the EU by establishing

global partnerships and providing access to the European market and to support the technology development activities of Korean companies for their overseas expansion into Europe.

Therefore, KIST Europe supports EU framework programs such as FP7 KESTCAP, KOORIDOR and ECO-INNOVERA and - for the future - plans to especially strengthen the mutual support for universities, research institutes and companies from Korea and Europe in order to ease their way and to perform joint research projects and international cooperation activities themselves.

KIST Europe pursues three main objectives which are:

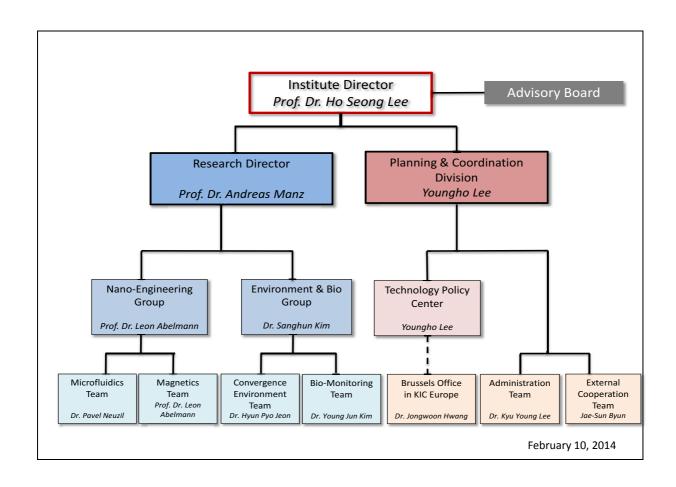
- to improve the expertise of Korea in the field of science and technology by conducting research activities in Europe (EU On-Site Research),
- to gain a key position for international technology exchange and joint research with Germany, Eastern Europe and Europe as whole (Korea-EU Cooperation Hub) and
- to support the technology development activities of Korean companies for their overseas expansion into Europe (Industry Support).

KIST Europe's goals for the future are clear: becoming the Center of Excellence in environmental and integrative nano-bio-engineering research and establishing the platform for effective S&T collaboration between Korea and the EU.

### Towards the Excellence.

# Introduction

### Organization





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### Introduction



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### Introduction

### **Advisory Board**

### **Chairman:**

### Dr. Byung Gwon Lee

President of Korea Institute of Science and Technology, Seoul (Korea)

### **Korean Members:**

### Dr. Kinam Kim

former President of Samsung Advanced Institute of Technology

### Prof. Dr. Soo-Won Kim

Professor at Korea University, Dept of Computer and Engineering

### Jae-Hong Lee

Director General of International Cooperation Bureau MSIP

### **Seung-Wook Yang**

Managing Director of Hyundai Kia Motors Europe Technical Center

### **German Members:**

### Prof. Dr. Günther Fuhr

Director of Fraunhofer Institute for Biomedical Engineering IBMT

### MinR Christian Jörgens

Head of Division Asia and Oceania, German Federal Ministry of Education and Research

### Jürgen Lennartz

Head of State Chancellery of Saarland

### Prof. Dr. Volker Linneweber

President of University of Saarland

### Prof. Dr. Dieter Rombach

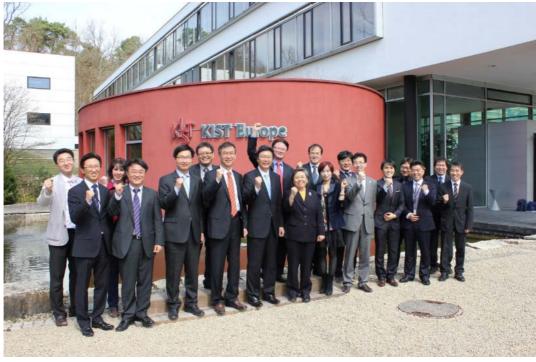
Director of Fraunhofer Institute for Experimental Software Engineering IESE

April 17, 2013

### Korean Embassy (EU) visits KIST Europe

KIST Europe had the honour to welcome Mr. Chang-Beom Kim (Ambassador), Mr. Hyuk-Chae Koo (Science Attaché) and Mr. Woojoon Kang (First Secretary).





### **Visits**

### **April 26, 2013**

### **KIST Europe Advisory Board Meeting**



### July 23, 2013

In July, the Korea Institute of Construction Technology (KICT) opened an European Office at KIST Europe.

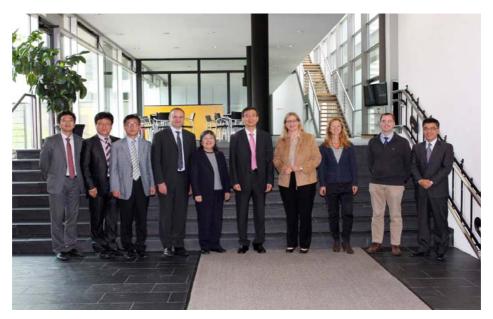


The unveiling ceremony

### **September 11, 2013**

### **Minister for Environment visits KIST Europe**

In September, Anke Rehlinger, the Saarland Minister for Environment & Consumer Protection, visited KIST Europe.



From left to right: Dr. Tae Kun Kim, Dr. Sanghun Kim, Mr. Young Chul Joo, Dr. Rainer Hanselmann, Dr. Myung Hee Jung, Prof. Dr. Ho Seong Lee, Ms. Anke Rehlinger, Ms. Anja Petschauer, Dr. Kilian Smith, Dr. Hyun Pyo Jeon

### October 14, 2013

### MoU with Leibniz Institutes for New Materials (INM) and KIST Europe

Leibniz Institutes for New Materials (Director Prof. Dr. Eduard Arzt) and KIST Europe (Director Prof. Dr. Ho Seong Lee) signed the MoU for advanced research collaboration and joint project development.



### **Visits**

### November 18, 2013

### **UST Students visit KIST Europe**

A group of students from the University of Science and Technology (UST) visited KIST Europe which is overtaking the role as European Hub Institute. The UST aims at fostering highly qualified R&D professionals in the field of new convergence technology throughout on-site R&D education distinguished from other R&D institutes. KIST Europe overtakes the role as a hub in Europe for the training of outstanding R&D personnel.



# Workshops / Conferences

### February 23-24, 2013

### ETMN 2013 - International Conference on emerging technologies: micro to nano 2013 in Goa (India)

Prof. Dr. Andreas Manz was invited speaker at the micro to nano conference 2013 in Goa. The conference brought together the academicians, researchers, industrialists, peers and resources with the objective of mutual benefit in this scientific area.







# Workshops / Conferences

### March 14, 2013

### KIST Europe participates in special seminar

Prof. Dr. Lee (Institute Director), Dr. Sanghun Kim (Leader of Environment Energy Center) and Dr. Sangwon Kim (Leader of Green Technology Team) participated in the special seminar of Korean Council of Environmental Industry, Technology & Policy hosted by LG Display in Wroclaw (Poland) with the theme "Compliance of Korean companies to emission trading system in Europe".



### April 18, 2013

### **INM-KIST Europe Workshop**

Researchers of KIST Europe and INM presented their current on-going research projects and discussed the actual and potential research subjects of both institutes joint project.



# Workshops / Conferences

### July 11, 2013

### **Attending the 11th NANO KOREA 2013**

The consortium of gwSaar, INM and KIST Europe conducted a Germany-Korea Nanotechnology workshop during "NANO KOREA 2013" Conference held at COEX Convention Center in Seoul.





### Workshops / Conferences

### September 11-13, 2013

# 2nd International Workshop on Microsystems Technologies for African Health u-Med-A in Pilanesberg National Park, South Africa

The Workshop was focused on defining the challenges of microsystems based health diagnostics, addressing the topics of HIV/Aids, malaria, TB and water monitoring. Topic specific sessions addressed the appropriate microsystems technologies - currently available or in the process of development - to target these areas. The workshop provided a forum for researchers, technologists, entrepreneurs and funding bodies to interact in the latest developments and future trends in the multidisciplinary field of microsystems technology. All speakers were by invitation only. **Prof. Dr. Andreas Manz** gave a talk about "Lab on Chip approach to clinical diagnostics".

Besides the workshop, the participants had the opportunity to visit the Pilanesberg National Park.



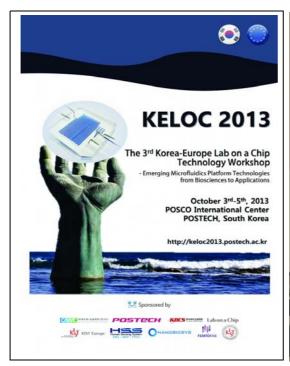


# Workshops / Conferences

### October 03-05, 2013

### 3rd Korea-EU Lab on a Chip Technology Workshop in Pohang, Korea

The 3rd Korea-Europe Lab on a Chip Technology Workshop In October 2013 - hosted by the journal "Lab on a Chip" - was focusing on "Emerging Microfluidics Platform Technologies: From Biosciences to Applications". KIST Europe was one of the sponsors of the conference. There were a lot of international speakers as **Prof. Dr. Andreas Manz**.







### Workshops / Conferences

### October 9-18, 2013

### **Executive Training Program for Korea-EU International Collaboration**

15 participants from the Korean government-funded research institutes took part in the training program for the purpose of searching for Korea-EU R&D cooperation which was held at KIST Europe in Saarbrücken (Germany), in Brussels (Belgium) and Paris (France).

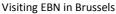
The involved institutes besides KIST Europe were:

- Max Planck Institute for Informatives and Software Systems (MPII & MPI-SWS) Saarbrücken
- German Research Center for Artificial Intelligence (DFKI), Saarbrücken
- Joint Research Centre, Brussels
- European Business & Innovation Centre Network, Brussels
- Centre National de la Recherche Scientifique (CNRS), Paris
- OECD, Paris
- Centre de Recherche sur la Conservation des Collections (CRCC), Paris



In the KIST Europe conference room







Visiting KRC in Brussels

### October 11, 2013

### 2nd Annual Meeting of IPGG in Paris (France)

During the 2nd Annual Meeting of IPGG in Paris (Institut Pierre Gilles de Gennes pour la microfluidique), Prof. Dr. Andreas Manz as invited speaker gave a talk about "Microfluidics - from integrated ,lab on chip' technology to simple droplets, from clean room technology to biomimetic microfabrication".







# Workshops / Conferences

### October 15-16, 2013

Nano Workshop "Future Materials and Safety: for establishing European-Korean Joint Research Projects"

KIST Europe held a Korea-EU joint workshop in collaboration with INM and gwSaar in Saarland, Germany.

This is the first cooperative workshop with KIST, KRIBB, KRICT, KRISS as Korean side and INM, University of Saarland, MPI-FKF, Tudor, KIST Europe as EU side.

The themes of the workshop were nano safety, nano medicine and pharmacy as well as nano materials. During the workshop, 30 speakers (Korea 14, EU 10, KIST 6) presented and 94 registered researchers participated.





# Workshops / Conferences

### October 29, 2013

### Participation in the 2nd Hungarian-Korean Technology Day

KIST Europe participated in the 2nd Hungarian-Korean Technology Day at the Hungarian Academy of Sciences which was also attended by Prof. Jozsef Palinkas (President of the Hungarian Academy of Sciences) and Prof. Istvan Barsony (Director of the Institute for Materials Science MFA).

The Institute Director, Prof. Dr. Ho Seong Lee, introduced the research activities of KIST Europe and discussed potential cooperation partnerships with several institutes.



Prof. Dr. Ho Seong Lee introduces KIST Europe



# Workshops / Conferences

### October 28-31, 2013

### MicroTAS Conference in Freiburg, Germany

The 17th International Conference on Miniaturized Systems for Chemistry and Life Sciences was held in Freiburg (Germany). MicroTAS 2013 continued a series of conferences that are the premier forum for reporting research results in microfluidics, microfabrication, nanotechnology, integration, materials and surfaces, analysis and synthesis and detection technologies for life science and chemistry. The conference offered plenary talks as well as contributed oral presentations and posters selected from submitted abstracts. There were also live demonstrations of microfluidic equipment and hands-on experiments.

**Prof. Dr. Andreas Manz** was one of the Vice Chairmans of the conference.







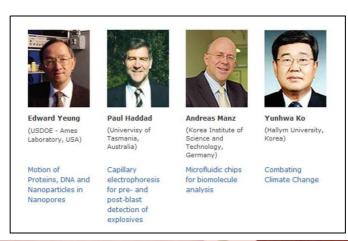
# Workshops / Conferences

### November 3-6, 2013

### 13th APCE Asia Pacific Symposium on Microscale Separation and Analysis in Jeju Island, Korea

Prof. Dr. Andreas Manz was plenary speaker at the 13th Asia Pacific Symposium in Jeju Island in November 2013. He gave a talk about "Microfluidic chips for biomolecule analysis".

The APCE conference was held in Korea for the second time and was jointly organized by the Korean Chemical Society, the Korean Biochip Society, the Korea Society for Environmental Analysis and the Korean Society of Analytical Sciences.





Group photo with Korea's former minister of environment, Mrs. Young Sook Yoo, who was Honorary Chairwoman of the conference.

# Workshops / Conferences

### November 07-08, 2013

### Participation in the Korea-EU Scientists & CEO's Meeting

KIST Europe participated in the Korea-EU Scientists & CEO's Meeting in Brussels (Belgium) which was also attended by the President of South Korea, Ms. Park Geun-hye.



The Institute Director, Prof. Dr. Ho Seong Lee, also joined the opening ceremony of the Korea Research & Innovation Center Europe hosted by Mdm President.



### February 14, 2013

### Presentation Day of the Internship Students at KIST Europe, Saarbrücken

Before returning to their home country after several months for traineeship at KIST Europe, the Internship Students presented the experience they have gained during their stay and received their certificate of completion.



### April 23, 2013

### **Korean Artist donates artwork**

The well known Korean artist Prof. Eun Nim Ro donated 5 pieces of her artwork to KIST Europe and travelled to Saarbrücken to supervise and strongly support the hanging procedure in person. Eun Nim Ro was born in Chun-Zu (South Korea) and studied at the Medicine Faculty of University of Seoul. After her mother died, she began her painting. She came to Germany as nurse assistant and studied at the University of Visual Arts in Hamburg (Germany). She was announced Professor for Painting at the technical college in Hamburg (Germany). Since 1994, she heads the international academy "Pentiment" in Hamburg (Germany).



### Miscellaneous



"I thought that nature was already the whole universe, of which I am a part of. Now I notice that there is something which rules over nature called time.

I observe this time like through the eye of a needle as it leads from the past to the present and into eternity. Whenever I experience such a moment, where time meets eternity, I do not know: What was be-fore? Who is standing here now? What will come later? I cannot differentiate.

Time makes me restless. Eternity makes me pure - and simple.

Good art demands purity. It can only live if everything complicated is thrown over board - and the simple thing has occurred. Only during such a moment does the door open, and a rare visitor stands there. He stays a while, and soon thereafter he leaves the room.

The metaphysical visitor - that is what I call art."

Eun Nim Ro, 1986

# Miscellaneous

### June 22, 2013

### Open Day of the University of Saarland, Saarbrücken

On June 22, 2013, KIST Europe participated in the Open Day of the Saarland University.

The team onsite informed about the latest research results of the Convergence Bioscience Group, provided information about Korean culture and offered typical Korean food.





### Miscellaneous

### August 21, 2013

### Groundbreaking Ceremony at Helmholtz Institute for Pharmaceutic Research, Saarbrücken

Directly in front of the buildings of KIST Europe in Saarbrücken, the Helmholtz Institute for Pharmaceutic Research is building a new research center at Saarland University Campus.

During the Groundbreaking Ceremony, Prof. Dr. Ho Seong Lee and Prof. Dr. Andreas Manz were able to talk to Saarland's Prime Minister Annegret Kramp-Karrenbauer and the German Federal Minister for Research, Prof. Dr. Johanna Wanka, and to get in touch with various representatives of Saarland Government, Saarland University and the Helmholtz Institute.





from left to right: Annegret Kramp-Karrenbauer, Prof. Dr. Ho Seong Lee, Prof. Dr. Johanna Wanka, Prof. Dr. Andreas Manz

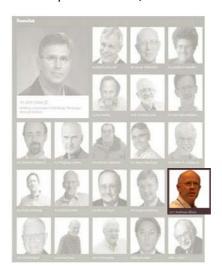
### **November 2013**

### **The Analytical Power List 2013**

"The Analytical Scientist", a scientific journal founded in 2013, published the "Analytical Scientist Power List 2013"with the 100 most influential people in the analytical sciences.

Prof. Dr. Andreas Manz, Research Director at KIST Europe since 2009, was voted No. 17.





