FRANCE-JAPAN WORKSHOP

Bio-inspired approaches: Micro- & Nano- Architectures, Materials & Imaging

October 11th-12th, 2011

Institut Européen de Chimie et Biologie - Bordeaux

Welcome

Dear all,

In 2010, shortly after the creation of the Japanese "Nano-Macro Materials, Devices and Systems Research Alliance", I was contacted by Prof. Shimomura and Prof. Saito to discuss the possibility of a France-Japan partnership in the field of biomimetic supramolecular chemistry, structural biology, bioimgaging and biomaterials. Upon their invitation, I went to Japan in February 2011 to attend a symposium on biomimetic chemistry. In April 2011, three scientists from the Tohoku University came to Bordeaux for a first contact with the IECB, the CBMN and the University of Bordeaux. Our discussions led us to organize the present workshop, with the aim of fostering France-Japan scientific exchanges.

As a result, the France-Japan workshop *"Bio-inspired approaches: Micro- & Nano- Architectures, Materials & Imaging"* is not focused on a single scientific area and is not intended to be an exhaustive presentation of the many scientific expertise and resources available in Japan and in Aquitaine. Rather, it is designed to facilitate interdisciplinary interactions and to provide a global picture of the main institutions, in Japan and Aquitaine, that could be involved in bilateral collaborations. Through oral presentations, participants will have the opportunity to learn more about the actual research environment of their French/Japanese counterparts. In addition, a large amount of time will be devoted to posters and social events, so that attendees can get to know each other and discuss specific scientific issues in a more informal context.

I hope you will find this workshop both exciting and useful and I sincerely thank you for your participation. I would like to address a very special thanks to our Japanese guests for accepting to come to IECB and I wish them a very enjoyable stay in Bordeaux.

Dr. Reiko Oda

Organizing committee: Dr. Reiko Oda, Dr. Brice Kauffmann, Pierre-Emmanuel Gaultier

Sponsors: Université Bordeaux 1, SFR TecSan, GIS Advanced Materials in Aquitaine, C'Nano GSO, Elexience, Beckman Coulter, Explora Nova, Ville de Bordeaux, Japanese Strategic Alliance Project for Creation of Nano-Materials, Nano-devices and Nano-systems

About the Japanese Strategic Alliance Project for Creation of Nano-Materials, Nano-devices and Nano-systems

Prof. Akihito Yamaguchi
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The "Strategic Alliance Project for the Creation of Nano-Materials, Nano-devices and Nano-systems" was initiated at the end of 2010 with the aim of promoting cooperation between five institutes scattered across Japan:

1. Research Institute for Electronic Science (RIES) - Hokkaido University,
2. Institute of Multidisciplinary Research for Advanced Materials (IMRAM) - Tohoku University
3. Chemical Resources Laboratory - Tokyo Institute of Technology,
4. The Institute of Scientific and Industrial Research (ISIR) - Osaka University
5. Institute for Materials Chemistry and Engineering, Kyushu University.

The mission of the alliance is to foster the development of materials, devices and systems in 4 strategic fields:

1. Next generation electronics (research group 1 – G1)
2. New energy harvesting materials and devices (research group 2 – G2)
3. Biomedical materials and devices (research group 3 – G3)
4. Environmental harmonized materials and devices (research group 4 – G4)

In this context, we are keen to promote scientific cooperation and technology transfer at an international level, through international collaborative projects, student and young researcher exchanges and the joint-organization of scientific events. We are glad to be participate in the present workshop, which should serves as a kick-off meeting for bilateral France/Japan cooperation in the frame of the Alliance.

29 Japanese researchers from the five institutes involved in research group 3 are here to meet their French counterparts and promote scientific exchanges. I hope this will be the starting line of a long-lasting collaboration between the two communities.

Prof. Akihito Yamaguchi

Workshop programme

Tuesday, October 11th

08.30 – 09.00	Registration
09.00 – 09.10	Welcome address
09.10 – 10.30	Session 1
09.10	· *Japanese Nano-Macro materials, Devices and System Research Alliance, G3* Prof. Akihito Yamaguchi
09.30	· *Institut Européen de Chimie et Biologie (IECB) - Interbio* Dr. Jean-Jacques Toulmé
10.00	· *Research Institute for Electronic Science, Hokkaido University* Prof. Takeharu Nagai
10.30 – 11.00	Coffee break
11. 00– 12.30	Session 2
11.00	· *Federate Research Structure Technologies for Health (SFR TecSan) / Technological Platform for Biomedical Innovation* Dr. Pierre Dos Santos
11.30	· *Institute of Multidisciplinary Research for Advanced Materials, Tohoku University* Prof. Masao Ikeda-Saito
12.00	· *Bordeaux Imaging Center (BIC), Bordeaux University* Dr. Marc Landry
12.30 – 14.00	Lunch & posters
14.00 – 15.30	Session 3
14.00	· *Chemical Resources Laboratory, Tokyo Institute of Technology* Prof. Toru Hisabori
14.30	· *Institute for Materials Chemistry and Engineering, Kyushu University* Prof. Atsushi Maruyama
15.00	· *GIS Advanced Materials in Aquitaine* Prof. Jean-Pierre Desvergne
15.30 – 16.00	Coffee break

16.00 – 16.45	Session 4
16.00	· *Federation for Soft Matter in Aquitaine* Dr. Annie Colin
16.15	· *Institute of Scientific and Industrial Research, Osaka University* Prof. Kazuhiko Nakatani
16.45 – 18.45	Posters
18.15 – 19.15	Cocktail & guided tour of IECB
19.30 – 23.30	Gala Diner (upon invitation only)

Wednesday, October 12th

09.00 – 09.30	Registration
09.30 – 10.00	Session 5
	· *Biomimetic approaches in France and Japan* Dr Ivan Huc / Prof. Masatsugu Shimomura
10.00 – 10.30	· *C'Nano GSO* Dr. Jean-Pierre Aimé
10.30 – 11.00	Coffee break & Posters
10.30 – 12.30	Posters
12.30 – 12.40	Closing address
	· *Institute of Multidisciplinary Research for Advanced Materials, Tohoku University* Prof. Junichi Kawamura
12.40 – 14.00	Lunch & Posters
14.00 – 15.00	Roundtable : *Taking forward France/Japan cooperation*
18.00 – 19.00	Cocktail at the Mairie de Bordeaux