# Summary

# MoUs

| Leibniz Institutes for New Materials (INM) | October 14, 2013 |
|--|------------------|
|--|------------------|

# **Visits**

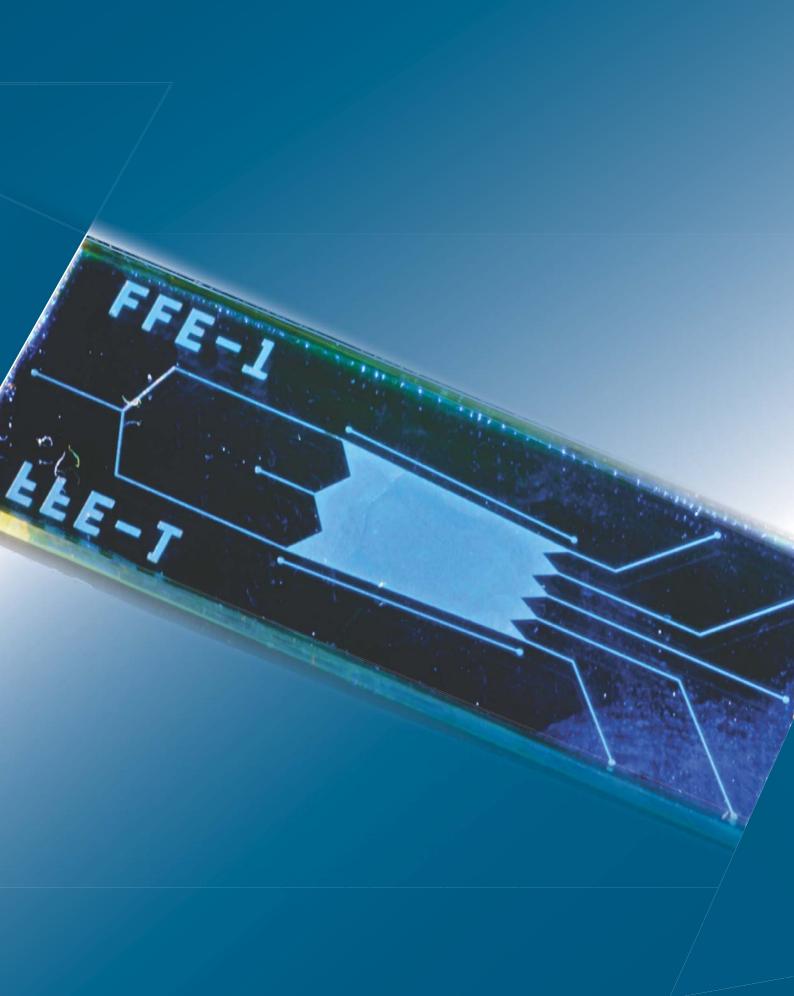
| Korean Embassy (EU) - Ambassador Mr. Chang-Beom Kim, Science Attaché Mr. Hyuk-<br>Chae Koo, First Secretary Mr. Woojong Kang | April 17, 2013     |
|--|--------------------|
| KIST Europe Advisory Board Meeting   | April 26, 2013     |
| KICT Korea Institute of Construction Technology  | July 23, 2013      |
| Saarland Minister for Environment & Consumer Protection Anke Rehlinger   | September 11, 2013 |
| UST Students   | November 18, 2013  |

# Workshops / Conferences

| ETMN International Conference on Emerging Technologies: Micro to Nano 2013 in Goa (India)                                      | February, 23-24,<br>2013 |
|--|--------------------------|
| Special Seminar of Korean Council of Environmental Industry, Technology & Policy in Wroclaw (Poland)                           | March 14, 2013           |
| INM-KIST Europe Workshop in Saarbrücken (Germany)  | April 18, 2013           |
| 11th NANO KOREA 2013 in Seoul (Korea)  | July 11, 2013            |
| 2nd International Workshop on Microsystems Technologies for African Health u-Med-A in Pilanesberg National Park (South Africa) | September 11-13,<br>2013 |
| 3rd Korea-EU Lab on a Chip Technology Workshop in Pohang (Korea)   | October 03-05, 2013      |
| Executive Training Program for Korea-EU International Collaboration (Saarbrücken, Brussels, Paris)                             | October 09-18, 2013      |
| 2nd Annual Meeting of IPGG in Paris (France)   | October 11, 2013         |
| Nano Workshop "Future Materials and Safety: for establishing European-Korean joint research projects" in Saarbrücken (Germany) | October 15-16, 2013      |
| Participation in the 2nd Hungarian-Korean Technology Day in Hungary  | October 29, 2013         |
| MicroTAS Conference in Freiburg (Germany)  | October 28-31, 2013      |
| 13th Asia Pacific Symposium on Microscale Separation and Analysis APCE in Jeju Island (Korea)                                  | November 3-6, 2013       |
| Participation in the Koea-EU Scientists & CEO's Meeting in Brussels (Belgium)  | November 07-08,<br>2013  |

# Miscellaneous

| Presentation Day of the Internship Students at KIST Europe, Saarbrücken (Germany)                                       | February 14, 2013 |
|---|-------------------|
| Korean Artist Prof. Eun Nim Ro donates artwork at KIST Europe, Saarbrücken (Germany)                                    | April 23, 2013    |
| Open Day of the University of Saarland, Saarbrücken (Germany)   | June 22, 2013     |
| Groundbreaking Ceremony at Helmholtz Institute for Pharmaceutic Research in Saarbrücken (Germany)                       | August 21, 2013   |
| Publication of Handbook "Land of Ideas, The Science & Technology Policy and Trend of Research & Debelopment in Germany" | October 2013      |
| The Analytical Power List 2013 of "The Analytical Scientist" - Prof. Manz voted No. 17                                  | November 2013     |



Chip device for free flow electrophoresis (FFE), microfabricated from silicon at KIST Europe, Saarbrücken

# Nano-Engineering Group

## Nano-Engineering Group

#### Introduction



Prof. Dr. ir. Leon Abelmann **Group Leader Nano-Engineering** 

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Leon Abelmann (1965) received his MSc degree in Electrical Engineering at the University of Twente in 1990, The Netherlands, with a specialization in the area of integrated optics. He spent the first two years of his PhD study at CNRS in Meudon, France, studying oblique evaporation of thin magnetic films. He continued this research at the University of Twente, where he obtained his PhD degree in 1994 with Professor Lodder. After his PhD, he continued research in magnetic force microscopy in Twente. In 1997 he received a grant from the Royal Dutch Academy of Sciences, allowing him to spend one year as a postdoctoral researcher at the Data Storage Systems Center at the Carnegie Mellon University, Pittsburg, USA in 1999, where he worked on micromagnetic simulations. In 2000 he received an Innovation Grant from the Netherlands Organisation for Scientific Research (NWO), which allowed him to work on probe based data storage. He was appointed Assistant Professor in 2001, and Associate Professor and interim chairholder in 2004 after the retirement of Professor Lodder. In 2006 he joined with his team the Transducer Science and Technology group of Professor Elwenspoek, where he continued his work on novel, MEMS based, information storage and nanomagnetism. In 2010 he extended his research towards self-assembly, focussing on magnetic forces to steer the assembly process and self-assembly of magnetically functionalized units. In 2013, Leon Abelmann accepted a position as research group leader at the European branch of the Korean Institute of Science and Technology in Saarbrücken, where he moved his field towards nanotechnology and magnetism for life sciences. Simultaneously, he was appointed extraordinary professor at the University of Twente to strengthen the connection between KIST and MESA. In 2014, Leon Abelmann was appointed professor at Saarland University, in the department of Mechatronics, to strengthen the academic cooperation between KIST Europe and Saarland University. Leon Abelmann was very active in education in the Electrical Engineering and Advanced Technology programs, as well as the Honours Program of the University of Twente. He was nominated teacher of the year for Electrical Engineering from 2002 to 2011, only interupted in 2010, and was recipient of the prize in 2005. He received the best teacher award for the Advanced Technology bachelor program in 2010, and the award for excellent education by the TNW department in 2012. He is (co)author of over 90 peer reviewed publications, and holds two patents.

The speed of chemical reactions and separations in fluidic systems is often determined by diffusion processes. Downscaling the reaction devices called "lab-on-a-chip" result in extreme reduction of both, reaction times as well as sample consumption. Furthermore, number of units can be integrated on a single chip with the size of a few cm<sup>2</sup> leading to high throughput at low cost.

In combination with fast and sensitive detection methods, a micro total analysis system "µTAS" is built consisting of sample loading (injection), separation and detection units. All steps are performed automatically. Fluorescence and/or electrochemical-based detection technique, or ion mobility spectrometry are implemented. The goal of the microfluidics research is the application of analytical chemistry into clinical diagnostics, such as quantitative RNA assays and immunoassays for body fluids, i.e. blood, urine and sputum. In addition, extracellular metabolites of bacteria, cells, fungi, animals and humans are investigated by ion mobility spectrometry.

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Magnetism combines well with lab-on-a-chip analysis systems, since magnetic fields penetrate in most aqueous solutions and hardly interact with bio-chemical processes. Magnetic fields can for instance be used to apply forces and torques (using either magnetic particles or non-magnetic particles in magnetic fluids), to heat magnetic particles by induction or to detect the presence of magnetic particles. We combine magnetic fields with microfluidic systems to study fundamental behavior of magnetic particles in solutions, or non-magnetic particles in magnetic solutions and to apply this in lab-on-a-chip applications.

The major contributions are exemplary in personalized medicine in general. Research activities range from fundamental issues in microfluidics and magnetism all the way to instrument manufacturing and clinical diagnostics.



# Nano-Engineering Group

### Towards microfabricated magnetic particles for medical imaging

Magnetism, microfabrication, magnetic particle imaging

#### **Summary:**

Magnetic nano-particles are successfully applied in medical applications, such as separation, immunoassays, contrast agents for imaging, drug delivery and hyperthermia. Currently, these particles are prepared by bottom-up chemical routes and are always superparamagnetic, which severely limits design of their magnetic properties. This is especially detrimental for magnetic particle imaging (MPI), where the specific non-linear magnetic response is used to detect minute amounts of particles in the human body against a strong background signal. Unfortunately, the magnetic background is also superparamagnetic. Therefore, magnetic particles which are realized by low cost, top-down microfabrication techniques. In this case we can design particles that have hysteresis loops very different from the typical superparamagnetic response.

Using Laser Interference Lithography, billions of particles can be lithographically defined with exposure times below one minute with dimensions below 100 nm (Figure 1). By means of the ultra-sensitive Anomalous Hall Effect we have studied the magnetic reversal of these elements (Figure 2). By statistical analysis of the thermal fluctuation of the switching field of individual elements, we have been able to determine the energy barrier against magnetic reversal, as well as the switching field in the absence of thermal energy of individual dots in the array. We discovered that the energy barrier is higher for elements that switch at higher fields. This confirms the suspicion of the scientific community that the origin of the switching field distribution in this particles should be found in variation in magnetic anisotropy.

This result is of major importance for the design of magnetic particles with a highly uniform switching field, which is a prerequisite for magnetic particle imaging.

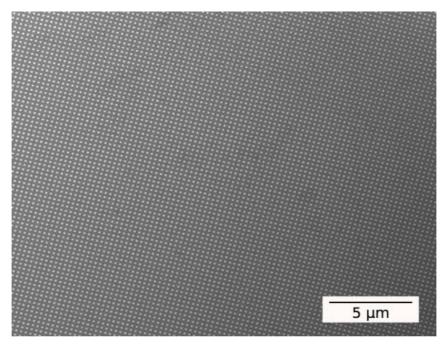


Figure 1: Magnetic particles with a diameter of 70nm prepared by Laser Interference Lithography.

# Nano-Engineering Group

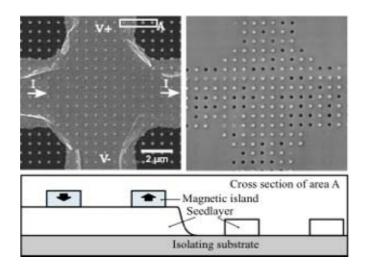


Figure 2: By means of Anomalous Hall Effect, the reversal of individual particles can be studied.

#### **Publications**

de Vries, J.; Bolhuis, T., Abelmann, L.: Energy barrier versus switching field for patterned Co80Pt20 alloy and Co/Pt multilayer films Journal of applied physics 17 (17B910), pp. 1-3



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## Nano-Engineering Group

#### Control of magneto-tactic bacteria

Microfluidics, magnetism, micro-robotics

#### **Summary:**

We study medical microrobots for drug delivery and minimally invasive surgery. Our approach is to use self-propelled systems and use magnetic fields only for steering.

As a first demonstration, we are currently experimenting with magneto-active bacteria (Figure 1).

Using advanced control algorithms, we were able to control magneto-tactic bacteria with a speed of approximately 30 micrometer per second to an arbitrary point in space, with a region of convergence of 10 micrometer.

These initial encouraging results open up the way towards the use of individual magnetotactic bacteria inside complex microfluidic networks, for instance as payload transporters.

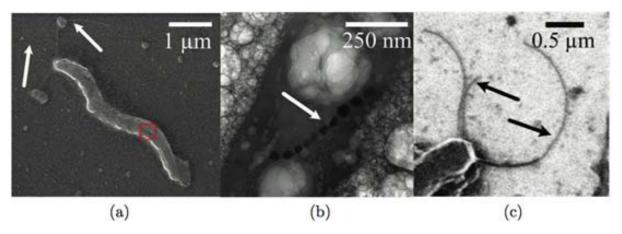


Figure 1: Electron microscopy of magneto-tactic bacterium:

- a) SEM image of Magnetospirilium Magnetotacticum (MS-1). The arrows indicate the flagella, the red square the location of the magnetosome.
- b) TEM image of the magnetosome chain which consists of iron-oxide particles of nanometer radius.

c) SEM image of the flagella which MS-1 uses for propulsion.

In close cooperation with the University of Twente, we study the behavior of magneto-tactic bacteria under application of external fields and in confined channels. We discovered that the movement of magneto-tactic bacteria can be accurately modeled by a competition between magnetic torque and rotational drag torque. From observation of the trajectory of single magneto-tactic bacteria under field reversal (Figure 2), the maximum magnetic torque can be determined, which agrees well with a micromagnetic model of the magnetosome chain.

# Nano-Engineering Group

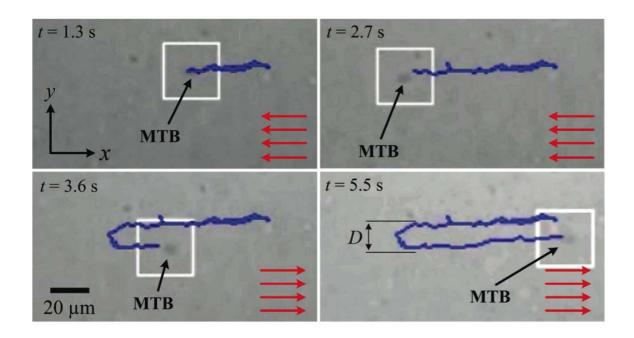


Figure 2: From the trajectory of a magneto-tactic bacterium under reversal of the external field, the ratio between drag and magnetic torque can be determined.

#### **Publications**

Khalil, I.S.M, Pichel, M.P., Abelmann, L., Misra, S.: Closed-loop control of magnetotactic bacteria The International Journal of Robotics Research 32 (6), pp. 637-649



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## Nano-Engineering Group

#### Free-flow isotachophoresis on a glass chip

free-flow, isotachophoresis, chip separation

#### **Summary:**

Isothachophoresis is a separation based on two buffers (leading and terminating) with ions of different mobility in the presence of electric field. This method enables focusing of the sample while allowing separation. Free-flow electrophoresis (FFE) separation methods have been developed and investigated for continuous sample preparation and mild separation conditions make it also interesting for online monitoring and detection applications. Here we have combined both methods into continuous free-flow isothachophoresis using microfabricated glass chip.

The microfluidic device was designed (see Figure 1) and its pattern was transferred by conventional photolithography and etched by HF/HCl mixture. The device consists of five inlets (I), a separation (main) chamber with the size of  $23 \times 15$  mm, two side chambers for connecting the electrodes and five outlets (O). The three middle inlets are split with a binary tree structure for equal distribution of samples and buffers. Two outer inlets are used to guide flow direction into the main chamber. The side chambers are separated from the main chamber by  $25~\mu m$  wide channels to prevent gas bubbles entering the main chamber. The outlets are designed in similar fashion as the inlets.

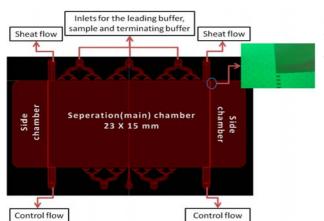


Figure 1: Chip schematic showing all chambers. The side chambers are separated from the main chamber by 25  $\mu$ m wide channels (see inset).

All buffers were prepared as follows with a resulting pH value of 9. The leading buffer was made by 50 mM NH4OH mixed with 20 mM HCl, the terminating buffer by 50 mM NH4OH mixed with 50 mM 2-(N-morpholino)ethanesulfonic acid. The sample was prepared by mixing 50 mM NH4OH with 2.5 mM O-acetylsalicylic acid and fluorescein and rhodamine 110, both with concentration of 50  $\mu M$ .

Chip was mounted on an inverted microscope equipped with fluorescent imaging system. Fused silica tubes with internal diameter of 100m were connected to the chip I/O using Upchurch connectors (see Figure 2).

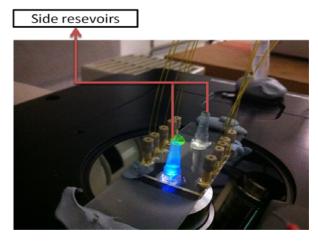


Figure 2: Photography of the chip setup illuminated by halogen lamp with blue filter. The side reservoirs have large volume to potentially generate gas bubbles far from the chip.

Steady flow of buffers and a sample was achieved by syringe pumps with flow rate of  $5\mu$ l/min. Once the separation voltage with amplitude of 500V was applied the rhodamine was separated from fluorescein which was focused.

# Nano-Engineering Group

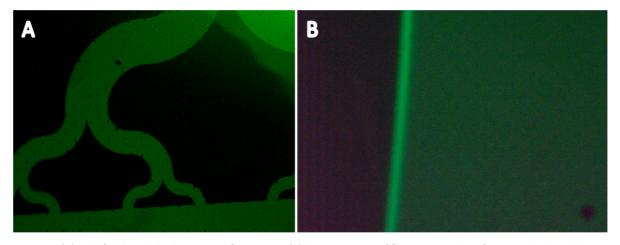


Figure 3: (A) Inlet filled with rhodamine and fluorescein, (B) the separation of fluorescein and its focusing. The bright line is fluorescein with 50 fold concentration compared to the input sample ones.

Figure 3A shows the 3mm wide sample inlet.

Figure 3B shows the separated stream of a fluorescein concentrated 500 fold compared to the original concentration. The concentrated fluorescein flow had width of  $60\mu m$ . This focussed stream was driven by positive pressure applied to two outer outlets. This configuration enables steering the focussed stream to any output of our choice.



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# Nano-Engineering Group

#### **Electrochemical Sensors for Biochemical Analysis**

Sensors, electrochemical detectors, chip

#### **Summary:**

The main target of this project is the development of an array of electrochemical detectors for detection and quantification of biosubstances by cyclic differential pulse voltammetry. This technique was described earlier and results from a combination of cyclic and differential pulse voltammetry. Optimization frequency and time of the pulses should result in superposing current responses for both increasing and decreasing voltage.

We have fabricated glass chip with 36 units grouped in 9 sets of four units (Fig. 1). Each unit has a working electrode and a combined reference/counter electrode. The chips were fabricated by conventional lithography process and covered with polymeric material to protect the connections leaving only the electrode area exposed to the solution.

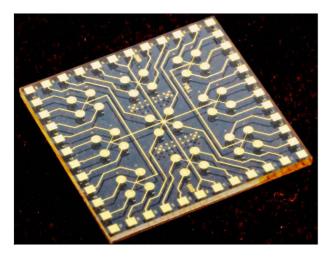


Figure 1: Glass chip with 36 units grouped in 9 sets of four units, each with working electrode and combined reference/counter electrode.

We have measured four sensors a time. Each sensor reference/counter electrode is powered by its own voltage pulse generator each with different frequency for differential pulse voltammetry. These pulses are superposed to a saw tooth signal for cyclic voltammetry. These pulses are distributed to a selected set of four units by four analog switches 1 x 16. An application for PC was developed to either select a single set or scan the entire chip (9 units) with a pre-defined time per unit. The system can be later on expanded to 64 sensors. Resulting currents from the electrochemical cell is collected at work electrodes in form of a composite current. It was converted into voltage and processed by demodulators and split into 4 individual channels. The amplitude of the DC signal in each channel corresponded to the current in the respective electrochemical cell (Fig. 2). This entire scheme is a lock-in amplifier with excellent signal to noise ratio.

The output voltage from the lock-in amplifier can be correlated with the saw tooth signal to extract a differential scanning voltammogram. Alternatively instead of saw tooth signal we could use only a DC bias thus the system would operate in potentiostatic mode. The communication between the signal generator and the board was conducted via an RS232 interface.

We have demonstrated the functionality of this platform. The chip was soldered to a printed circuit board (PCB) containing the multiplexer and the other electronic circuits.

We have performed measurements of the oxidation/reduction of FeCN ions, a widely-used calibration reaction to evaluate the behavior of electrochemical sensors. In the next step, the chip will be used to the analysis of complex matrices, as food and biomedical samples in handheld format.

# Nano-Engineering Group

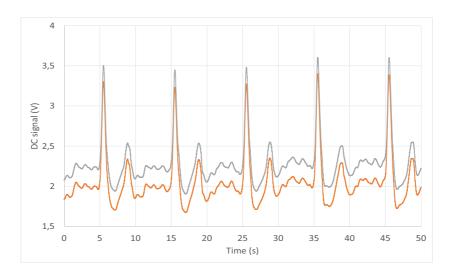


Figure 2: DC signal, as function of time from 3 channels.

#### **Publications**

F. Drake, R.P. Van Duyne, A.M. Bond: Cyclic differential pulse voltammetry; A versatile instrumental approach using a computerized system *Journal of Electroanalytical Chemistry, Vol. 89,* pp. 231-246, 1978



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# Nano-Engineering Group

# Development of micro in-vitro system to study response of liver to sepsis

#### in-vitro system, liver, sepsis

#### **Summary:**

Liver is one of the most important host's organs as it removes toxins from the body such as the ones produced during sepsis. These toxins can damage liver and especially hepatocyte cells. We are developing method based on microfluidic device to study response of liver cells to sepsis.

There is a requirement for predictive in-vitro models to improve drug discovery and development. One of the employed methods is mimicking in-vivo environments at the micro scale. But most studies are typically conducted using the conventional 2D cell-culture approach. It is simple but does reflect typical cell biology as cultures cannot maintain these differentiated functions. In our project we have decided to move from 2D cell monolayers to 3D to emulate real cell behavior. We were investigating extracellular matrix (ECM) as part of multicellular structure. This matrix typically provides structural support to the surrounding cells such as cell adhesion to the substrate. It also helps in cell-to-cell communication as well as stem cell differentiation.

Sepsis is a severe problem in public health care system due to its high mortality rate. It can cause multiple organ dysfunction syndrome (MODS) including liver failure. As of now prognosis of a liver failure is rather poor and its cause remains unclear. For our study we have used an in-vitro liver culture system based on a microfluidic chip device in form of 384 well plate from Leiden University (Fig. 1). The well plate glass bottom has a microfluidic system attached for 3D heterogeneous cell and tissue culture. The glass chip bottom is hydrophilic and corresponding capillary force can assist in filling microfluidic system with fluid (and cells) (Fig. 1) The bottom of the 384-well structures are merged into 40 cell culturing chambers. Each chamber has three inlets and three outlets (called flanking lanes) connecting with external tubing by corresponding wells (see Fig. 1 inset).

The cell culturing part of each chamber has length of 4.5mm, width of 200  $\mu m$  and height of 120  $\mu m.$ 

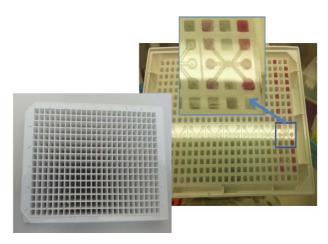


Figure 1: 384 well format 3D cell culture plate includes 40 culture chambers with three-lane in bottom plate

The experiment started with HepG2 liver cell culturing at conventional culturing flask. After that cells were harvested and resuspended in ECM and pipetted into the microfluidic chips. Here ECM was gelated and we have filled empty flanking lanes with culturing media by perfusion. The microfluidic device was kept at 37 °C with 5% CO<sub>2</sub> ambient. We have observed that the 3D liver cell culture was viable for several weeks. Next we have started to develop a sepsis model based on cell interaction with toxins and harmful bacteria. First we have administrated either a toxin Lipopolysacharide (LPS) or live bacteria E. coli transfected plasmid DNA of green fluorescent protein (GFP) and observed cell behavior. Observation was done using dead/live cell staining protocol.

# Nano-Engineering Group

In the E. coli experiment we were also able to see emitted green fluorescence from the GFP and monitor E. coli location and motion (see Fig. 2).

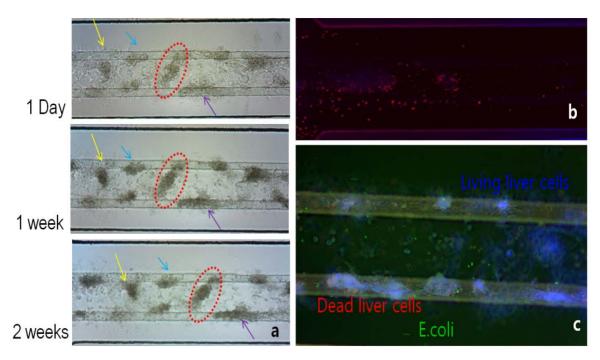


Figure 2: (a) Images of HepG2 liver cells in extracellular matrix gel in a middle lane microfluidic chamber; (b) cell viability fluorescent images for in-vitro sepsis model with LPS treatment; (c) GFP E. coli in 3D micro liver system followed by fluorescent live/dead staining (blue/red).

In conclusion, we are developing in-vitro micro liver model to study sepsis. We were able to maintain healthy cell population for few weeks in 3D format. We have used two models to induce sepsis, toxin and bacteria. This model can be used for sepsis understanding and thus help to prevent it.



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**Environment & Bio Group** 

### **Environment & Bio Group**

#### Introduction



**Dr. Sanghun Kim**Group Leader Environment & Bio Group

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Dr. Sanghun Kim is Group Leader of the newly founded Environment & Bio Group at KIST Europe since 2013. He studied Environmental Engineering at TU Berlin and joined KIST Europe in 2004. He focused his work on emerging pollutants monitoring & control and Chemical Risk Assessment. From 2006 to 2012 he worked as Teamleader in the Chemical Risk Management Team and performed several projects linked between Korea and the EU in the field of Chemical Safety Management, Regulation compliance and Environmental policy.

One team of the Environment & Bio Group, the <u>Convergence Environment Team</u>, focusses on investigating global environmental issues that are relevant for Korea and the EU. It conducts research in the field of emerging pollutants, particularly with regards to their monitoring and control, looking at aspects of their eco-toxicity and mixture toxicity in order to determine the influence of such chemicals and engineered nano-materials on the environment. Furthermore, it provides global regulation compliance with knowledge-based services for the Korean chemical Industry.

The outcome of these studies and the experience have led to a great knowledge in relation to environmental issues, which are relevant for the research of this team. As mentioned above the team works in the field of monitoring and control of environmental pollutants. During the last years it has developed a computational model to predict the effects of mixture toxicity, and for this purpose it has applied several in-vivo/-vitro bioassays to measure the toxicological effects of chemical mixtures.

Ongoing from the previous experience the team is performing research on developing screening methods for engineered nanomaterials (ENMs) and surface modified metallic nanoparticles (SMMNPs) to estimate their potential effect as a fate in the environment. The effects of nanoparticles are especially a concern in relation with aquatic environment. The aquatic environment is also main concern in one of the cooperation projects the team is currently performing with partners in Korea and Germany. The impact of green algae in rivers is steadily increasing. In this context, the team tries to determine the ecotoxicity of Cyanobacteria using in-vitro bioassays based on bacteria and luminescent bacteria, as well as the removal of the toxins of cyanobacteria by sonolysis.

Related to the above the team is also focusing on developing methods to better assess the effects of (emerging) organic pollutant mixtures in aquatic environments. These are based on combining passive sampling and dosing approaches using polymers and applying these in different toxicity bioassays covering different endpoints and levels of biological organization.

## **Environment & Bio Group**

Based on our knowledge on environmental issues the Convergence Environment Team also concentrates on the handling on global issues in relation to chemical regulation compliance. Starting with projects of the Korean Ministry of Environment it has established a large knowledge base in the field of regulation compliance, which plays an important role for the Korean economy. By supporting the industry, the team firstly helps them to comply with European regulations and secondly ensures a safe handling of dangerous substances, to contribute to overall well-being, which is the highest goal.



In order to provide innovative, new therapeutics and diagnostics for biomedical and pharmaceutical applications, the second team, the <u>Bio-Monitoring Team</u>, performs interdisciplinary, application-oriented R&D in biomaterial and biosensor development, cancer immunotherapy, drug delivery and molecular drug discovery. The usefulness of biomarker detection and cancer immunotherapy in tomorrow's personalized health care environment will facilitate early and precise diagnosis of many types of human physiological conditions and diseases. These research fields are considered as one of the most important topics in health and well-being which are becoming most important concern in an aging society. In detail, the development of Biomaterials for tissue engineering, the development of bio/medical devices using phage display technology and the development of strong tools for immune cell-mediated drug delivery are current ongoing research activities.



### **Environment & Bio Group**

#### New challenge on chemical regulation and our activities 2013

REACH, registration, lead registrant, K-REACH, cosmetic regulation, food contact material regulation, biocidal product regulation

#### **Summary:**

REACH stands for Registration, Evaluation, Authorisation and Restriction of Chemicals and entered into force on 1 June 2007. REACH is adopted to improve the protection of human health and the environment from the risks of chemicals. The REACH regulations also have a strong impact on K-REACH, legislated on May 2013. EU Cosmetic Regulation, entering into force on July 2013, protects consumers and makes sure that all cosmetic products on the EU market are safe. Regulatory support for the Korean chemical industry to have a market in the EU will be required.

#### REACH (EC No. 1907/2006)

The REACH deadline for registering substances manufactured or imported in quantities of 100 to 1,000 tonnes per year was on 31 May 2013. By that time, 3,215 companies had submitted 9 084 registration dossiers to ECHA. Since that date, 270 additional registration dossiers have already

been received which are linked to the 2013 deadline. 34 % of the companies declared themselves as micro, small or medium-sized companies, accounting for 19 % of all registrations. Since the 2010 registration deadline, 2,998 substances have now been registered for the 2013 deadline.

KIST Europe as the only representative for 6 Korean companies has done 28 registrations successfully and 2 substances also registered by KIST Europe as lead registrant who must act with the agreement of the other co-registrants and submit the lead dossier of the joint submission and also usually coordinates all activities within the SIEF.

#### EU cosmetic regulation (EC No. 1223/2009)

The new European cosmetic regulation, which came into force in July 2013, replaces the (EU) Cosmetic Directive 76/768/EEC and its regulatory requirements are strengthened. It is demanding many new aspects, i.e. Responsible Person (RP), Cosmetic Product Safety Report (CPSR), Product Information File (PIF), Serious Unexpected Effects and Cosmetic Product Notification Portal (CPNP).

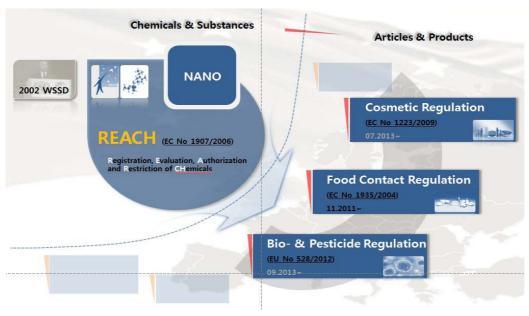


Figure 1: The relation of REACH and REACH-like regulation in EU; regulations for consumer products based on REACH regulation, such as cosmetic regulation, food contact materials regulation and biocidal product regulation, are important issues for chemical based product industry.

## **Environment & Bio Group**

#### Acts of K-REACH

- General provision (Art 1~7)
- · Registration of chemicals (Art 8~19)
- Evaluation of chemicals (Art 20~25)
- · Authorization and Restriction (Art 26~29)
- Communication on chemicals (Art 30~31)
- Supplemental & Penalties (Art 32~47)

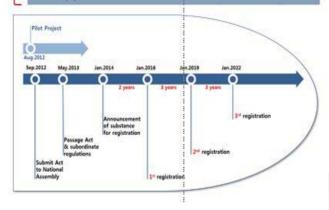


Figure 2: Time table for establishing Korea REACH with 3 registration steps like REACH, legislated on May 2013.

The relevant compliance solution to the new European cosmetic regulation had been prepared for supporting Korea cosmetic industry and published "Guidance of compliance EU cosmetic regulation for Korea cosmetic industry" in 2013.

#### Korea REACH (2013)

Korea introduced a so called K-REACH, the Registration and Evaluation of Chemical Substances Act, a similar piece of legislation to the European REACH Regulation, on May 2013.

Our expertise in the field of chemical regulation compliance makes us an excellent partner for the Korean government in supporting the introduction of chemical regulations in Korea. Three projects for bench-marking REACH and establishing subordinate regulation of K-REACH funded by Korea Ministry of Environment have been carried out successfully.

#### Other activities

In 2013, compliance strategy for Food Contact Materials Regulation (2011) had also been deve-

loped and 2 notifications for France Nanomaterial Regulation had been carried out, too.

#### **Publications**

H. Jeon, S.Kim:

The global trend and issues of Nano Materials regulation

Workshop of Council for Science and Technology/Korean Council of Environmental Industry, Technology & Policy, 2013

#### H. Jeon, S.Kim:

The trend and compliance issues of REACH-like regulation

Workshop of Council for Science and Technology/Korean Council of Environmental Industry, Technology & Policy, 2013

#### H. Jeon, S.Kim:

The compliance strategy to REACH and REACH-like regulation for Korea cosmetic industry

Institute of LG Health and Houses, 2013



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**Oliver Weiss** 

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### **Environment & Bio Group**

## QSAR-TSP: An in silico technique for predicting mixture toxicity

mixture toxicity, QSAR, two-stage prediction, integrated addition model

#### **Summary:**

As an alternative "In Silico" technique to calculate the mixture toxicity using knowledge on mixture components, a quantitative structure-activity relationship-based two-stage prediction model (QSAR-TSP), was developed in this study. This study highlights that the QSAR-TSP, integrating concentration addition and independent action models, showed a potential to effectively predict the mixture toxicity in the absence of data on the mode of toxic action of each component which are strongly required in conventional models.