Oral communications

INSTITUT EUROPEEN DE CHIMIE ET BIOLOGIE (IECB) - INTERBIO

Dr. Jean-Jacques Toulmé
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About the Institut Européen de Chimie et Biologie (IECB)
The Institut Européen de Chimie et Biologie (IECB) is a research team incubator placed under the joint authority of the CNRS, the Inserm and the Université de Bordeaux (UB1 and UBS). It was created in 1998 with the support of the Aquitaine Regional Council to provide promising European chemists and biologists with an environment designed to facilitate the development of first-class interdisciplinary research programs, in collaboration with international public and private research centres.
IECB's International Scientific Advisory Board guides the selection and periodic evaluation of the team leaders. After a probative period of two years, research teams are then hosted for a maximum of 10 years. During their stay at IECB, teams enjoy full financial and managerial autonomy and benefit from state-of-the-art facilities and dedicated technical expertise through IECB's technology platforms in structural biology and preparative and analytical techniques. Team leaders are strongly encouraged to transfer their research to the industry. The IECB is now the largest research team incubator in France recognized by the "Cellule Hôtels à Projets" of the CNRS, with 17 research teams accounting for more than 150 researchers and expert technicians. A company – Fluofarma – and a technology transfer unit – Novaptech –, both created by former IECB team leaders, also operate on site and currently employ 25 people.
Learn more at : www.iecb.u-bordeaux.fr

About Interbio
The IECB is a founding member of Interbio, an interregional cooperation programme sponsored by the European Commission which aims at fostering transnational cooperation, technology transfer and innovation in the fields of biotechnologies and life sciences in South-West Europe.
As an interdisciplinary network, Interbio brings together research centers, technology platforms and companies from the Barcelona, Bordeaux, Lisbon, Toulouse and Valencia regions. Since its launch in 2009, Interbio has given rise to 16 interregional collaborative projects as well as to a number of scientific symposia, summer schools and technology transfer events.
Learn more at : www.interbio-sudoe.eu

RESEARCH INSTITUTE FOR ELECTRONIC SCIENCE, HOKKAIDO UNIVERSITY

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About the Research Institute for Electronic Science (RIES)

Research Institute for Electronic Sccience (RIES), Hokkaido University is composed of 17 laboratories, the researches of which are ranging from mathematics, physics, chemistry, and biology. We are aiming to create a "Trans-disciplinary Nanoscience" by synergizing the areas related to photonics, molecular and biological sciences. The Nanotechnology Research Center (established in 2002) and Nikon Imaging Center (established in 2005) house domestic and foreign researchers, and the industries for scientific exchanges, which provide the coverage for research extending into various multi-dimensional regime, and function as a spearheading effort for novel and innovative trans-disciplinary research of higher dimension. In this symposium, 4 research groups, i.e. Coherent X-ray photonics, Molecule and life nonlinear science, Nanosystems physiology, and Molecular and cellular biophysics will attend to further promote trans-disciplinary collaborations with the researchers in France.

SFR TECSAN, BORDEAUX UNIVERSITY

Dr. Pierre Dos Santos
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About the Federate Research Structure (FRS) "Technologies for Health"

The FRS "Technologies for Health" brings together 11 laboratories in Aquitaine, thus covering several research fields, from fundamental research to clinical development. The FRS develops of a focused scientific program, organized according to four major axes covering the major domains of "Technologies for Health". These axes are: 1) Molecules and targets of therapeutic or diagnostic interest; 2) Bioengineering and nanotechnologies; 3) Bio-Imaging; 4) Interventional techniques and assistance to the patient. These axes derive from the integration of the experimental and technological assets of the various partners.
The technological resources of the various partners of the FRS ensure a continuum of research, from cellular and molecular aspects to clinical ones. The FRS maintains close ties with the others FRSs of the University Bordeaux Segalen, such as the one in "Public Health", "Neuroscience", or "Translational Biology and Medicine", and the Teaching Hospital of Bordeaux (THB) and its various poles, as well as the interfaces dedicated to these operations (i.e., the Centre of Clinical Investigation (CCI) and the Clinical Research Centre for Technological Innovation (CRC-TI) "Biomaterials" 0802).
Technology transfer within the FRS is facilitated as it includes facilities dedicated to start-up companies.

About the Technological Platform for Biomedical Innovation (PTIB)

The general objective of the PTIB is to foster technology transfer from academic labs holding clinical and experimental competences towards the industry, so as to develop or validate diagnostic or therapeutic tools in the fields of cardiovascular, pulmonary, osseous pathologies, biomaterials and medical devices.
Thus, the PTIB federates, on the same site, the many actors implied in this field. The implementation of the PTIB's mission is based on the availability, since September 2006, of a 3500m^2 space equipped with research laboratories offering facilities in chemistry, molecular biology, cellular biology, cellular imaging, in vivo imaging in small animals (micro-CT and micro-SPECT) and in vivo experimentation in large animals (two operating rooms, one equiped with a 1.5T Siemens MRI and one with a Toshiba numerized X ray) .
Research priorities:
4 main axis were selected, based on the available means and know-how. They are primarily centered on cardiovascular, pulmonary and osseous pathologies, as well as biomaterials; they relate to key public health issues and priorities.

1. Development (in vitro and in vivo) of new procedure of tissue reconstruction based on molecular and cellular assemblies to the surface of biomaterials intended for osseous and vascular substitution.
2. Development of the new procedures for cardiovascular and bronchopulmonary imaging
3. Development of new procedures for the treament of arrhythmias and heart pacing
4. Study of thrombosis and platelet pathologies

INSTITUTE OF MULTIDISCIPLINARY RESEARCH FOR ADVANCED MATERIALS, TOHOKU UNIVERSITY

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The Institute of Multidisciplinary Research for Advanced Materials (IMRAM) was founded on April 1, 2001 as a result of a merger of three research institutes, Institute for Chemical Reaction Science, Institute of Scientific Measurements, and Institute of Advanced Materials Processing, each of them was established around 1940's in Tohoku University. IMRAM has ~140 faculty members and ~300 graduate students housed in 48 laboratories covering a wide range of research areas including biological, organic, polymer and inorganic materials, process engineering, and scientific measurements. Our annual operating expenditure is ~€48M. To support our research endeavor, IMRAM has a machine shop, glass blowing service, a technical support section, in addition to Central Analytical Facility that manages shared major instruments. The mission of IMRAM is "To promote basic and applied material science, so as to educate next generations of international leading scientists and engineers, and to contribute to the global human communities."

IMRAM laboratories directed by Drs. Ishijima, Kinbara, Nagatsugi, Shimizu, Shimomura, Takahashi, Wada, and myself belong to Biological Materials, Device and System Research Group (G3) of the Strategic Alliance Project for Creation of Nano-Materials, Nano-devices and Nano-systems (Alliance). Dr. Nagatsugi develops intelligent molecules for regulation of gene expression, and Dr. Wada's major project is design and synthesis of artificial nucleic acids and proteins for controlling cellar function. Dr. Kinbara focuses on development of functional molecules inspired by biological systems, and Dr. Shimomura designs and prepares hierarchically structured materials based on nanotechnology and biomimetics. Dr. Ishijima utilizes optical microscope methods to elucidate mechanism of bio-molecules at single molecular level. Dr. Takahashi studies protein folding and functional dynamics through single molecule and ensemble observation. Dr. Shimizu works on structure-function relationships of heme-based gas-sensor proteins. My laboratory studies mechanism of heme enzymes and glutamate receptors by structural biochemistry methods. IMRAM G3 members' research interests cover areas including synthetic chemistry, bio-inspired chemistry, single molecular biophysics, bioinorganic chemistry, chemical biology, and structural biology.

SFR INFRASCTRUCTURE, BORDEAUX UNIVERSITY

Prof. Marc Landry
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The BIC (Bordeaux Imaging Center) offers resources in photonic and electronic imaging, mainly in life, health and plant sciences. It is a core facility identified at the national level as "Infrastructure en Biologie Sante et Agronomie" (IBISA). The BIC is the imaging component of the Center for Functional Genomics of Bordeaux and its different components of the BIC are: PHOTONIC imaging, ELECTRONIC imaging, PLANT imaging.

The Bordeaux Imaging Center offers access to the most advanced bio-imaging techniques for fixed and live cell imaging, video-microscopy, confocal microscopy, multiphoton imaging, transmission electron microscopy and scanning electron microscopy. It provides a unique set of high-end equipment for super-resolution microscopy such as confocal STED microscopy, FRAP video-microscopy, Fluorescence lifetime imaging FLIM for the measurement of molecular interactions. It also provides access to equipments for sample preparation such as ultramicrotoms, high pressure freeze and we can host live samples.

CHEMICAL RESOURCES LABORATORY, TOKYO INSTITUTE OF TECHNOLOGY

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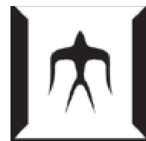

About the Chemical Resources Laboratory

It becomes commonly recognized that the natural resources are finite. The mission of Chemical Resources Laboratory is to carry out a research to improve the human life without contaminating the environments through the sophisticated utilization of the natural resources on the earth. The time when the mass-production and the mass-consumption are praised has passed and the efficient use and recycle of natural resources become an urgent issue.
Chemical Resources Laboratory has been established in 1937 affiliated to Tokyo Institute of Technology anticipating a demand of such an age and grown through the research on theory and application of the chemical utilization technologies of natural resources.
At the present, Chemical Resources Laboratory has 13 divisions, 1 research installation covering wide range of areas from basic to applied or developmental research besides common facilities and an administrative office. The faculties of Chemical Resources Laboratory are also lecturing and giving research guidance to doctoral or master level students under the cooperation with the interdisciplinary graduate school of science and engineering of Tokyo Institute of Technology.

Our Divisions

Inorganic Resources Division
Molecular Materials Design Division
Organic Resources Division
Bio-Resources Division
Catalytic Chemistry Division
Polymer Chemistry Division
Chemical Spectroscopy Division
Chemical System Synthesis Division
Process Systems Engineering Division
Chemistry for Inorganic Materials Division
Integrated Molecular Engineering Divison
Smart Material Division
Synthetic Organic Division
Resources Recycling Process Division
Materials for Energy Conversion (Toppan Printing) Division (Donated Division)

INSTITUTE OF MATERIALS CHEMISTRY AND ENGINEERING, KYUSHU UNIVERSITY

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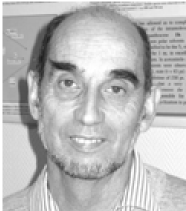
About the Institute of Materials Chemistry and Engineering (IMCE) at Kyushu University
IMCE was founded on April 1, 2003, following the merger and reorganization of the Institute of Advanced Material Study (a research institute attached to Kyushu University) and the Institute for Fundamental Research of Organic Chemistry (a joint education and research facility within Kyushu University). Since the reorganization, the IMCE has had two missions: to conduct cutting-edge research in areas from basic chemistry to process engineering, which concern the creation of highly functional substances and materials and the development of related engineering based on practical application; and to nurture young people through research. In particular, the objective of the IMCE is to advance "cutting-edge research in materials chemistry" which is necessary for the foundations of nanotechnology, information sciences, environmental and energy technology, bio/life sciences and other advanced industrial technologies. The IMCE is comprised of four divisions. In cooperation with research groups related to the synthesis of new functional molecules, the chemistry of new molecular assemblies, the chemistry of organic-inorganic hybrid materials, and the processing of advanced materials into devices, each of the divisions continues to work day and night to form a world-class core research based on the basic science and application of the structure and functions of materials from an atomic, molecular and nanoscale to a macroscale. Nanotechnology and materials are the common base of the entire IMCE, and are key focus areas of the government's Science and Technology Basic Plan. The priority goal of our research is for each of our researcher groups to strive to create materials chemistry that opens new frontiers in the fields of environmental and energy technology, life sciences and IT.

About the G3 activity in Nano-Macro Materials, Devices and System Research Alliance
Five researchers relating to bio/life/medical engineering have joined to the Group3 (G3), medical materials, device and system project group, in the Nano-Macro Materials, Devices and System Research Alliance, covering the following research themes: design and synthesis of bioactive-organic chemicals (Prof. M.Shido), medical engineering with functional biomacromolecules (Prof. A.Maruyama), bioimaging application of plasmon resonance in nanostructured metal crystal system (Prof. K.Tamada), precision design of surface chemistry and structures for biomedical engineering (Prof. A.Takahara), and development of cell manipulation materials based on cell mechanobiology (Prof. S.Kidoaki).