The concentration addition (CA) and independent action (IA) models are frequently employed to estimate the additive toxicity of mixture components, and were basically established on opposite assumptions: a mixture consists of components having either similar or dissimilar modes of toxic action (MoAs), respectively. However, the predictability of both models can be limited where accurate MoAs for all components are not readily available. In addition, the knowledge on MoAs of diverse chemicals still lacks.

The objectives of this study were to develop and evaluate a QSAR-TSP as an integrated addition model (IAM) for non-interacting mixtures using clustering methods that classify based on the structural similarity between chemical substances. In addition, the relative important molecular descriptors for the chemical clustering were provided by applying Random Forest (RF) Analysis. The predictive performance of the QSAR-TSP was validated by two datasets which were previously published: Dataset 1 for the mixture toxicity of ten pesticides (five herbicides, four fungicides and one insecticide) on Vibrio fischeri; and Dataset 2 for a realistic pesticide mixture consisting of 23 pesticides on Scenedesmus vacuolatus strain 211-15.

For the validation datasets, the QSAR-TSP overall outperformed the other reference models, the CA, IA, and conventional TSP models which require the information of MoAs.

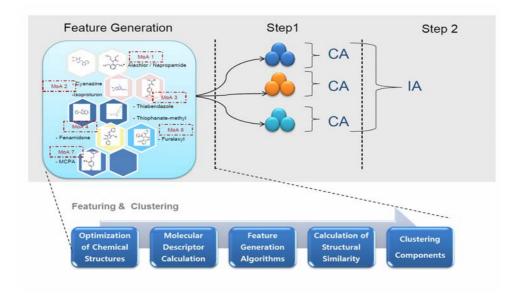


Figure 1: The QSAR-TSP approach for determining the toxicity of mixtures.

## **Environment & Bio Group**

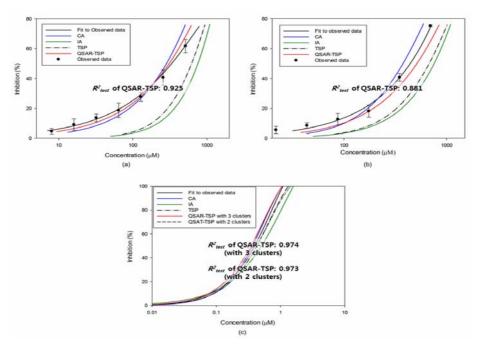


Figure 2: Overview on the comparison of CA, IA, TSP and QSAR-TSP predictions against observed toxicity for validation datasets: (a) Mixture 1: different modes of action groups (ten pesticides) at 50% effect concentration of each component in Dataset 1; (b) Mixture 2: different modes of action groups (ten pesticides) at 10% effect concentration of each component in Dataset 1; (c) Mixture 3: different modes of action groups (23 pesticides) at 50% effect concentration of each component in Dataset 2 reflecting a realistic pesticide mixture (modified from Kim et al., SAR QSAR Environ. Res., 2013).

This study also highlights the QSAR-TSP's high potential to minimize the required information and resources for estimating the toxicity of complex mixtures as it only needs a set of data on dose-response curves of components on a commonly employed test organism.

In addition, the top 20 relative important descriptors, mainly related with Barysz matrices, in calculations of structural information for clustering chemicals in the three target mixtures were found by the RF analysis in this study. Barysz matrices are symmetric weighted distance matrices explaining the presence of both heteroatoms and multiple bonds in the molecule. Further studies on the validation of the QSAR-TSP need to be conducted with toxicity data based on different types of mixtures and test organisms.

#### **Publications:**

J. Kim, S. Kim, G. E. Schaumann: Development of QSAR-based two-stage prediction model for estimating mixture toxicity SAR and QSAR in Environmental Research, 24/2013, p. 841-861

#### J. Kim, S. Kim, G.E. Schaumann:

Reliable predictive computational toxicology methods for mixture toxicity: Toward the development of innovative integrated models for environmental risk assessment

Reviews in Environmental Science and Biotechnology, 12/2013, p. 235-256



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### **Environment & Bio Group**

#### Application of sonication system to control cyanobacteria

Sonication, cyanobacteria, algal toxin, toxicity effects

#### **Summary:**

The proliferation type and the density of algal cell were investigated by scanning electron microscope (SEM) and UV-Vis spectrometer. The results suggested that UV absorbance at 684 nm has a linear relationship with algal cell counts for two different cyanobacteria, Microcystis aeruginosa and Microcystis sp. Sonication systems with two different frequencies, 20 kHz and 1100 kHz, were then applied to control those cyanobacteria and the sonication system with high frequency showed better results on the control of tested cyanobacteria regrowth.

Two different Microcystis species, Microcystis aeruginosa and Microcystis sp., were selected as tested models to understand the proliferation type of cyanobacteria. Their morphological characteristics were observed by scanning electron microscope (SEM) with absorbance at 684 nm of wavelength at which algae were shown to have a high absorbance due to the presence of chlorophyll a.

The morphological characteristic and the correlation between cell density and UV absorbance at 684 nm of both tested Microcystis species are shown in Fig. 1.

Interestingly, although 2 different Microcystis species were similar in the morphological characteristic, cell size, cell division type, the proliferation type were clearly distinct from each Microcystis species. Microcystis aeruginosa (Fig. 1A) formed a group of algal cell with cell division, whereas Microcystis sp. (Fig. 1B) proliferated multiply by cell division. Fig. 1C shows the relationship between UV absorbance at 684 nm and algal cell density as cell/ml. From this figure, it is known that the sensitivities of two graphs for Microcystis aeruginosa and Microcystis sp. were significantly different.

For the cases of the highest cell densities for Microcystis aeruginosa and Microcystis sp., 6.6X10E6 cell/ml and 7.6X10E6 cell/ml, respectively, UV absorbance at 684 of Microcystis aeruginosa is significantly higher than that of Micro-

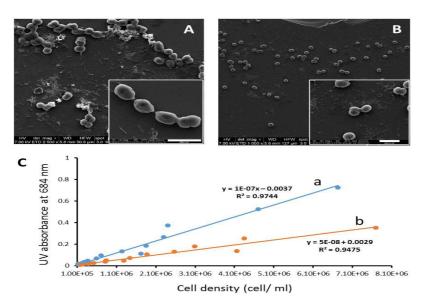


Figure 1: Proliferation type of two different Microcystis species (A and B) and comparison of UV absorbance at 684nm between those two species (C); A: SEM image of Microcystis aeruginosa (scale bar = 5  $\mu$ m), B: SEM image of Microcystis sp. (scale bar = 5  $\mu$ m), C a): UV absorbance at 684 nm versus cell density for Microcystis aeruginosa, C b): UV absorbance at 684 nm versus cell density for Microcystis sp.

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crystis sp., despite the cell density of Microcystis aeruginosa is lower than that of Microcystis sp.

Sonication systems with different frequencies, 20 kHz and 1100 kHz, were applied to control those two cyanobacteria species, and quantification of tested cyanobacteria were analyzed by UV absorbance at 684 nm. Sonication systems were operated for 60 min for both species though different sonication powers were applied, 0.285 W/cm3 and 0.114 W/cm3 for 20 kHz and 1100 kHz, respectively. After 60 min of sonication, both species were investigated for their inhibition of regrowth and they are depicted in Fig. 2.

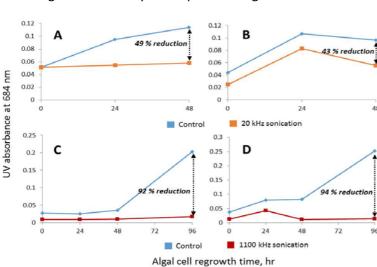


Figure 2: Cyanobacteria regrowth after 60 min of sonication with 20kHz (A and B) and 1100 kHz (C and D); A: Microcystis sp. with 20kHz sonication, B: Microcystis aeruginosa with 20 kHz sonication, C: Microcystis sp. with 1100 kHz sonication; D: Microcystis aerugniosa with 1100 kHz sonication

As this figure, it is known that 92% and 94% reductions for the regrowth of Microcystis sp. and Microcystis aeruginosa, respectively, with 1100 kHz sonication. Those reduction results were two times higher than those with 20 kHz sonication. Consequently, high frequency sonication, 1100 kHz in this project, was more efficient to inhibit cyanobacteria regrowth compared to low frequency sonication, 20 kHz in this project. Cyanobacteria including Microcystis species tested in this project are of concern due to algal toxins and their toxicity effects on human and the environment. Sonication systems were shown effective to control cyanobacteria apparently, but algal toxins with sonication systems should be considered in future.

This work was supported by the KIST ORP Institutional Program (Project No. 2E24280).

#### **Publications:**

S. Baik, C. Park, J. Kim, Y. Jung, J. Choi, S. Lee, S. Kim: Development and application of sonication on the removal of harmful cyanobacteria SETAC North America 34th Annual Meeting, Nashville TN (USA), 2013



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### Environment & Bio Group

# Toxicity screening test of surface coated silver nanoparticles on multiple bacteria

Surface coated AgNPs, luminescent bacteria, ecotoxicological screeening tool

#### **Summary:**

Luminous microbial array for risk assessment (LumiMARA), using multi-strains of luminescent bacteria, was applied for toxicity screening test of differently surface coated silver nanoparticles (AgNPs). Toxicological sensitivities of 9 marine bacteria were shown to differently depend on physicochemical properties of AgNPs. Compared to toxicity tests with single strain of bacteria, this would be a suitable toxicological screening test for real environmental samples since the tool using multi-strains of bacteria provides the wide ranges of toxicity results.

LumiMARA test kits (NCIMB, Scotland), containing 11 bioluminescent bacteria, were used for toxicological assessment of surface modified AgNPs in this study. 4 different AgNPs with average particle size of 20 nm were selected as target AgNPs based on their surface charge and salt stability, and achieved from Nanocomposix, U.S.A.

They are classified as the surface coating materials of citrate, tannic acid, polyethylene glycol (PEG) and branched polyethyleneimine (BPEI).

Ecotoxicological effect of surface coated AgNPs was evaluated by measuring reduction of light output from luminescent bacteria as exposing them to AgNPs for 15 minutes at 28 °C. Among all tested bacteria, 9 marine bacteria were selected to compare their median effective concentration (EC50) values and they are depicted in Figure 1. It is known that toxicity effects based on EC50 values of AgNPs on 9 marine bacteria have the following order, BPEI-AgNP > PEG-AgNP > tan-AgNP > cit-AgNP. Especially, strain #3, Vibrio fischeri (NCIMB 30268), showed the most sensitivity with EC50 for BPEI-AgNP as lower than 1.25 mg/L which was the lowest dose concentration in this study.

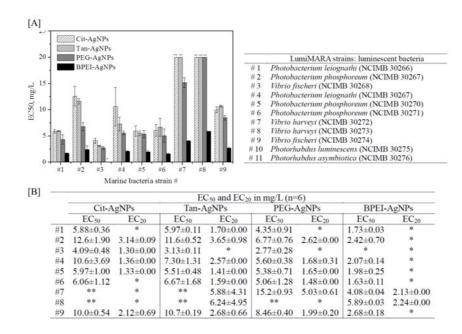


Figure 1: [A] Median effective concentration (EC50) of 9 marine luminescent bacteria for the surface coated AgNPs. EC50 values of cit-AgNP and tan-AgNP for strain #7, and cit-AgNP, tan-AgNP, and PEG-AgNP for strain #8 were higher than initial (highest) dose concentration for this toxicological assessment. [B] Table of EC50 and EC20 of 9 marine luminescent bacteria strains for the surface coated AgNPs.

<sup>\*</sup>EC50 and EC20 are lower than 1.25 mg/L which was the lowest dose concentration.

<sup>\*\*</sup>EC50 is higher than 20 mg/L which was the highest dose concentration.

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All values of EC50 and 20% effective concentration (EC20) achieved from dose-response curves were summarized in table in Figure 1. As comparing salt stability properties of AgNPs, BPEI-AgNP and PEG-AgNP with moderate and high salt stability, respectively, were more toxic than cit-AgNP and tan-AgNP with low salt stability, since AgNPs with low salt stability were aggregated easily and lost nano-characteristic which influence on the toxicity. Regarding surface charge of AgNPs, toxicity on marine bacteria were in order of BPEI-AgNP > PEG-AgNP > tan-AgNP, with zeta potentials of 12.6 mV, -16.8 mV, and -41.4 mV, respectively, since luminescent bacteria which are negatively charged on cell wall may have an ionic interaction with AgNPs positively charged on the surface.

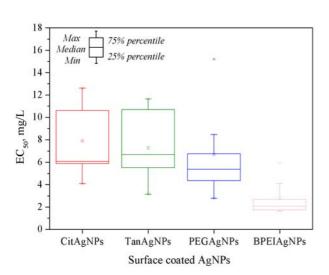


Figure 2: Box plot of EC50 data obtained for 4 target surface coated AgNPs in LumiMARA after 15 minutes incubating time at 28°C. EC50 values not achieved from LumiMARA due to higher than 20 mg/L which was the initial concentration for this assay were not included in this box plot.

As a result of using multi-strains of bacteria, ranges of toxicological impacts for target AgNPs on luminescent bacteria were achieved and depicted in Figure 2, a set of box plot which shows EC50 values from all tested luminescent bacteria for each AgNP. Within the box, 25% to 75% percentile of EC50 values can be compared and toxicity trends for tested NPs can be easily explained. Since toxicological screening tool using multi-strains of bacteria provides the ranges of toxicity results, LumiMARA would be suitable as a toxicological screening tool for chemicals or en-

vironmental samples as giving more realistic results from its toxicological assessment.

#### **Publications:**

Y. Jung, S. Baik, J. Kim, H. Jeon, S. Kim: Characterization and in-vitro toxicity test of surface modified metallic nanoparticles *Euronanoforum 2013, Dublin (Ireland), June 2013* 

#### S. Baik, Y. Jung:

Ecotoxicity screening test with multiple strains of luminescent bacteria for surface modified metallic nanoparticles

Future Materials and Safety, Saarbrücken (Germany), October 2013



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### Environment & Bio Group

# Passive dosing: The Application of polymers for handling organic contaminants in toxicity tests

passive dosing, polymers, toxicity tests

#### **Summary:**

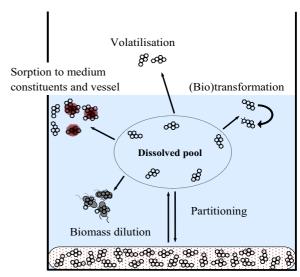
Environmental toxicity results from exposure to mixtures of pollutants, making understanding of mixture toxicity a priority. One important group of environmental pollutants comprises the hydrophobic organic contaminants. However, it is difficult to reliably determine the toxicity of such contaminants and their mixtures, since they are difficult to dissolve, have low aqueous solubilities and are prone to sorptive and other losses during testing. This results in poorly defined and decreasing exposure concentrations, making it hard to relate the bioassay response to a well-defined effect concentration. This is a particular issue when using solvent spiking to introduce the test compound(s), and new approaches for improved exposure control are needed.

In this regard, passive dosing shows considerable promise. Here, a reservoir of polymer-sorbed compound acts as a partitioning source in equilibrium with the dissolved pool of compound in the test medium. Loss processes depleting this dissolved pool are compensated for by further partitioning, enabling toxicity testing at defined and constant dissolved concentrations (Figure 1).

As part of a wider drive to establish passive dosing for the proper control of exposure in toxicity bioassays, a format suited for routine application in the Microtox® assay has been developed and tested. This widely applied bioassay uses the marine bacterium Vibrio fischeri, where exposure to the toxicant(s) leads to metabolic inhibition as determined by a decrease in the inherent luminescence.

Polydimethylsiloxane (PDMS) silicone disks (1 cm diameter, 76  $\mu$ m thickness) were loaded with decreasing concentrations of four model hydrophobic compounds (acenaphthene, phenanthrene, fluoranthene and benzo(a)pyrene).

These were then used to passively dose the testmedium, to give dissolved concentrations ranging from aqueous solubility down to 2 orders of magnitude below this. A parallel test was per- formed where the equivalent nominal concentrations were solvent spiked using DMSO.



Reservoir of polymer sorbed HOC

Figure 1: Passive dosing for defined and constant dissolved concentrations of hydrophobic organic compounds in toxicity tests.

Figure 2 shows the resulting concentration-response curves for passive dosing versus solvent spiking. Acenaphthene and phenanthrene resulted in full concentration-response curves, with a significant difference in the calculated 50% effect concentrations (EC<sub>50</sub>) observed between passive dosing and spiking. For acenaphthene these differed by a factor of two (EC<sub>50</sub> 231 and 412  $\mu$ g L<sup>-1</sup> for passive dosing and spiking, respectively). For phenanthrene these differed by a factor of 15 (EC<sub>50</sub> 9.5 and 139  $\mu$ g L<sup>-1</sup> for passive dosing and spiking, respectively).