GIS MATÉRIAUX IN AQUITAINE



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AMA is a cluster created in 2007 located in the Aquitaine Region of France with Bordeaux as its capital. It aims to promote Basic Research and Higher Education in the field of Advanced Materials.

The key objectives of AMA (which have been achieved) are to:

- **create** a new dynamic among the scientific community to generate ambitious and innovative projects
- **strengthen** the international visibility of a critical number of prominent scientists working on top level equipment, localised in Aquitaine but belonging to different scientific organisations, universities and engineering schools
- **welcome** senior and junior researchers, for short or long periods of time
- **support** scientific collaborative projects
- **develop** a policy of scientific and cultural exchange and promote a world-wide recognized scientific label
- **foster** international attractiveness (grants…)
- **promote** emerging novel research themes (grants…)
- **upgrade** scientific and technical platforms
- **encourage** diffusion of information leading to additional socio-economic benefits

The main successes of AMA are:

- A strong structuration of the research on Materials in Aquitaine Region with the emergence of 8 high level interdisciplinary projects involving ca 300 researchers and 22 different laboratories and 10 technical platforms.
- The welcoming and integration of 3 internationally renowned senior researchers together with their team and a promising junior researcher (for instance Prof. G. Hadziioannou for implementation of a pole of organic electronics, and Dr. M. Blanchard-Desce for advancing ionic liquids research…)
- The reinforcement of links with industry and start-ups.
- The participation in the establishment of strong connections (research, formation and teaching with foreign laboratories and institutions (e.g. Waterloo University, Canada…)
- Additionally, thanks to the aforementioned achievements, AMA has strongly contributed to the success of the 'Investment for the Future' of the National Plan on the Bordeaux site, which was awarded one Equipex* (ELORPrinTec), one Labex* (Amadeus) and the Idex Label (Initiative of Excellence).

*AMADEus (Prof. E. Duguet): Advanced Materials by Design (goal of becoming a worldwide-recognized major cluster in material science, engineering and technology) *ELORPrinTec (Prof. G. Hadziioannou): High-tech Facility for Flexible Printed Organic Electronics (for design and integration of new materials in electronic devices components, with numerous potential markets applications: lighting, display, health, photovoltaics, etc. with exploration of technologies beyond silicium.)

FEDERATION FOR SOFT MATTER IN AQUITAINE

Dr. Annie Colin
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The *Fédération de la matière molle* gathers teams of 9 laboratories involved in soft matter studies.

Soft matter deals with fragile materials. These materials respond highly to thermal and mechanical solicitations.

Foams, emulsions, polymers, cells, granular media are examples of soft matter we meet everyday.

These teams develop various approaches ranging from mathematics, to physics, from chemistry to biology. The aim of this structure is to link the people of this community and to promote new projects. Workshops, seminaries are organized regularly.

The main projects deal with the following questions:

- How do soft matter self assemble?
- How can we modify or induce these assemblies?
- How do fragile materials flow?

The main applications encompass : enhanced oil recovery, tissue engineering, vectorization, chemical engineering.

INSTITUTE OF SCIENTIFIC AND INDUSTRIAL RESEARCH, OSAKA UNIVERSITY

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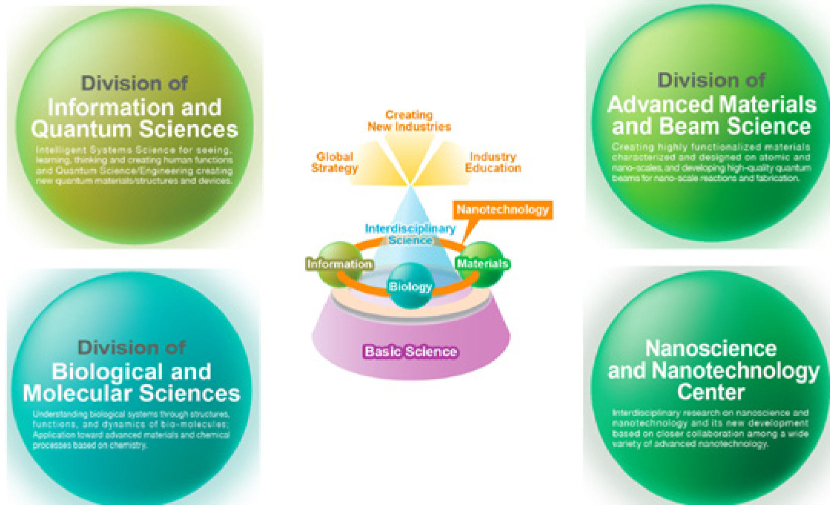
About the ISIR



The Institute of Scientific and Industrial Research (ISIR) was founded in 1939, as a part of Osaka University, based on the strong desire of the business leaders of private enterprises in Osaka area. The purpose of the Institute is to study science necessary for industry and their applications. Since then, the institute had developed into one of the leading research organizations for science and engineering in Japan.

In 2009, we have made a great restructuring since 1995 in order to develop the novel interdisciplinary research fields and exercise leadership in nanotechnology research field into 3 great divisions (Division of Information and Quantum Sciences, Division of Material and Beam Sciences, and Division of Biological and Molecular Sciences) and expand the Nanoscience and Nanotechnology Center into that containing 6 full-size laboratories.

In 2010, in order to establish a core for academia-industry collaboration and open innovation, we have constructed the 5-storied SANKEN Incubation Building This building contains Osaka University's first on-campus rental laboratory space (Company Research Park) for private corporations, Nanoscience Techno-Core for project research, SANKEN Manufacturing Factory, and Osaka University Renovation Center for reusing the used research facilities.

BIOMIMETIC APPROACHES IN FRANCE AND JAPAN

Dr. Ivan Huc
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Prof. Masatsugu Shimomura
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The importance of "Learning from Nature" is a prevailing knowledge in each field of science and technology. Since the turn of the century, research and development on nature-inspired science and technology, generally referred to as "biomimetics," have been coming to the fore. At the molecular level (1-10 nm) - we are entering an era in which biopolymers and their synthetic mimics have a level of complexity that can be routinely addressed and engineered. This is highly significant because biopolymers are the tools nature has selected to carry out its most elaborate functions such as information storage and replication, energy capture, storage and conversion, molecular recognition and catalysis. Biomimetics have also had a fast development in the micrometer scale with, for example, artificial muscle technology, and in the millimeter-centimeter scale (insect-like robots, sonar inspired by bats,...). Some missing links have been identified between these areas where future developments are expected. In the 10 nm to 1000 nm range, molecular self-assembly plays a major role. The design of complex artificial self-assembled systems has been identified as one the great scientific challenge for the future.

Session 5

C'NANO GSO

Dr. Jean-Pierre Aimé,
Chair of C'Nano GSO / UMR 5248 CBMN CNRS - Université Bordeaux
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Bioinspired fabrication method aims at creating materials, functions and algorithm, opening new area in material and computing science. For instance, DNA is an emblematic example and an attractive tool for nanoscience and nanotechnology; Watson-Crick base pairing can be translated into binary sequences (0,1) to organise nanomaterials in programmable way. Conception and fabrication of complex arrangements of functional materials and nanodevices is thought to be a key step towards overcoming Moore's Law. A key aim is to assemble and organise functional materials and systems at increasing levels of complexity. Designing multi-scale structures to create hierarchical order requires the cooperative development of new experimental tools that record information at different scales. Key challenges have been identified, most of them being well known in the fields of nanosciences and nanotechnology and will be detailed in this presentation.

Combination of top down and bottom up fabrication methods is an important step that requires fertile links between small research teams and centres of nanotechnologies. In this presentation we will describe the networks that have been built in the SW of France that support the development of nanosciences and nanotechnologies and at the European level. In particular network cooperation with the centre of technology LAAS has allowed Cnano GSO to build a European network COST "BioInspired Nanotechnologies: from concept to applications" gathering 20 countries and 70 teams. The network aims at developing Bioinspired nanotechnologies initiatives at the European level with which the present project INANOC is strongly correlated.

www.bioinspired-nano.eu/en/

w3.cost.eu/domains_actions/cmst/Actions/TD1003

www.cnano.fr

Poster session - Day 1

1 - ANALYTICAL STRATEGIES FOR THE CHARACTERIZATION OF PROTEIN COMPLEXES BY MASS SPECTROMETRY

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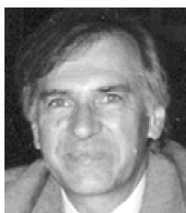

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Three analytical strategies are currently used at the Functional Genomics Centre (CGFB, Bordeaux), to characterize protein supramolecular assemblies. The first one uses Blue Native-SDS Polyacrylamide Gel Electrophoresis (BN/SDS-PAGE) for the separation of complexes, prior to a bottom-up proteomic approach. The second one relies on the use of Hydrogen/Deuterium eXchange monitored by Mass Spectrometry (HXMS). The third strategy uses cross-linking for the stabilization of complexes and identification of the subunits of the assembly. These methodologies have been applied to various types of protein complexes, and described examples will include yeast ATP synthase, the model prion Het-s, and complexes from Escherichia coli or Helicobacter pylori. The BN-PAGE methodology is two-dimensional. Electrophoresis in the first dimension allows separating complexes that are kept under native conditions. Constituting subunits are then separated in a second dimension under denaturating conditions. Examples will be given for the analysis of protein complexes from Escherichia coli. In the HXMS strategy, amide hydrogen atoms from proteins are exchanged under native conditions with deuterons. This spontaneous exchange is quenched by lowering pH and temperature (0°C, pH 2.5). The maximum number of incorporated deuterons equals the number of amino acids minus the number of proline residues. The incorporation of deuterons can be mapped after proteolytic cleavage, still effected under conditions of quenched H/D exchange, and mass spectrometric analysis. Application to the yeast ATP synthase complex and a model prion will be presented. The third approach uses cross-linkers with various reactive functions to link proteins that are in close neighbourhood within a complex. Complexes stabilized in this way can be analyzed with a MALDI mass spectrometer equipped with a high mass detector. Further, after cleavage by a protease, mass spectrometry is used for the identification and mapping of interaction sites. Results obtained with the yeast ATP synthase complex will be discussed.

Marc Bonneu, Jean-Marie Schmitter

2 - ROTOR ARCHITECTURE IN THE F1C10 SUB-COMPLEX OF THE YEAST F1F0-ATP SYNTHASE

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F1Fo-ATP synthase is of the Mg.ADP-inhibited state of the yeast F1c10-ATP synthase, solved at 3.4 Å resolution, an ADP molecule was in both betaDP and betaTP catalytic sites [1]. The alphaDP-betaDP pair is slightly open and resembles the novel conformation identified in the yeast F1 [2], whereas the alphaTP-betaTP pair is very closed and resembles more a DP pair. In the Fo rotor ring, the essential cGlu59 carboxylate group is only surrounded by apolar residues. Its closest hydrogen bond acceptor, the cLeu57 carbonyl oxygen of the adjacent c-subunit, is too far away to make a direct hydrogen bond. The proton binding has specific features compared to the bacterial Na+-transporting or the cyanobacterial and chloroplastic H+-transporting F-type ATP synthase rotor structures. In the crystal, significant interactions of the c10-ring with the F1-head of neighboring molecules affect the overall conformation of the F1-c-ring complex. The symmetry axis of the F1-stator and the inertia axis of the c-ring are tilted near the F1-Fo rotor interface, resulting in an unbalanced machine. Recently, we have solved a new crystal form of the yeast Saccharomyces cerevisiae F1c10 complex, named yF1c10(II), inhibited by adenylyl imidodiphosphate (AMP-PNP) and dicyclohexylcarbodiimide (DCCD), at 6.5 Å resolution in which the crystal packing has a weaker influence over the conformation of the F1-c-ring complex [3,4]. Despite a low resolution, the overall fold is clearly visible with a more straight C-terminal helix of subunit gamma. Though the F1-stator is 8° tilted relative to the rotor axis, its center of mass is located approximately on this axis. Therefore, yF1c10(II) provides a model of a more efficient generator. The present yeast yF1c10(II) and the bovine bF1c8 [5] models are comparable and together provide accurate models of the F1-c-ring domain in the intact F1Fo-ATP synthase. [1] A. Dautant, J. Velours, M.-F. Giraud, J. Biol Chem 2010, 285, 29502-29510. [2] V. Kabaleeswaran, N. Puri, J.E. Walker, A.G.W. Leslie, D.M. Mueller, EMBO J.. 2006, 25, 5433–5442. [3] M.-F. Giraud, P. Paumard, C. Sanchez, D. Brèthes, J. Velours, A. Dautant, J Struct Biol, 2011, accepted. [4] A Dautant, J Velours, J-C Talbot, C Stines-Chaumeil, D Brèthes, M-F Giraud Acta Cryst. 2011, A67, C262-C263. [5] I.N. Watt, M.G. Montgomery, M.J. Runswick, A.G. Leslie, J.E. Walker, PNAS USA. 2010, 107, 16823-16827.