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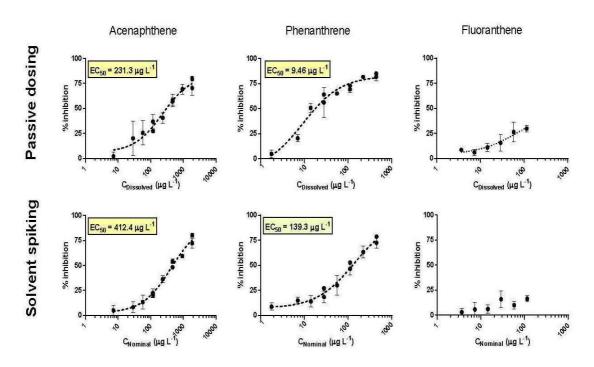


Figure 2: Comparison of passive dosing and solvent spiking for the control of exposure in the Microtox® bioassay.

For fluoranthene, a reliable trend with increasing concentration was only obtained with passive dosing, and aqueous solubility concentrations did not result in full inhibition. Benzo(a)pyrene did not result in any toxicity even at aqueous solubility (data not shown).

These observations have rather fundamental implications because (i) such effect concentrations are important in chemical regulation and (ii) poor exposure control might mean that mixtures of such compounds are deemed to be non-toxic when this is not the case. Therefore, the application of polymers such as silicone as a passive dosing phase forms an useful approach for improving the toxicity testing of hydrophobic compounds and their mixture. Efforts are underway to develop passive dosing formats for other assays (e.g., acute and chronic Daphnia magna bioassays, algal growth inhibition assay). Ultimately the goal is to use polymers such as silicone to first sample the bioavailable fraction of environmental mixtures via passive sampling, and then to directly dose these mixtures at the correct levels in different toxicity bioassays using the above passive dosing approach.

The above study forms the basis of a scientific manuscript being prepared for submission.



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### Environment & Bio Group

#### Biotechnology applied electrode research

#### Metal oxide, filamentous bacteriophage, electrode

#### **Summary:**

The mono dispersity and long rod shape of the phage, bearing specific recognition motifs, enable the organization of various nano materials (metal oxide) such as TiO<sub>2</sub>, WO<sub>3</sub>, V<sub>2</sub>O<sub>5</sub> and Al<sub>2</sub>O<sub>3</sub> into periodically ordered hierarchical structures that could be useful for electronic, optical and biotechnological applications. In addition, it is benefit to utilize filamentous phage in terms of environmentally friendly reactions. In order to know the organization of bacteriophage in conjunction with metal oxide, we used The Quartz Crystal Microbalance with Dissipation Monitoring (QCMD), UV-Vis absorption spectra measurements and scanning electron microscopy (SEM) imaging showed that metal oxide binding peptide aids phage binding to metal oxide precursors. The objective of this research is to create new and efficient electrode surface via environmentally friendly organic electrode.

PDMS-Surface was respectively patterned by the bacteriophage and nano particles ( $TiO_2$ ). The patterned PDMS can be used as electrode and sensor.

To begin with, the bacteriophage and nano particles (TiO<sub>2</sub>) were arrayed on PDMS-surface. Afterwards, they were deposited by sputtering Pt. coating cycle of sputtering at once, coating time of sputtering is 20 seconds, and the target current of sputtering is 120mA. Images of SEM were conducted with an FEI Quanta 250 FEG scanning electron microscope at 5 kV in high vacuum. Presence of bacteria phage and nano particles was confirmed by SEM.

As PDMSs of Fig.1 were compared at 1 $\mu$ m and 5 $\mu$ m of width of PDMS, the PDMS of 5 $\mu$ m was well arrayed than that of 1 $\mu$ m. This result suggests that the place which can be occupied by something should be more than enough to input over 1 $\mu$ m in terms of PDMS-width. That's because the length of bacteriophage is approximately 1 $\mu$ m.

Fig. 2 shows that nano particles ( $TiO_2$ )(100nm) were entirely arrayed well on surface according to width, but the cracks occurred after PDMS-surfaces longer than that of 40 $\mu$ m had been treated with nano particles ( $TiO_2$ )(100nm).

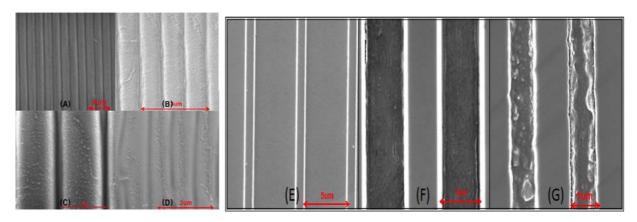


Figure 1: SEM images of PDMS treated with bacteriophage (1.7654 X  $10^{13}$  /ml). The width of Figure 1(A)~(D) is  $1\mu$ m and the width of Figure 1(E)~(G) is  $5\mu$ m. Imaging SEM was conducted with an FEI Quanta 250 FEG scanning electron microscope at 5 kV in high vacuum.

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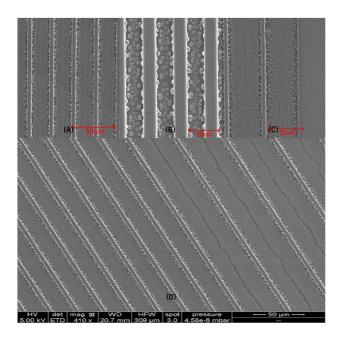


Figure 2: SEM images of PDMS-surface treated with  $TiO_2$ -nano particles (100nm) at at different width of PDMS. PDMS-surface was treated with  $TiO_2$ -nano particle (100nm) (width = 20 $\mu$ m) is shown in Fig (A). PDMS-surface treated with  $TiO_2$ -nano particle (100nm) (width = 10 $\mu$ m) is shown in Fig (B). PDMS-surface treated with  $TiO_2$ -nano particle (100nm) (width = 50 $\mu$ m) is shown in Fig (C) and PDMS-surface treated with  $TiO_2$ -nano particle (100nm) is shown in Fig (D) according to width. Imaging SEM was conducted with an FEI Quanta 250 FEG scanning electron microscope at 5 kV in high vacuum.

Until now, we had PDMS-surface respectively patterned by the bacteriophage and nano particles (TiO<sub>2</sub>). The next experiment will be performed following binding both bacteriophage and metal oxide. We will make a periodically patterned PDMS with bacteriophage and metal oxide alike. After that, it will be possible to make efficient electrodes.



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### **Environment & Bio Group**

# Development of TiO<sub>2</sub>-phage complex by using TiO<sub>2</sub> nanoparticles and a water-soluble precursor (TiBALDH)

Titanium dioxide, Biomineralization, Titanium (IV) bis(ammonium lactato) dihydroxide, phage surface technique, DSSC system

#### **Summary:**

Phages can express various peptides and bioactive protein on their surface for biomineralization of TiO<sub>2</sub> as a prerequisite for the development of DSSC system. Compared to chemical and thermal process of TiO<sub>2</sub> nanoparticle manufacturing, the process of the phage based TiO<sub>2</sub> biomineralization can be attribute to easy cost at relatively low temperatures using TiBADLH, a water-soluble and nonhazardous titanium complex, which is stable at near neutral pH and ambient conditions. In the present study, using the phage surface technique for biomineralization of TiO<sub>2</sub>, two serines were genetically engineered on a p8 of f88 phage (STB1-f88 phage) and p8 of phage (p8SSG-phage), respectively. The interaction of STB1 and two serines with TiO<sub>2</sub> precursor was probed using a different pH and UV illumination.

The binding of STB1-f88 was shown in Figure 1. It result in different size of  ${\rm TiO_2}$  / STB1-f88 complex due to their overlapping of interaction with size of extended length ranging from 2 to 12  $\mu$ m which is more than two orders larger than the size of single phage.

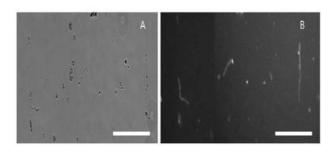


Figure 1: Bright field (A) and fluorescence microscopy images (B) of STB1-f88 phage were mixed with  ${\rm TiO_2}$  nanoparticles for 1 day (at pH 9.0). Immunofluorescence labeling of phage was performed with an anti-p8 antibody linked to biotin. Fluorescence images were taken avert incubation the antibody treated phage with cy3 conjugated streptavidin molecules. Scale bar = 10  $\mu$ m.

The affinities of STB1-f88 to  ${\rm TiO_2}$  particles were assessed again in the present study shown in Figure 2. By using BCA assay, it is shown that  ${\rm TiO_2}$  addition to STB1-f88 is concentration dependant. Our observation indicates that STB1-f88 induces complexation with  ${\rm TiO_2}$  with specific affinity at pH 9.0.

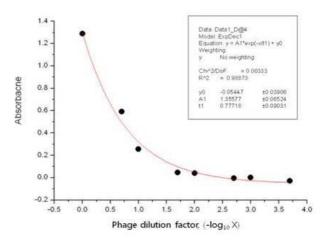


Figure 2: Measurement of UV absorbance in 560 nm according to phage concentration. Different concentrations of STB1-f88 phage with TiO<sub>2</sub> particle; concentrations of STB1-f88 phage was stepwise decreased with fixed concentration of TiO<sub>2</sub> particle (0.1 g/ml).

We further investigated the diameter STB1-f88 distribution of TiO<sub>2</sub> / complexation by DLS measurements after mixing of TiO2 and STB1-f88 phage solutions shown in Figure 3. The average particle size of TiO<sub>2</sub> and STB1-f88 phage were determined as 450 and 120 nm, respectively (Figure 3A and 3B). When TiO<sub>2</sub> was mixed with STB1-f88 phage, the mean diameter shows a bimodal distribution of TiO<sub>2</sub> / STB1-f88 phage complex 170 and 460 nm after 5 min incubation (Figure 3C). As shown in highly scattering TiO<sub>2</sub> / STB1-f88 phage were obtained after 1 hour incubation at a mean of 700 nm in diameter which is notably larger than that of STB1-f88 phage and corresponding to size of was dramatically decreased (Figure 3D).

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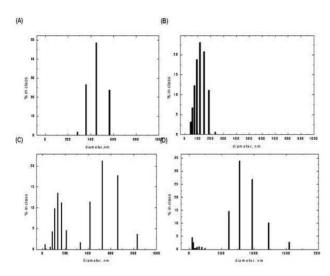


Figure 3: Dynamic light scattering measurement result. (A) only  $TiO_2$  particle (0.5  $\mu$ g/ml); (B) only f88-STB1 phage (1x10<sup>12</sup> cfu/ml), (C) f88-STB1 phage +  $TiO_2$  particle, incubation for 5 min; (D) f88-STB1 phage +  $TiO_2$  particle, incubation for 1 hour.

UV-induced mineralization is triggered shown in Figure 4. The biomineralization of bacteriophage experiment consists of mixing of 0.5 % of TiBALDH with 5 X  $10^{11}$  cfu/ml of phage concentration. After irradiation of samples are transferred to dialysis bag to remove reminder of precursor solution. The biomineralization of phage to TiO $_2$  was observed using bright field microscope and SEM (Figure 4).

Figure 4A shows nanorode shapes of TiO<sub>2</sub> mineralized phage on Si wafer at low magnification obtained bγ bright field microscopy. The biomineralized phage was deposited phage on Si film observed by bright microsopy and SEM. EDS analysis shows Ti elements which is good agreement with successful formation of TiO2 crystalline (Figure 4E). The average single size of the crystalline of phage / TiO<sub>2</sub> complex is 2 μm. One can notice that distal of ends a single crystalline grows to extend and distinguishible morphology.

In this study, phage as template can have many advantages in comparisons to synthetic nanomaterials, like have fewer safety concerns with environmentally-friendly approaches. Our experiments suggest that fabrication of phage based nanostructure will satisfy our most common biotechnology applications.



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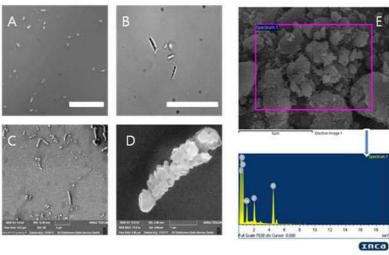


Figure 4:  $TiO_2$  and phage nanocrystalline obtained from TiBALDH by using UV-illumination. Bright field microscopic images of the biomineralized phage showing the microstructure of the phages (A and B). SEM images of biomineralized phage showing the details of the microstructure of a single phage (C and D). Samples were measured in 5kV, X 10,000 (C) and 15 kV, X 74,900 (D). (D) is EDS analysis of  $TiO_2/STB1$  f88 phage complexes. Each scale bar indicates 10  $\mu$ m.

# **Environment & Bio Group**

#### Recombinant phages for nanowire templating

Bacteriophage, genetic engineering, metallization, gold, nanoparticle, SEM

#### **Summary:**

Bacteriophages are nano-sized virion particles infecting bacteria. In this study, it is shown that metal binding properties of filamentous fd-bacteriophages can be enhanced by genetic engineering. Quartz crystal microbalance (QCM) analyses, UV-Vis absorption spectra measurements and Scanning Electron Microscopy (SEM) imaging revealed that expression of MMM short amino acid sequence on major coat protein p8 facilitates recombinant MMM- phage binding to Au surfaces and nanoparticles (NPs) via gold-sulfur (Au-S) interaction. Electroless deposition of Au particles on phage assemblies was investigated upon chemical reduction reaction with NaBH<sub>4</sub> at different HAuCl<sub>4</sub> precursor concentrations. Energy dispersive X-ray spectroscopy (EDX) measurements confirmed the presence of Au on both AuNP decorated and chemically metallized phage structures. Further studies on patterning and controlled immobilization of recombinant bacteriophages on specific surfaces may contribute to bio-templated nanowire development field and biosensor application studies.

In this study, wild type fd-bacteriophages and genetically modified MMM-phages displaying methionine (M) amino acids at N-terminus of 2500 copies of p8 major coat protein were compared in terms of their binding affinities to Au substrates. Engineered MMM-phages were shown to bind on either Au sensor surfaces or to 20 nm AuNPs by UV-Vis spectroscopy, QCM and SEM analyses (Fig. 1).

However, wild type fd-phages did not show a remarkable binding affinity against Au substrates (please refer to the publication for detailed information). These results pointed at the functionality of Au-S interaction provided by the available sulfur (S) containing functional groups displayed on bacteriophage surface. Many biological molecules like DNA, microtubules, actin filaments and S-layer proteins were already applied as templates for metal deposition. The most common way to metallize biotemplates is to incubate them with metal precursor solutions and then let the bound metal ions be reduced upon reducing agent treatment.

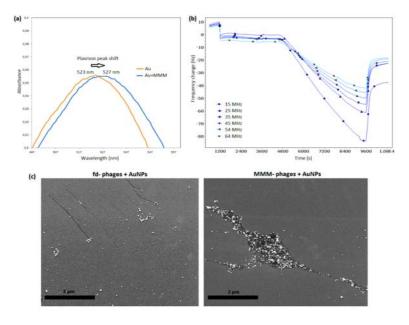


Figure 1: UV-Vis spectra of AuNPs mixed with MMM-phages show a 4nm peak shift indicating conjugation (a). Frequency decrease observed during QCM analyses of MMM-phages on Au sensor surfaces implicates bacteriophage binding and accumulation on gold coated sensor (b). SEM images show fd- and MMM-phages after incubation with citrate stabilized (c).

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Similarly, MMM-phages were first mixed with different concentrations of HAuCl<sub>4</sub> precursor solution (3 mM, 30 mM and 60 mM). Bacteriophages were observed to form bundles upon precursor treatment as seen in Fig. 2. When the precursor concentration was increased, attractive forces between phage filaments got stronger forming more intertwined and larger structures which can be described by Manning's theory of counterion condensation. Phage bundles were next treated with a strong reducing agent, NaBH<sub>4</sub>, resulting in electroless deposition of Au clusters on bacteriophage filaments (Fig. 2). EDX analyses conducted on metallized samples clearly demonstrated the presence of C and Au peaks corresponding to bacteriophages and Au clusters. Sonication samples after metallization resulted 1-2 in long μm filamentous structures decorated with Au clusters.

#### **Publications**

N. Korkmaz:

Recombinant bacteriophages as gold binding biotemplates

Colloids Surf B. Biointerfaces, 112C, p. 219-228



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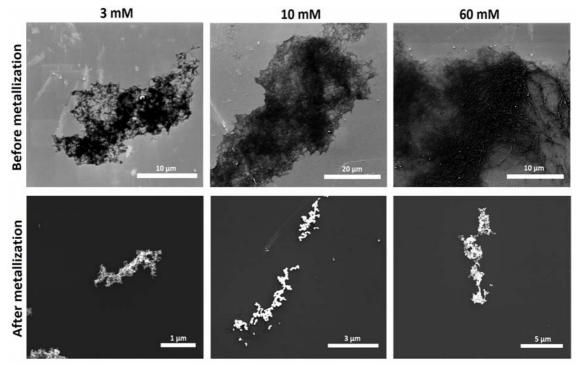


Figure 2: SEM images of MMM-phages after HAuCl<sub>4</sub> treatment (3mM, 10mM and 60mM) and chemical reduction. Metallized samples were sonicated before immobilization on SiO<sub>2</sub> wafers.

# Environment & Bio Group

# BsAb increases photosensitizer transfer in T cell-mediated drug delivery

Photodynamic therapy, cell-mediated drug delivery, cytotoxic T cells, cancer

#### Summary:

Photodynamic therapy (PDT) and adoptive T cell transfer (ACT) are emerging treatment modalities for cancer. However, the photosensitizers (PS) administered in PDT lack high specificity for neoplasms. We pursue a new approach, using bispecific antibody (bsAb) redirected T lymphocytes as vehicles to specifically deliver PS to cancer cells. We have demonstrated that in the presence of bsAb, PS-transfer from drug loaded carrier cells to targeted cancer cells is indeed increased. Our findings support the idea that a living drug delivery system is suitable to improve the specificity of an anti-tumor drug.

We pursue a new targeted photodynamic therapy (PDT) modality by using bispecific antibody (bsAb) redirected drug-loaded T lymphocytes to transport photosensitizer (PS) molecules to sites of malignancies. The bsAb used is dual specific for a tumor-associated antigen expressed by carcinoma cells and the epsilon-chain of the CD3 antigen expressed by T cells. In our approach, bispecific antibodies cross-link ex vivo activated polyclonal human T lymphocytes with target cells, which then triggers the cytolytic function of killer T cells against aberrant cells. In the context of adoptive cell transfer, the concept intends to increase the selectivity and efficacy of PDT, while decreasing effects like eve adverse and photosensitivity. Particularly, the approach is meant to combine the cytotoxicity of tumorspecific T lymphocytes (T cell effect) with the phototoxicity of delivered PS (drug effect) to achieve synergistic anti-tumor effects.

In 2012 we reported on a study, which demonstrated the proof of our cell-mediated drug delivery concept in vitro (Annual Report 2012). We showed that in co-cultures bsAbguided loaded T cells were superior to redirected unloaded T lymphocytes in killing cancer cells.

Notably, within the first 16 hours of coincubation retargeted loaded T cells were more effective in impairing cancer cell growth than bsAb-guided unloaded T cells plus freely applied drug. Thus, we confirmed that the combination cytotoxicity and phototoxicity indeed exhibited synergistic effects. In 2013 a detailed case study was conducted, in which the drug transfer from carrier T cells to target cancer cells was investigated. For this purpose, tumor cells were co-cultivated with unloaded or drugloaded T lymphocytes with or without bsAb. We showed that in the presence of bsAb significantly more **PS-molecules** were transferred (Figure 1).

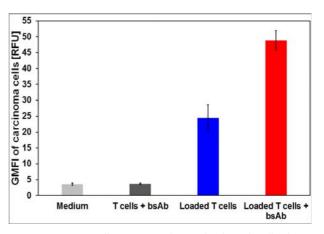


Figure 1: Cancer cells were co-cultivated only with cell culture medium (grey bar), with T cells + bsAb (dark grey bar), with loaded lymphocytes in combination with bsAb (red bar) or without bsAb (blue bar) for 6 h at  $37^{\circ}$ C. Geometric means of fluorescence intensities (GMFI) obtained from gated cancer cells populations are expressed as relative fluorescence units  $\pm$  SD. In general, significantly more PS is transferred in the presence than in the absence of bsAb.

## **Environment & Bio Group**

In co-cultures loaded T cells sediment on top of or between tumor cells and the drug is passed from one cell to another (Figure 2). However, in the presence of bsAb the contact duration between carrier cells and target cells is prolonged. As a consequence, more drug is transferred. This major finding probably explains the observed synergistic phenomena and supports the idea that a pharmaceutical can be specifically delivered by bispecific antibody redirected loaded T lymphocytes. The available data suggest that our cell-mediated drug delivery approach could be feasible.

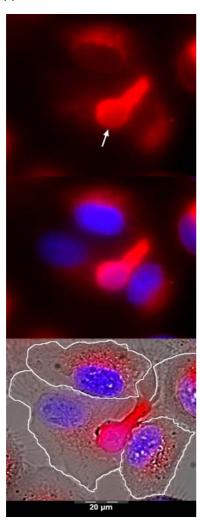


Figure 2: A bright red fluorescent drug loaded T cell (white arrow) attached to three carcinoma cells after co-cultivation in the presence of bsAb is shown. Tumor cells have incorporated the red fluorescent photosensitizer (upper panel). Accumulated PS-molecules are located around the cell nuclei (upper panel and blue fluorescent in the middle panel). Bright lied image and corresponding fluorescence images are shown as overlay in the lower panel. (white lines = tumor cell membranes).



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### Environment & Bio Group

# Establishment of an electrochemical Cytochrome P450 biosensor with direct electron transfer detection

Biosensor, cytochrome P450, dendrimer, electrochemistry, drug discovery, biocatalysis

#### **Summary:**

Here we present an electrochemical Cytochrome P450 (P450) biosensor that employs dendritic molecules as connective layer between transducer and the biological sensing element. Being involved in detoxification as well as in biocatalytic processes where -in general- oxygen is introduced to the target molecule, the heme-containing P450s are an interesting target for sensor research. Assembly was carried out layer-by-layer via electrostatic attraction of the involved species and monitored by QCM-D. Currently, the B.subtilis CYP109 is used for the establishment of the sensor and the electrical response in presence of substrate Testosterone is probed by cyclic voltammetry.

Cytochrome P450s are a diverse superfamily of heme-thiolate enzyme proteins involved in the removal and inactivation of potential harmful or toxic substances from cells or from the body as well as in biocatalytic processes like the steroid pathway. This makes them an interesting target for the pharmaceutical industry where they are a standard test subject in drug discovery and also offer the possibility for highly-specific cata-

lytic reactions. So biosensors as well as reactors incorporating those enzymes are an interesting target for research and an increasing amount of effort is put into the establishment of electrochemical sensors employing a direct unmediated electron transfer from an electrode to the heme group of the enzyme (Schneider and Clark 2013).

Performance of biosensors is mainly depending on the connection between the transducer, which is usually a metal electrode and the biological sensing element, the P450. In this project, a new group of polymers was used as connective layer. Dendrimers are characterized by their branched, tree-like structure, exhibiting a high amount of surface groups and internal porosity. Here, dendrimers of generation 4 with a Polyamidoamine (PAMAM) core were used for their good biocompatibility and their functional surface groups (-NH3). Because of these groups a layer-by-layer assembly (LBL) method could be used (Figure 1).

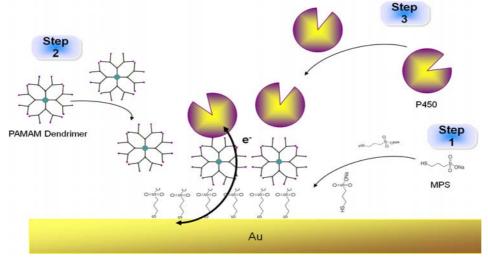


Figure 1: Biosensor LBL Assembly Strategy. Step 1 represents the introduction of charge to the Au electrode surface by 3-Mercapto-1-Proganesulfonate (MPS). Step 2 is the dendrimer adsorption at pH below dendrimer pl. Step 3 is the SYP109 adsorption at pH above pl. The result is a biosensor with stacked monolayers of the respected molecules.

## **Environment & Bio Group**

Furthermore, Au nanoparticles were included by a mineralization process inside the dendrimer molecules cavities to increase conductivity of the dendrimer layer and to decrease the distance between electron source and enzyme (Zhang and Hu 2007).

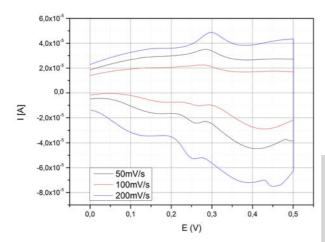


Figure 2: Cyclic voltammogram of the (Au/MPS/PAMAM G4-Au/CYP109) biosensor response in presence of  $15\mu M$  testosterone at scanrates 50,100 and 200 mV/s. Increasing  $I_{pc}$  &  $I_{pa}$  values can be explained by the scanrate variation and are in good agreement with theory. Peak separation between anodic and cathodic current hints at a 1 elctron transfer reaction.

To test the electrical response of the biosensor, cyclic voltammetry (CV) was used to probe the transfer of electrons to and from the heme group of the enzyme (FeII/FeIII), where, during substrate conversion, one electron is consumed to introduce oxygen to the substrate, in this case Testosterone. Figure 2 shows a typical voltammogram at different scanrates (50, 100, 200 mV/s) of the (Au/MPS/PAMAM G4-Au/ CYP109) biosensor which is in good agreement with expected data. Peak separation between anodic and cathodic peaks hints at a one electron transfer and is close to the expected 57 mV and the differences in peak height (Ipc>Ipa) can be attributed to the electron consumption during substrate conversion. Testosterone samples between 25 and 0.5 μM were prepared, used as substrate for the biosensor and, even at the lowest substrate concentration, a definite reductive current peak could be clearly observed.

Concluding, it can be said that a working P450 biosensor could be constructed. Further optimization of the sensor could even yield a larger electrochemical response from the immobilized enzyme and thus making it possible to realize even lower detection limits than 500 nM. Further testing of the influence of Aunanoparticle modification of the PAMAM dendrimers on the electrical connectivity between a metal electrode and an electroactive enzyme is also regarded to be highly interesting and will be looked into in the near future.

#### **Publications**

Schneider, E., Clark, DS:

Cytochrome P450 (CYP) enzymes and the development of CYP biosensors

Biosensors and Bioelectronics, 39/2013, pp. 637-649

Zhang, H., Hu, N.:

Conductive Effect of Gold Nanoparticles Encapsulated inside polyamidoamine (PAMAM) Dendrimers on Electrochemistry of Myoglobin (Mb) in {PAMAM-Au/Mb}n Layer-by-Layer Films

The Journal of Physical Chemistry B 111/2007, p. 10583-10590



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Technology Policy Center

## Technology Policy Center

#### Introduction



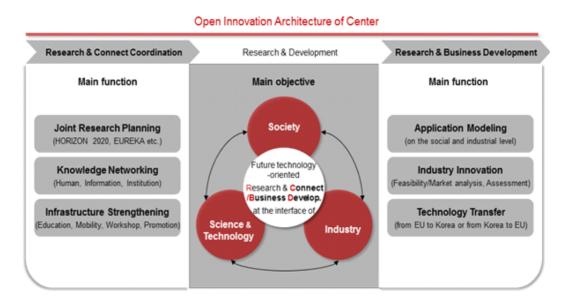
**Dr. Jongwoon Hwang**Center Leader Technology Policy Center

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Dr. Jongwoon Hwang was the head of the Technology Policy Center at KIST Europe since 2006. He joined KIST Europe in 2000 and is in charge of international cooperation especially between EU and Korea. He has involved in many national and international projects such as KESTCAP, KORRIDOR, KECO, Global Tech, European ICT Trends and Infrastructure analysis etc. He also leads studies such as Sustainable Waste Management Strategy for Green Printing Industry Business of ECO-INNOVERA (EU CIP). He has lead research projects such as development of energy management system for smart energy services. He got his doctoral degree in Business Administration at Technical University of Berlin in Germany and has relevant and interdisciplinary experiences in Science and Technology Cooperation. Dr. Jongwoon Hwang was transfered to KIC-Europe (Korea Innovation Center) in Brussels in February 2014.

The Technology Policy Center sees its main activities in contributing to solve societal challenges on the Korean and European level by establishing strategic concepts as well as technical solutions for reliable market applications in Korea and EU industries.

The main objective of the Technology Policy Center is future technology-oriented collaboration & policy development based on investigation of core & fusion technology, and providing technical & strategic solutions. The center is especially interested in planning European R&D project such as HORIZON 2020 and EUREKA, and supporting the EU framework program such KONNECT, ECO-INNOVERA that improves the international link between Korea and the EU by establishing a global partnership and providing a European market access. Furthermore, the center sees its activities in order to achieve sustainable, smart and healthy ageing society.



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## Technology Policy Center

In addition to the aforementioned R&D activities, the Technology Policy Center has taken a conclusive role and activities as a base for extending R&D networks between Korea and EU in terms of personnel and information exchange, and technology transfer for enhancing the international cooperation in science and technology with European countries which is a central issue and a strong factor for achieving the main objective. Furthermore, the center is conducting organization of international conferences to establish joint research projects in future technologies to build a personnel network and to extend the cooperation of the European-Korean research society and, organization of human resource development programs for Korean students and experts in cooperation with the Korean Council for University Education (KCUE) and Korean NRF (National Research Foundation).

Besides, the Technology Policy Center is building database about European-Korean S&T policy and information and publishing a handbook on European/German S&T Policy, research programs and partner institutes. This on-line network and off-line publication can give a motivation for more research cooperation and can extend the S&T cooperation between Korea and Germany.



## Technology Policy Center

## A survey analysis on the wellness human-care platform

#### eHealth, survey analysis, wellness

#### Summary:

This research analyzes policies and technology related to eHealth and best practices of wellness business models in Europe. Many advanced countries have been tried to solve legal issues about use of ICT in healthcare system and have invested in the research related to eHealth. In particular, member states of European Commission have been preparing policies and strategy of eHealth since 1999. This research surveys European leading strategies and technologies, and thus might support the development of wellness human-care platform in Korea and the construction of a cooperation network between Korea and Europe.

On the other hand, the health maintenance has been affected by information and communications technologies (ICT) in the digital age since 2000. World Health Organization (WHO) defines eHealth as the cost-effective and secure use of ICT in support of health and health-related fields, including health-care services, health surveillance, health literature, and health education, knowledge and research.

In many advanced countries such as the US and the EU, eHealth research and related products based on ICT have been increasing. This research analyzes policies and technology related to eHealth and best practices of wellness business models in Europe. Figure 1 shows the scope of the study.

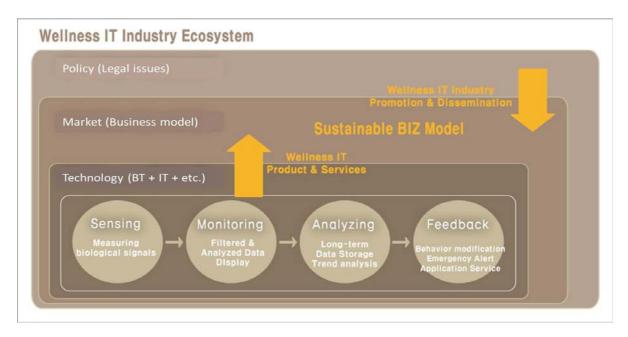


Figure 1: Sustainable wellness IT industry: Wellness products and services are developed by technological connvergence and the political support for the development and the development are essential.

Wellness is an active process of becoming aware of and making choices toward a healthy and fulfilling life. Especially, the health maintenance paradigm changed from treatment to prevention according to aging causes rapid increase in demand on wellness.

In order to sustain wellness IT industry, the products and services developed by various technologies have to be promoted by appropriate policies. Eventually, it is expected to support the construction of a cooperation network between Korea and Europe.

## Technology Policy Center



Figure 2: Dr. Hwang making a presentation

The wellness global forum concerning this research was held on 2nd December 2013 in Seoul, Korea. Four panels attended the forum, and they introduced research and technology of eHealth system as well as institutional issues of Korea and Europe. Figure 2 shows Dr. Hwang's presentation under the title of 'A Survey Analysis on Wellness R&D and Business Models in EU.' In continuous panel discussions, he talked about German health insurance system and European health-care system integration.

For the further work, policies and legal issues of Germany, France and the UK of 28 EC member states will be analyzed. In addition, more research programs and best practices have to be surveyed for analysis of European eHealth research status. This research will be continued until April 2014.



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## Technology Policy Center

### **Cooperation with Korean Government**

#### JSTCC, KIC-Europe, R&D Forum

#### Summary:

These are to support Korean government activities in the 50th anniversary year of EU-Korea relations. In June, Korea-EU R&D Forum and 4th EU-Korea Joint Science and Technology Cooperation Committee (JSTCC) were held in Brussels to share research areas of common interest and discuss the cooperation between Korea and the EU. As a follow-up step of the JSTCC, new 'Research & innovation centre -Europe (KIC-Europe)' is established in European Brussels, district of the systematic as collaborative infrastructure. It will information and human resource network in the EU and support political dialogues. With opening ceremony of the KIC-Europe, a round table meeting hosted by Mdm. President was also held in Brussels to celebrate it.

Korea and the EU play a key role in the areas of Science, Research and Innovation at an international level and cooperation in these domains constitutes a key element of the overall partnership. The science and technology agreement between Korea and the EU entered into force in April 2007. In June 2013, Korea-EU R&D Forum hosted by Korean Mission to the EU was held in Brussels. Objectives of the forum are sharing the status of the four science and technology areas - ICT, BT, NT, and Energy - of Korea and the EU.

With this forum, 4th EU-Korea Joint Science and Technology Cooperation Committee (JSTCC) hosted by Ministry of Science, ICT, and Future Planning (MSIP) were held in Brussels (Figure 1). This committee discussed research areas of interest common and mechanisms cooperation with a view to further strengthen cooperation in research and innovation. In addition. this committee contributes to supporting cooperation between both sides and explored mechanisms by way of working groups in thematic areas.



Figure 1: 4th EU-Korea Joint Science and Technology Cooperation Committee

As a follow-up step of the JSTCC, the foundation of Korea Research & Innovation Centre - Europe (KIC-Europe) had been prosecuted since June 2013. The KIC-Europe contributes as a strategic platform, makes a commitment to facilitate a regional policy dialogue to include a full spectrum of Korean & European actors and accompanies a pivotal role in disseminating momentous information and promoting Korean-European science and technology partnership. KIST Europe has supported everything related to KIC-Europe including finding an establishing a plan, etc. The KIC-Europe is established in November, and opening ceremony was held on 8th November. A round table meeting hosted by Mdm. President was also held in Brussels to celebrate opening KIC-Europe.