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3 - MIMICKING PROTEIN SECONDARY STRUCTURES WITH NON NATURAL OLIGOMERS

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Protein functions (e.g. molecular recognition, sensing, transport, catalysis) largely depend on the ability of the polypeptide chain, to fold correctly into unique, well-ordered and compact structures. Understanding folding and self assembly processes –essentially governed by non-covalent forces– at work in proteins has led to significant progress in designing de novo protein secondary structures and folds from sequence information alone. Concurrently, approaches, at the interface between biology, synthetic organic and polymer chemistries, to elaborate bioinspired synthetic systems with protein-like structures and functions are being developed. Foldamers are discrete artificial oligomers with predictable and well-characterized folding patterns akin to naturally occuring helices, turns and linear strands(Guichard and Huc, Chem Commun 2011). Our group has investigated enantiopure oligomers consisting of urea bridging units and bearing proteinogenic side-chains(Fischer and Guichard, OBC 2010). These oligomers show a high propensity to fold into stable helical secondary structures reminiscent of the α-helix(Fischer et al., ACIE 2010; Violette et al., JACS 2005). Because of their folding predictability, diversity in side chain appendages, and also their resistance to enzymatic degradation, urea-based helical foldamers are promising scaffolds for biomolecular recognition and biomedical applications. We will show that short sequences designed to mimic globally amphiphilic α–helical host-defense peptides disrupt bacterial membranes and display broad antibacterial activity with some selectivity for prokaryotic versus mammalian red blood cell membranes(Claudon et al., ACIE 2010; Violette et al., Chem Biol 2006).

Gilles Guichard

4 - STRUCTURAL BIOLOGY OF PROTEIN COMPLEXES INVOLVED IN EUKARYOTIC RNA MATURATION

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Structural Biochemistry The lab studies molecular details of RNA maturation in eukaryotes with a special focus on 3' end processing of mRNA and pre-ribosomal RNA maturation. We primarily use X-ray crystallography to solve protein and complexes structures. We also complement X-ray crystallography with small angle X-ray scattering (SAXS), Electron Microscopy (EM) for large complexes and with NMR (in collaboration with Dr C. Mackereth) for smaller complexes or proteins. Our aim is to gain insight into the molecular details underlying optimal generation of mature mRNAs and rRNA and their realtionship with human disease appearance when appropriate.

S. Fribourg, L. Minvielle-Sebastia, N. Perebaskine, C. Monfoulet, A. Dupin.

5 - RECONSTITUTED P. AERUGINOSA EFFLUX PUMP BY CRYO-ELECTRON TOMOGRAPHY

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Complexes of OprM and MexA, two proteins of the MexA–MexB–OprM multidrug efflux pump from Pseudomonas aeruginosa, an opportunistic Gram-negative bacterium, were reconstituted into proteoliposomes by detergent removal. Stacks of protein layers with a constant height of 21 nm, separated by lipidbilayers, were obtained at stoichiometry of 1:1 (w/w). Using cryo-electron microscopy and tomography, we showed that these protein layers were composed of MexA–OprM complexes self-assembled into regular arrays. Image processing of extracted sub-tomograms depicted the architecture of the bipartite complex sandwiched between two lipid bilayers, representing an environment close to that of the native whole pump (i.e. anchored between outer and inner membranes of P. aeruginosa). The MexA–OprM complex appeared as a cylindrical structure in which we were able to identify the OprM molecule and the MexA moiety. MexA molecules have a cylindrical shape prolonging the periplasmic helices of OprM, and widening near the lipid bilayer. The flared part is likely composed of two MexA domains adjacent to the lipid bilayer, although their precise organization was not reachable mainly due to their flexibility. Moreover, the intermembrane distance of 21 nm indicated that the height of the bipartite complex is larger than that of the tripartite AcrA–AcrB–TolC built-up model in which TolC and AcrB are docked into contact. We proposed a model of MexA–OprM taking into account features of previous models based on AcrA–AcrB–TolC and our structural results providing clues to a possible mechanism of tripartite system assembly. Trépout S., Taveau J.C., Benabdelhak H., Granier T., Ducruix A., Frangakis A.S., Lambert O. (2010). Structure of reconstituted bacterial membrane efflux pump by cryo electron tomography. Biochim. Biophys. Acta. – Biomembranes 1798, 1953–1960

Jean-Christophe Taveau, Laetitia Daury, Sylvain Trépout, Thierry Granier, Arnaud Ducruix, Martin Picard Achilleas S. Frangakis, Olivier Lambert

6 - NMR SPECTROSCOPY OF PROTEIN-NUCLEIC ACID COMPLEXES

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The lab studies molecular details of large protein-nucleic acid macromolecules using a variety of new NMR techniques as well as established biophysical approaches. For large complexes, we combine small angle neutron or X-ray scattering (SANS/SAXS), NMR paramagnetic spin labelling to acquire information on long-range contacts, as well as in vitro mutational analysis and other binding assays. For smaller proteins and domains, standard NMR-based approaches are used, but with additional insight gained from complementary techniques. Equally important to the lab is the traditional strength of NMR as a tool to probe the dynamics of biological samples, the characterization of transient interactions, and the possibility to look at structures that exhibit a significant amount of unstructured elements. Specific projects include investigating the molecular details of constitutive and alternative splicing, as well as post-translational modification and regulation of histone structure and dynamics.