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## Technology Policy Center

## **Investigation of Science Culture Programme of Europe**

#### Science & culture of the EU, science in society in the EU

#### Summary:

This project investigates scientific and cultural activities of European and Korean governmental-funded research institutes. In addition, the contents from investigation will be compared for looking for suggestion. The results of this project contribute to various and effective scientific and cultural program planning in Korea institute. In addition, this project build a foundation for the Korea-EU scientific and cultural program cooperation network.

The scientific and cultural activity is not a culture of scientists or research process. It is the program for relation to science and public. The scientific and cultural activities are demanded by social responsibility and reliability to science and technology. And the more various and effective programs are needed with regard to improved science and technology.

This project investigates scientific and cultural program contents and managing process of European and Korean research institutes. This project focuses on program contents and managing process at governmental-funded research institute of EU, Germany, Great Britain, France, Sweden. The EU has the one of the category "Science in Society(SIS)" since 2001, and now it is conducting in research and development program Capacities, FP7. This program has the 6 objectives that choose together, unlock the full potential, creative learning fresh ideas, share results to advance, do the right thing and do it right, and design science for and with society and 3 main action plans that responsible governance, human potential and horizons, and science and society communicate. For example, the active program in SIS is "European Union Contest for Young Scientists" (EUCYS) and is held every year for making interest of science and technology to young people. In this project, the contents, participants and planning, running, evaluating process of this contest will be investigated.

There are many type of science and cultural program contents and managing process at

European research institute will be analyzed to looking for various and effective future plan.

Also, the program contents and managing process of Korean research institute will be investigated for understanding their status and needs. The contents from investigation will be compared and analyzed. The project conducting basic method is report review of European and Korean program. Also, the European contents are investigated on-line, off-line interview for the deep-understanding. On the other side, conference meeting will be held for listening to program manager's opinion for demand survey in Korea. This project is funded by Korea research council of fundamental science and technology(KRCF). And the investigation of Korean program and managing process will be conducted by Korea institute of science and technology information(KISTI).



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## Technology Policy Center

# Strengthening STI Cooperation between Korea and the EU, promoting innovation and the enhancement of communication for technology-related policy dialogue (KONNECT)

Korea-EU, STI cooperation, STI policy, cooperation roadmap, Horizon2020

#### Summary:

The KONNECT project, funded by EU Framework Programme 7, will strengthen STI cooperation between the EU and Korea, promoting and the enhancement innovation communication for technology-related policy dialogue. This project brings together seven organizations from the EU and Korea to improve and sustain communication between the two regions at the research and policy level and increase the expansion of the scope of Science, Technology, and Innovation (STI) networks and activities.

The KONNECT project will be committed to adhering to the following broad objectives: 1) Developing Knowledge-based Infrastructure, 2) Improving Strategic Communication, 3) Raising Awareness to Facilitate Cooperation between the EU and Korea, 4) Enhancing Networking between Science, Technology, and Innovationfocused Actors, and 5) Fostering Innovationfocused Joint Activities. The WP I for research, analysis and monitoring, which is led by KIST Europe, conducts a guiding role for all the other project activities and supports the project theoretically and strategically to achieve its ultimate objectives. WP I facilitates a greater understanding of the current state of STI activities in Europe and Korea and associated strategies currently being implemented in both regions. Based on this information, practical strategies for future S&T collaboration leading towards Horizon 2020 and beyond will be developed. Information from WP I provide evidence-based knowledge for recommendations on the future design of funding and management programs as well as policies that will function as a pivotal foundation for longterm S&T collaboration between Korea and Europe.



Consortium meeting October 3, 2013

Through the WP I, a database of EU-Korean Science and Technology (S&T) Collaborative activities will be constructed and baseline reports for all the project's other activities will be produced.

Cooperative S&T activities supported by both European countries and Korea will be monitored and analyzed using data provided by governments, funding agencies, and research institutions from both sides. An in-depth study will be conducted of best practices provided by stakeholders, including private enterprises, to understand the current state of research and innovation collaboration. S&T political strategies will be recommended for both sides based on a long-term collaborative roadmap which will be developed utilizing the constructed database and results produced from analysis.

The main activities of the KONNECT project will work towards progressing research in four central fields of mutual interest: Information and Communications Technology (ICT), Nano, Materials, and New Production Technologies (NMP), Green Technology and Secure, Clean,

## Technology Policy Center



General Assembly, October 3, 2013

and Efficient Energy (GT), and Biotechnologies (BT).

The project's consortium will organize important activities within the set parameters of the project and expanding joint activities under thematic areas. Due to the newly launched EU R&D Framework Program (Horizon 2020) and Korea's government changeover in 2013, initiating new collaborative activities between the two sides takes on even greater importance.



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## Technology Policy Center

### **Experts Networking: Workshop and Training Program**

#### International collaboration, researcher networking

#### Summary:

Workshop for S&T researchers and training program for executive officers were held in October, 2013 to extend R&D collaboration and improve the efficiency of the participation to R&D programs. Workshop, co-organized with German research institute INM, was purposed to establishing Korean-European research collaboration and attending the R&D Programs in EU and Korea, furthermore. As a first achievement of it, INM and KIST Europe signed and exchanged MoU.

16 Korean executive officers in international collaboration learned about EU R&D programs and visited several institutes responsible for the programs.

To extend the research collaboration with European institutes, KIST Europe and INM (Leibniz Institute of New Materials, Germany) co-hosted a workshop: "Future Materials and Safety: for establishing European-Korean research", with sponsorship from gwSaar, Saarland, Germany from 15 to 16 October, 2013. The 6 sessions and 30 lectures were conducted about nano technology based areas covering materials, medicine & pharmacy, safety, and special lectures about HORIZON2020 and Korean R&D Program.

There were 94 registered participants from 19 institutes from 6 countries. Korean research experts participated from four main national research institutes (KIST, KRIBB, KRICT and KRISS). After the presentation program, researchers from Korea and KIST Europe were parted in 4 major fields for intensive discussion with European partners to establishing collaboration and finding out the research topics in order to join in R&D programs. One of the main achievements of it was signing MoU between KIST Europe and INM, on 14 October, 2013.

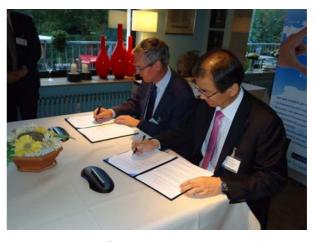


Figure 2: Signing of MoU between KIST Europe and INM



Figure 1: Group photo of the workshop participants "Future Materials and Safety".

## **Technology Policy Center**



Figure 3: Group photo of the executive training program.

The executive training program was held from 8 to 19 October, 2013 in Saarbrücken, Brussels, and Paris in order to understand EU R&D programs in depth and learning the process to join into them. Total 16 Korean officers working on international collaboration attended from governmental sectors, national research institutes, and universities. The program focused on EU frame work programs such as HORIZON2020, EUREKA etc. from introduction to participation guide. After the lecture schedules, they visited the hearts of the European programs in Brussels and Paris.



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## Technology Policy Center

## Develop business plans and policy suggestions for recycling waste-ink

Recycle, sustainability, waste, ink, printing, flexographic, life cycle assessment

#### Summary:

The flexographic and gravure industry generates large amounts of waste ink during the cleaning of machines which is incinerated causing highly toxic waste. In this project, through Olax 22 technology developed by University of Alicante, ink and solvent would be recovered from waste ink and reused as raw materials for the relevant industrial process. The strategy for recycling business shall be developed from the data of the current status of the industry obtained through interviews and survey and the results of LCA. Then, the demonstration of recycling process will be established to produce the data for LCA.

The project is EU project in collaboration with University of Alicante (UA) and École Polytechnique Fédérale de Lausanne (EPFL). University of Alicante develops the technology as well as research the market in depth while EPFL conducts Life Cycle Assessments (LCA). KIST Europe is responsible for the overall management as well as developing the business model and policy suggestions based on data from UA and EPFL as well as surveys and interviews. The survey will be conducted to companies in printing and waste management industry to gain information and insights of the current and future market.

#### Progress during the year 2013:

3 consortium meetings have taken place:

16.04.2013 EPFL, Lausanne (Swiss): Kick-Off Meeting

11.06.2013 UA, Alicante (Spain): Consortium Meeting

21.11.2013 UA, Alicante (Spain):
Consortium Meeting & Company Meeting



Group photo from Kick-off Meeting in April 2013

The first meeting was held in the form of kick-off meeting to plan strategies and processes of tasks. The consortium agreed on project activities, divided the tasks and the time plans were set up. Current issues on technical matters were discussed with explanations on the progress and technical background of the recycling process. Matters on Life Cycle Assessment (LCA) were also covered from defining the indicators for LCA to software implementations with the software provider. The members gave a review on financial status shared feedbacks on Consortium Agreement which were then incorporated in finalized document. Communication method and terms throughout the project were also discussed.

The second meeting was between EPFL and UA to carry out an in-depth discussion on technical matters for better understanding and execution of the project. University of Alicante gave a detailed explanation of flexographic printing technic and ink used in the process as well as the holistic life cycle approach which would be used in the implementation of LCA by EPFL. The minutes of each meeting were taken by one of the participating organizations, then revised and agreed to by all participants.

## Technology Policy Center

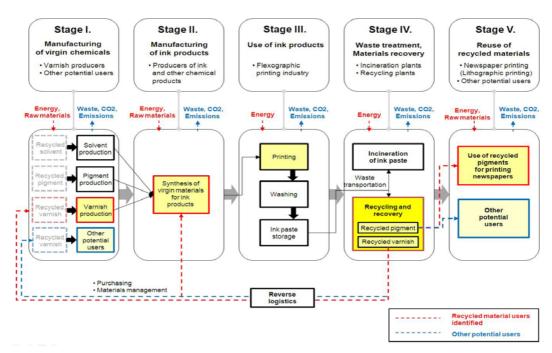


Figure shows supply chain involved in printing and recycling industry.

The third meeting was between KIST Europe and UA as well as KIST visiting partner companies in Spain. The purpose was to gain a deeper understanding of the market and technology in order to get input and feedback designing questionnaires for the company survey.

#### Deliverables

30.09.2013 1st Periodic Report & Company List of Printing Industry

1st periodic report and company list (for conducting survey) was prepared and written by KIST Europe. University of Alicante and EPFL provided data and information regarding the task of each responsibility. Company list for the survey has been derived from Orbis database using specific keywords and target based on research of the industry.

#### **Designing Survey**

Company list has been developed based on deep understanding of the technology and market. Then, survey questionnaires were developed by KIST Europe before the visit to University of Alicante. In November, during the visit the questionnaires were reviewed by University of Alicante.

After that, interviews with companies in printing and ink manufacturing industry were held together with pre-survey of the questionnaires.



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## **Technology Policy Center**

## Handbook "Science & Technology Policy and Research & Development Trends in Germany"

Handbook, technology policy, trend of research

#### **Summary:**

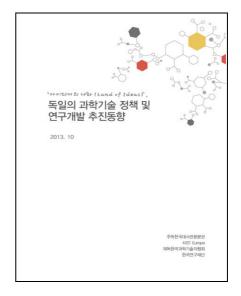
In cooperation with the Korea Research Foundation, the Germany Embassy of the Republic of Korea and VeKNI (Verein Koreanischer Naturwissenschaftlicher und Ingenieure in Deutschland), the International Cooperation Team published a handbook in Korean Language with the title:

"Science & Technology Policy and Research & Development Trends in Germany"

For memorial of the 130th anniversary of Korea-Germany diplomatic relations KIST Europe published this handbook of Germans S&T (Science and Technology) policy with:

- detailed informations about the major German Research Institutions (Max-Plank-Gesellschaft, Fraunhofer Gesellschaft, Helmholtz Gesellschaft, Leibniz-Gemeinschaft),
- the current status of S&T collaboration promo tion between Korea and Germany
- and R&D trends in Germany.

It includes information about the Federal Institutes and the Technical Universities (TU) besides the major German research institutions. Through distributing the handbooks to S&T stakeholders in Korea and Europe they could have an overview about German research and development infrastructure based on precise information about German S&T policy (High-Tech Strategy 2020) and R&D trends, which contributes to enhanced reciprocal S&T collaboration and reinforced R&D network between Korea and Germany.





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## **Technology Policy Center**

## **PEM Fuel Cells with Graphene-supported Pt Catalysts**

#### Functionalized graphene, Polymer Electrolyte Membrane

#### **Summary:**

performance of polymer membrane fuel cells (PEMFC) is directly related to the interfacial resistance between the membrane and Gas Diffusion Electrodes (GDE), the catalyst availability in the three phase zone and the micro structure of the catalyst containing porous layer. Therefore we have deposited a hydrophobic porous layer onto a Gas Diffusion Layer (GDL), and we have prepared GDEs particularly suited for fuel cells with graphene-supported Pt catalyst (Pt/G) as electrodes. Characterization of the prepared electrodes was demonstrated by powder X-ray diffraction and electron microscopy. The electrochemical performance of the Pt/G was found to be superior as compared to the commercial carbon supported platinum catalyst by using cyclic voltammetry.

An ongoing challenge in low temperature fuel cells is the lowering of the catalyst loading by a more efficient usage of this mostly platinum containing electrocatalyst. For this reason carbon supported nanocatalysts are in use for polymer electrolyte membrane fuel cells (PEMFC) to increase the specific catalyst surface. But there are limits for the reduction of the catalyst particle size because a particle growth takes place under fuel cell conditions anyway. This is one aspect of the catalyst degradation in low temperature fuel cells. Electrochemically active are only those electrocatalysts which are located in the three phase boundary region. The contact to this region can be improved with a graphene layer on larger areas.

Graphenes consist of a few graphite layers which are characterized by wide lateral dimensions. The surface area of such flakes can be several microns. The chemical surface structure of the graphenes (e.g. OH-functionalities) enables the bonding of metal ions, complexes and also organic compounds with functional groups. This special property can be used for immobilization of metal or alloy catalysts - in this manner the particle agglomeration can be reduced.

A further advantage of this material class is based on the graphene structure: The mass-transport of educts and products through these flake-like structures is very efficient and also the electronic conductivity should be improved by an electron transfer through a wide extended carbon layer instead of percolated single carbon nanoparticles used for conventional catalyst supports.

In this contribution we will show the use of functionalized graphene structures as catalyst support for the application in fuel cells. The influence of the graphene morphology on the catalyst activity will be determined for structurally functionalized graphene materials which differ strongly in their characteristic graphene properties (flake size, flake thickness, number of layers). For structural characterization of the graphenes, catalysts and supported catalysts X-ray diffraction, electron microscopy and electrochemical methods (CV) were used.

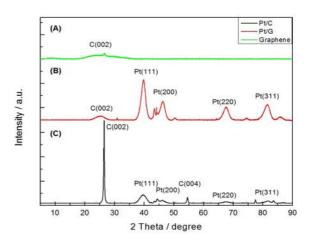


Figure 1: Powder XRD of (A) functionalized graphene, (B) graphene-supported platinum catalyst (Pt/G) and (C) carbon-supported platinum catalyst (Pt/C).

The XRD pattern of the catalyst in Fig.1 (A) indicates that graphene sheets have a broad (002) Bragg peak centered at 25.75°, which is slightly shifted from the graphite (002) Bragg peak at 26.5°. The shifting of the peak signifies that the c-axis of graphite layer is exfoliated and carbon sp<sup>2</sup> bonds are reconstructed in graphene.

## Technology Policy Center

From Fig. 1 (B) and (C), the catalysts show the presence of crystalline phases of platinum. Pt/C catalyst has strong Pt (111) peak, while Pt/G is much weaker because of the smaller Pt nanoparticles size, showing that the higher Pt loading was successful in replicating the Pt particles on Pt/C.

The TEM image in Fig. 2 (A) shows the flakes to appear of good quality with on average ~3 monolayers per flake. The Fig. 2 (B) and (C) show that the distribution of Pt particles is well distributed on the carbon black and graphene, respectively.

Catalyst particles were deposited on the surface of the graphene flakes. The supported catalysts were used to prepare membrane electrode assemblies (MEA's) for the testing in  $\rm H_2/O_2$ -fuel cells (50 cm²). Further topics of this research will be the preparation of graphene dispersions, the application on gas diffusion layers, the different techniques for metal precursor reduction and also the MEA characterization in a fuel cell test station.

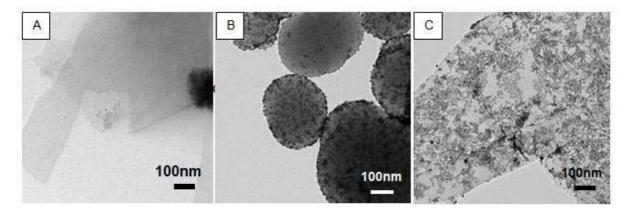


Figure 2: Electron microscopy of (A) functionalized graphene, (B) 19 wt.% carbon deposited platinum catalyst (Pt/C) and (C) 19 wt.% graphene deposited platinum catalyst (Pt/G).