Cameron Mackereth, Samir Amrane, Yoan Monneau, Sarah Bourbigot

7 - G-QUADRUPLEX STRUCTURES FOR DNA NANOAPPLICATIONS

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G-quadruplex nucleic acids are of interest due to recent demonstrations of their biological relevance and to applications in nanobiotechnology. G-quadruplexes result from the association of four guanine-rich DNA or RNA strands. These structures are formed by the interaction of four guanines organized in a cyclic Hoogsteen hydrogen bonding arrangement termed a G-quartet, and by the stacking of several G-quartets. Because of their high thermal stability and rigidity, they are used as building blocks for DNA nanoconstructs. In our projects, we design novel DNA nanoapplications with G-quadruplexes as follows: (1) L-DNA quadruplex. L-Nucleic acids, the mirror image of natural D-DNA and RNA, have found a number of interesting applications in biotechnology1 and nanotechnology2. One of the advantages of L-nucleic acids is their nuclease resistance, allowing L-nucleic acids to enter clinical trials3. We demonstrate that a short guanine rich L-DNA strand forms a tetramolecular quadruplex with the same properties as a D-DNA strand of identical sequence. L- and D-strands self exclude when mixed together4, showing that the controlled parallel self-assembly of different G-rich strands can be obtained through L-DNA use. (2) G-wires. G-wires are DNA superstructures based on the intermolecular interactions of four Guanine bases. They allow the fabrication of structures reaching the micrometer scale using only short DNA oligonucleotides, what makes them potentially interesting for molecular electronics5. Our group constructs G-wires made of poly-dG with short DNA sequences and observes their structures using AFM and EM. (3) Controlling G-quadruplex folding. G-quadruplexes tend to fall into kinetically trapped unstable structures because of their conformational polymorphism. Once meta-stable quadruplexes are assembled, their re-assembly into the most stable quadruplex is extremely slow. Using four different strands, we achieve unitary G-quadruplex formation with three parallel-stranded duplexes. The resulting G-quadruplex core serves as a «Square knot» due to its predicted high stability. The ends of three parallel stranded duplexes present convenient points of attachments for desired DNA sequences prone to formation of specific secondary folds.

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Rui Moriyama, Phong Lan Thao Tran, Lionel Beaurepaire, Gaëlle Labrunie, Liliya A. Yatsunyk, Olivier Peitrement, Eric Le Cam, Delphine Albrecht, Atsushi Maruyama, Bernard Rayner & Jean-Louis Mergny

8 - OLIGONUCLEOTIDE APTAMERS FOR REGULATING, SENSING OR IMAGING

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We are interested in the design of aptamers i.e. RNA or DNA structured oligonucleotides able to bind any pre-determined target (proteins, nucleic acids, small molecules, living cells ...). Their properties -strong affinity, high specificity- make them rivals of antibodies. These oligomers are selected from a randomly synthesized pool of sequences containing up to 1015 different molecules through a combinatorial process termed SELEX (Systematic Evolution of Ligands by EXponantial enrichment). Our activity deals with various aspects relevant to genetic engineering and bio/nanotechnology.

1 - Regulation of gene expression: Numerous RNA structures play a key role in the regulation of gene expression. We have used SELEX against functional viral RNA structures from the HIV (TAR: trans-active responsive element) and from HCV (IRES: internal ribosome entry site) in order to perturb the multiplication of the virus. We identified RNA or DNA hairpins binding with high affinity and specificity to their viral cognate hairpin targets through loop-loop interactions. The anti-TAR aptamer reduces the TAR-dependent transcription in cultured cells. The structure of this complex has been solved by NMR spectroscopy and X-Ray cristallography.

2 - Bio-inspired nano-scaffolds: Aptamers can be associated to synthetic polymers in order to generate scaffolds with properties that combine peculiarities of the two partners. For instance, in collaboration with I. Huc (IECB, Pessac, France) we identified and characterized four-stranded DNA aptamers (quadruplexes) targeting aromatic oligoamide helical foldamers. These aptamers do show chemical and enantiomeric specificity. «Foldaptamer» chimeras might be of interest for the recognition of hydrophobic targets or for the design of higher order scaffolds.

3 - Biosensors : Type A influenza causes acute respiratory infections that are highly contagious. The identification of the most pertinent viral strains for the production of vaccines against seasonal flu and to anticipate the onset of influenza pandemics is necessary. In collaboration with D. Desmecht and F. Cornet (Univ Liège, Belgium) we have raised aptamers against matrix protein 1 that shows the highest phylogenetic stability in influenza viruses. Anti-M1 aptamers have been identified and used for M1 detection with aptamer-based arrays.

4 - Probes for imaging tumors: Extracellular matrix metalloproteases (MMPs) are involved in several pathological processes (tumor progression, cerebral ischemia) making them relevant targets for either diagnostic or therapeutic. We identified high affinity aptamers to the human MMP-9; one of them was truncated, chemically-modified and 99mTc labelled. In collaboration with clinicians (M. Allard, CHU, Bordeaux, France) this aptamer was used for imaging human glioblastoma slices by scintigraphy.

5 - Aptamer technology developments: We have carried out methodological improvements of SELEX. Notably, an automated platform has been assembled in the laboratory that speeds up the selection process. We recently developed a high throughput screening assay termed HAPIscreen that potentially allows the identification of orphan candidates. We also set up an SPR(BIAcore)-based selection procedure. These innovative methodologies are currently used by Novaptech, a unit for technology transfer associated to our team, for the development of aptamer-based tools for academic labs and industry.

Carmelo Di Primo, Laurent Azéma, Laurence Delaurière, Eric Dausse, Emilie Daguerre, Thinhinane Chaou, William Palau and Jean-Jacques Toulmé Inserm U869, ARNA laboratory, IECB.
Sonia Da Rocha Gomes, Laetitia Evadé-Farrugia, Amandine Duphil, Novaptech, IECB

28 - CONFORMATIONAL REDUCTION OF CATECHOLAMINES IN THE GAS PHASE STUDIED BY LASER DESORPTION SUPERSONIC JET LASER SPECTROSCOPY

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The conformational reduction in catecholamine neurotransmitters was studied by resonance enhanced multi photon ionization (REMPI), ultraviolet-ultraviolet (UV-UV) hole burning and infrared (IR) dip spectroscopy with applying a laser desorption supersonic jet technique to DOPA, which is one of the catecholamine neurotransmitters and has one more phenolic OH group than tyrosine. It is concluded that DOPA has a single observable conformer in the gas phase at low temperature. Quantum chemical calculations at several levels with or without the dispersion correction were also carried out to study stable conformations. >From the comparison between the computational IR spectra and the experimental ones, the most stable structure was determined. It is strongly suggested that the conformational reduction is caused by electrostatic interactions, such as a dipole–dipole interaction, between the chain and OH groups.