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Oh, E.-J.; Kim, SW., Natter, H.; Hempelmann, R.\*: Graphene based electrode materials for PEM fuel cells and electrochemical oxygen reduction reaction Doktorandentag Saarland University, Saarbrücken (Germany), November 13, 2013



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## Technology Policy Center

## Test of ionic exchange membrane for Vanadium redox flow battery

#### Vanadium redox low battery, membrane, ionic conductivity

#### **Summary:**

A redox flow battery is a good candidate device for stationary energy storage. There are various functional materials like redox couples, electrodes and membranes to construct a system for redox flow battery. The ideal membrane should have good chemical stability under strong acidic conditions, low permeability to the vanadium ions, high permeability to hydrogen ions which carry charge and low cost. Especially we test the permeability of vanadium ion across various membranes.

Ionic exchange membranes are classified into cation exchange membranes and anion exchange membranes by the type of ionic functional groups which are attached to the polymer backbone of the membrane elements. Cation exchange membranes mostly contain negatively charged functional groups such as -PO<sub>3</sub><sup>2-</sup>, -SO<sub>3</sub><sup>-</sup> and -COO. They enable the passage of cations mostly such as H<sup>+</sup>. Anion exchange mem- branes involve positive functional groups like -NH3+, -NR<sub>2</sub>H<sup>+</sup>, and -SR<sub>2</sub><sup>+</sup> and they allow the passage of anions such as -Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup>. Here we deal with 10 membranes (VX-20, F-1850, FX-7050, FAP-450, FAP-5, FAP-420, FAP-0, FAP-PP-475, F-9120, Nafion-115) which are made by Fumatech GmbH except Nafion-115 which is made by DuPont. The diffusion rate of vanadium ions (IV) is measured by using a dialysis cell.

The same volume (60mL) of 2 M VOSO $_4$  in 3 M H $_2$ SO $_4$  and 2 M MgSO $_4$  in 3 M H $_2$ SO $_4$  was filled into each side of the dialysis cell. The MgSO $_4$  solution used for blank solution prevents the osmotic pressure and eliminates the water transport across the membrane. The absorbance of the vanadium ions having permeated the membrane in the magnesium sulfite solution was measured periodically for one week by UV-Visible spectroscopy photometry.

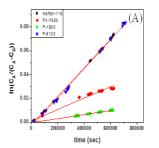
By plotting abs vs. concentration, we can get a calibration curve of  $VO^{2+}$  (4+) which enables to interpolate the unknown concentration of solution for every measurement.

By using Fick's law of diffusion and Beer's law, the diffusion coefficient of vanadium ion is determined.

$$\ln\left(\frac{C_A}{C_A - C_B}\right) = \frac{DA}{V_B L} t$$

Where D is the diffusion coefficients of vanadium ions (cm $^2$ s $^{-1}$ ); A is the effective area of the membrane (cm $^2$ ); L is the thickness of the membrane (cm);  $C_A$  is the concentration of vanadium ions of raw solution;  $C_B$  is the concentration of vanadium ions in blank solution; and t is the test time (sec). Assuming the volume of deficiency side ( $V_B$ ) is a constant. The value of  $C_A$  can be approximately regarded as a constant.

In Figure 1, F-1850 ( $3.23 \times 10^{-10}$  cm<sup>2</sup>s<sup>-1</sup>) and FX-7050 ( $9.06 \times 10^{-10}$  cm<sup>2</sup>s<sup>-1</sup>) which are cationic exchange membranes show the least permeability of VO<sup>2+</sup>. Especially, VX-20 shows no permeability of VO<sup>2+</sup> which means the diffusion coefficient of VO<sup>2+</sup> is zero.



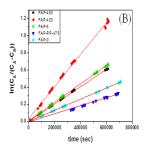


Figure 1: Plots of  $\ln(C_A/(C_A-C_B))$  vs. time; cationic exchange membrane (A), anionic exchange membrane (B).



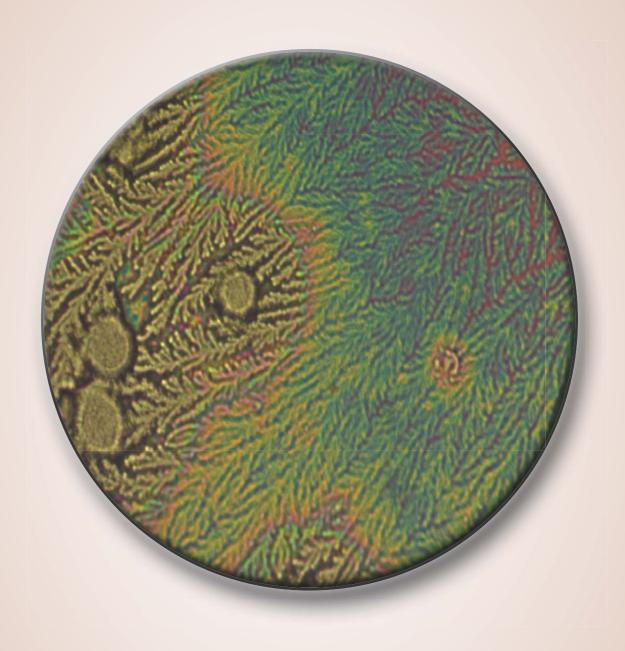
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## **Citations / Impact Factor**

## **ACTUAL CITATIONS OF 2010/2011 PAPERS (KIST EMPLOYEES)**

| Journal                       | Year | First Author*               | Citations |
|-------------------------------|------|-----------------------------|-----------|
| Anal Chem                     | 2010 | Arora, A. et al.            | 162       |
| Anal Chem                     | 2010 | Salieb-Beugelaar, G. et al. | 75        |
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| Nanotechnology                | 2010 | Engelen, J.B.C. et al.      | 14        |
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| Lab Chip                      | 2011 | Vulto, P. et al.            | 12        |
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## HIGHEST IMPACT FACTOR OF JOURNALS of 2012/2013 papers (KIST Employees)

| Journal                       | Year | First Author*        | Impact-Factor |
|-------------------------------|------|----------------------|---------------|
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| Environ Sci Technol           | 2012 | Smith, K.E.C. et al. | 5.2           |
| Environ Sci Technol           | 2012 | Smith, K.E.C. et al. | 5.2           |
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| J Biol. Chem                  | 2013 | Hilbers, F. et al.   | 4.7           |
| Analyst                       | 2012 | Arora, A. et al.     | 4.2           |

<sup>\*</sup> author with other co-authors incl. actual KIST employees

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Control of magnetotactic bacterium in a micro-fabricated maze

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#### H.H. Tran, W. Wu, N.Y. Lee \*

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Magnetic control of potential microrobotic drug delivery systems: nanoparticles, magnetotactic bacteria and self-propelled microjets

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Characterization and control of biological microrobots

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#### J. Kim

Prediction of mixture toxicity using computational toxicology methods: Towards integrated model for environmental risk assessment

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#### J. Park

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#### **MASTER THESIS**

#### C. Blattner

Etablierung einer dreidimensionalen Tumorzellenkultur zur Validierung neuer Immuntherapieansätze University of Saarland, Germany, August 2013

#### **INVITED LECTURES**

#### A. Manz

Microfluidics & Miniaturization for clinical diagnostics ETMN 2013, International Conference on emerging technologies: micro to nano 2013, Goa (India), February 23-24, 2013

#### H. Jeon

The trend of global chemical regulation and the compliance strategy of Korea chemical industry Kumho Petrochemical Laboratory, Asan (Korea) February 2013

#### H. Jeon

Establishment of sub-ordinate regulation of Korea REACH Korea Ministry of Environment, Sejong (Korea) February 2013

#### A. Manz

Microfluidics & Miniaturization for clinical diagnostics 6. Charité Entrepreneurship Summit, Berlin (Germany) April 8-9, 2013

#### N. Korkmaz

Bacteriophages for nanobiotechnological applications INM & KIST Europe Workshop, Saarbrücken (Germany), April 18, 2013

#### A. Manz

Microfluidic chips for biomolecule analysis

IMTB, 2nd International Conference on Implementation of Microreactor Technology in Biotechnology,

Cavtat (Croatia)

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#### N. Korkmaz

Nano-Bio materials for convergence applications

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May 31, 2013

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#### H. Jeon

Global trend of nano-material regulation

1st half 2013 Workshop of Council for Science and Technology/Korean Council of Environmental Industry, Technology & Policy, Bonn (Germany)

June 2013

#### N. Korkmaz

Bacteriophages as engineered templates for nanobiotechnological applications

Nano Convergence Technology International Workshop KRICT, Daejeon (South Korea)

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#### A. Manz

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#### A. Manz

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#### A. Manz

Microfluidics - from integrated "lab on chip" technology to simple droplets, from clean room technology to biomimetic microfabrication

Challenges in Soft Matter Research: From Modelling to Structures and Applications, Freiburg (Germany) October 7-11, 2013

#### A. Manz

 $\label{lem:microfluidics-from} \begin{tabular}{l} Microfluidics-from\ integrated\ {\it "lab}\ on\ chip"\ technology\ to\ simple\ droplets,\ from\ clean\ room\ technology\ to\ biomimetic\ microfabrication \end{tabular}$ 

2nd Annual Meeting of IPGG, Paris, (France)

October 11, 2013

#### A. Philippi

Immune cells as drug carriers in cancer therapy
Future Materials and Safety Workshop KIST Europe, Saarbrücken (Germany)
October 15-16, 2013

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Bacteriophages as programmable bionanomachines Germany-Korea Joint Workshop on Future Material and Safety KIST Europe, Saarbrücken (Germany) October 16, 2013

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#### P. Löthman

Nanotechnology in the archaeal virosphere Institute of Microbiology, Archaea Centre, University of Regensburg, Regensburg (Germany) October 31, 2013

#### A. Manz

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#### J. Hwang

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Microfluidic chips for biomolecule analysis

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#### J. Kim

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#### **CONTRIBUTED LECTURES**

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Magnetic control of potential microrobotic drug delivery systems: nanoparticles, magnetotactic bacteria and self-propelled microjets

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Challenges in predicting mixture toxicity using computational toxicology methods: Toward integrated environmental hazard assessment

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Recombinant phages for nanowire templating NANO KOREA 2013, Seoul (South Korea)
July 10-12, 2013

#### C.D.M. Campos, F.G.R. Reyes, J.A.F. da Silva \*

Development of capillary electrophoresis based method to the analysis of protein hydrolysate products 3rd ICAP, Sao Paulo (Brazil)
July 28-31,2013

#### E.J. Oh, S.J. Hwang

Soft chemical routes to the 2D nanosheets and thin film of layered manganese cobalt nickel oxide Energy Research Symposium INM Leibniz Institute for new materials, Saarbrücken (Germany) August 08, 2013

#### P. Löthman, E. Favret

Seeing Surface Architecture by novel optical and digital methods - a study of biological and technological surfaces by ULOI, RIMAPS and Variogram Analysis

EUROMAT Conference, Sevilla (Spain)

September 2013

#### R. Arenal, P. Löthman, M. Picher, V. Jourdain \*

Atomic Structure of ultra-long carbon nanotubes HeteroNanoCarb 2013, Castelldefels/Barcelona (Spain) September 22-26, 2013

#### P. Neuzil

MicroPCR Development - Towards hand-held tool for infectious disease diagnostics Micro Med A workshop, Pilanesburg (South Africa) September 11-13, 2013

#### Y. Jung, S. Baik, J. Kim, H. Jeon, S. Kim

Ecotoxicity screening test of surface modified silver nanoparticles in pure water 7th SETAC Europe Special Science Symposium, Brussel (Belgium)
October 2-3, 2013

#### D.K. Kim, Y.S. Kang, S.W. Kim

Synthesis of tetragonal LaVO4:Dy by hydrothermal methods and its application for dye-sensitized solar cell

VeKNI Academic Seminar, Essen-Heidhausen (Germany) October 26, 2013

#### E.J. Oh, S.W. Kim

Graphene-supported Pt catalyst as electrode material for PEMFCs VeKNI Academic Seminar, Essen-Heidhausen (Germany) October 26, 2013

#### **Publications**

#### P. Neuzil

MicroPCR Development - Towards hand-held tool for infectious disease diagnostics Lab on a chip Asia, Singapore November 11-13, 2013

#### E.J. Oh, S.W. Kim, H. Natter, R. Hempelmann

Graphene based electrode materials for PEM fuel cells and electrochemical oxygen reduction reaction Doktorandentag Saarland University, Saarbrücken (Germany) November 13, 2013

#### K. Hatakeyama et. al (L. Abelmann) \*

Batch fabricated scanning Hall probes ICMR 2013, Akita (Japan) November 20-22, 2013

#### Y. Jung, S. Baik, J. Kim, H. Jeon, S. Kim

In-vitro screening test and understanding the mechanism on the ecotoxicity: adverse effect of surface modified silver nanoparticles on luminescent bacteria

Nanosafety 2013, Saarbrücken (Germany)

November 20-22, 2013

#### PROCEEDINGS, OTHERS

#### J. Kim

Predicting cocktail effects of chemical mixtures: challenges and a way forward
The Korean Scientists and Engineers Association in the FRG (VeKN), Essen-Heidhausen (Germany)
October 25-27, 2013

#### C. Park

Ecological effects assessment of treated wastewater using bio- and biological responses in aquatic organisms

The Korean Scientists and Engineers Association in the FRG (VeKN), Essen-Heidhausen (Germany) October 25-27, 2013

#### Y. Jeong

Developing a consolidated exposure scenario for mixture products using ES-modifier under REACH The Korean Scientists and Engineers Association in the FRG (VeKN), Essen-Heidhausen (Germany) October 25-27, 2013

#### J. Yoo, S. Kim, H. Jeon

Challenges in New European cosmetic regulation

The Korean Scientists and Engineers Association in the FRG (VeKN), Essen-Heidhausen (Germany) October 25-27, 2013

#### **Publications**

#### S. Baik

Sonolysis with high and low frequencies on the removal of cyanobacteria

The Korean Scientists and Engineers Association in the FRG (VeKN), Essen-Heidhausen (Germany)

October 25-27, 2013

#### Y. Jung

Toxicity effect of surface modified silver nanoparticles on multiple luminescent bacteria The Korean Scientists and Engineers Association in the FRG (VeKN), Essen-Heidhausen (Germany) October 25-27, 2013

#### S. Baik

Ecotoxicity screening test with multiple strains of luminescent bacteria for surface modified metallic nanoparticles

Future Materials and Safety, Saarbrücken (Germany) October 2013

#### S. Baik

Development and application of sonication on the removal of harmful cyanobacteria SETAC North America 34th Annual Meeting, Nashville (USA) November 2013

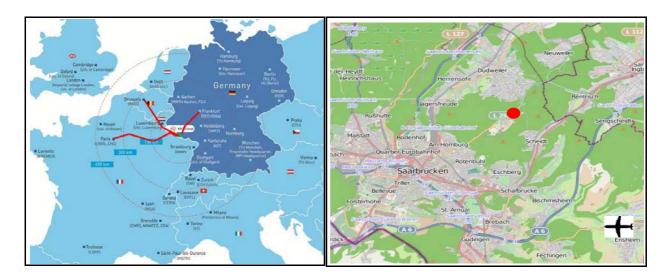
#### **PATENT FILING IN 2013**

#### J.I. Baumbach, T. Rabis, U. Sommerwerck, G. Weinreich

Mittel und Verfahren zur Diagnose von Pseudomonas aeruginosa filed in Germany
Priority Date: June 07,2013

## **Appendix**

## How to reach KIST Europe



#### Address:

KIST Europe Campus E7.1

D - 66123 Saarbrücken phone: +49 681 9382-0 fax: +49 681 9382-109

info@kist-europe.de www.kist-europe.de

#### **Distances:**

| Brussels - Saarbrücken   | 320km |
|--------------------------|-------|
| Cologne - Saarbrücken    | 280km |
| Frankfurt - Saarbrücken  | 200km |
| Luxemburg - Saarbrücken  | 90km  |
| Paris - Saarbrücken      | 390km |
|                          |       |
| Strasbourg - Saarbrücken | 110km |

#### 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

<u>By Taxi</u>: 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

<u>Information for the navigation system:</u> 66123 Saarbrücken, Stuhlsatzenhausweg 97

<u>From east</u> (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".

## How to reach KIST Europe

#### 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/ICF
- 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

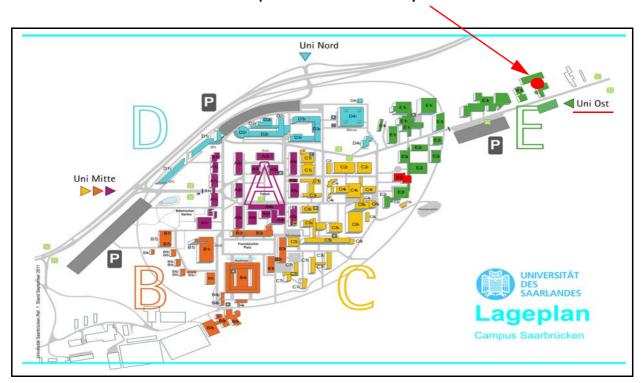
More information:

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 Campus of Saarland University**



## **Imprint**

#### **Publisher**

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