Shun-ichi Ishiuchi, Mitsuhiko Miyazaki, Makoto Sakai and Masaaki Fujii

29 - EXTRACTING THE MOST UNBLIASED KINETIC SCHEME FROM SINGLE MOLECULE TIME

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Complex dynamics of a wide range of biophysical systems, such as the opened/closed gating of ion channels, the On/Off blinking of nanoparticles, the bound/unbound kinetics in cell signaling processes, are often probed experimentally in the form of time series with finite discrete levels. Statistics of the dwell-time time series, the stationary state distributions (SSDs) associated with the chronological sequence of the lengths of time that the system dwells at each level, have been studied to infer the underlying dynamics and kinetics of the system. However, it is well known that the underlying kinetic scheme, a hidden Markov model (HMM) composed of states and state transitions, cannot be identified uniquely from the SSDs of dwell-times because some states and the associated transitions of the underlying HMM are hidden and cannot be resolved by finite-level measurements. Here, we present an information-theoretic framework to quantify the amount of excessive information contained in a given HMM that is not warranted by the measured dwell-time statistics. In this framework, the HMM with minimum excessive information can be uniquely identified and it is regarded as the most objective representation one can extract from the observed data. The minimum excessive information enables us to compare the degree of identifiability of the underlying HMM for measurements of the same system using different observables. The method is applied to a single molecule (SM) enzymatic turnover experiment, and the origin of dynamic disorder is discussed in terms of the network properties of the HMM.

Chun Biu Li and Tamiki Komatsuzaki

30 - TOWARD COHERENT IMAGING USING X-RAY FREE-ELECTRON LASER

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X-ray free-electron laser (XFEL) has recently emerged as a new type of light. LCLS at Stanford succeeded in the world's first x-ray laser in 2009, and SACLA in Japan has achieved first lasing in June, 2011. European and other XFEL projects are also in progress. XFEL is billion times stronger in peak brilliance compared to thus far strongest synchrotron radiation. It also features almost perfect spatial coherence and ultrashort pulse duration in femtosecond scale. With these excellent properties, XFEL will open a new frontier in science. We will present our plan of coherent imaging experiments using XFEL. By using synchrotron radiation, coherent x-ray imaging has been demonstrated to be a powerful tool to visualize cellular organelles [1]. XFEL will further extend its ability and will enable structural analysis of uncrystallized biomolecules. Because the pulse duration of XFEL is shorter than the time scale of radiation damage process, coherent diffraction occurs before sample biomolecules are destroyed with a single shot exposure of XFEL. With this "diffract-and-destroy" concept, XFEL can for the first time overcome the radiation damage problem, which has been setting a limit on resolution in conventional high-resolution bioimaging. We are planning coherent solution scattering of biomolecules using XFEL. In the experiment, nano-focusing of x-ray beam is essential to obtain sufficient scattering signal from biomolecules with a single shot [2]. The ultrashort pulse duration of XFEL will also enable to take atomic movie. We have been developing techniques of ultrafast coherent imaging by using extreme ultraviolet FEL from the SCSS test accelerator in Japan [3]. By using XFEL, we are planning to visualize strain dynamics in nano-crystals by coherent x-ray diffraction in the Bragg geometry [4].

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Yoshinori Nishino, Marcus Newton, Takashi Kimura

31 - PHOSPHOPEPTIDE-DEPENDENT FLUORESCENCE LABELING OF 14-3-3 ZETA PROTEIN BY FUSICOCCINS

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14-3-3 proteins are critically involved in Ser/Thr kinase-dependent signaling pathways through protein-protein interactions with multiple phosphorylated protein ligands. Ligand-dependent 14-3-3 detection would provide a desirable technique for elucidating 14-3-3-related intracellular signaling networks. In this presentation, we describe phosphopeptide-dependent fluorescent labeling of 14-3-3zeta by cell permeable probes derived from a diterpene natural product, fusicoccin. In vitro evaluations demonstrated that these compounds site specifically attached a fluorescent tag onto the protein surface as a result of ternary complex formation with the 14-3-3 and a phosphopeptide ligand. The BODIPY-attached probe labeled human endogenous 14-3-3 in cancer cells under hyper-phospholylation condition, proving that 14-3-3 is a primary target of the fusicoccins in mammalian cells. This cell permeable labeling agent would be a useful tool to explore mechanistic insight of antitumor activity of the fusicoccin-related antitumor agents

Michiko Takahashi, Akie Kawamura, Nobuo Kato, Tsuyoshi Nishi, Itaru Hamachi, and Junko Ohkanda

32 - SINGLE MOLECULE INVESTIGATION OF PROTEIN FOLDING DYNAMICS IN THE TIME DOMAIN FROM 30 μs TO SECONDS

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Dynamics of protein folding involves a myriad of molecular events that lead the heterogeneous unfolded proteins to the specific native conformation. To investigate the heterogeneity of protein folding, two methods were developed to detect fluorescence intensities for extended time period from single molecules diffusing freely. In the first method, a unique optical system was designed that enabled us the imaging of molecules in a large observation volume for more than several seconds. In the second method, a new type of confocal microscopy was constructed, allowing us to obtain fluorescence data with a time resolution of ~30 μs. The single-molecule measurements demonstrated the presence of a slow dynamics in the unfolded state of cytochrome c. We suggest that the free energy landscape of the denatured state of cytochrome c is rugged, whose implications will be discussed.

Satoshi Takahashi, Hiroyuki Oikawa, Kiyoto Kamagata

33 - NANOBIO IMAGING ON AG NANOPARTICLE 2D CRYSTALLINE SHEET

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Recently we have been studying collective localized surface plasmon resonance (LSPR) in 2D crystalline films composed of metallic nanoparticles [1]. The films are fabricated by self-assembly at air-water interface and deposited on hydrophobic substrates by Langmuir-Schaefer method. Both the experimental data and FDTD simulation data revealed an unique collective property of LSPR in 2D sheet, e.g., a long-distance interaction between particles in the 2D sheet, which is more than 6 times longer compared with the particle pairs (1D). The homogeneous coupling of LSPR in 2D crystalline sheet results in not only a significant red-shift of sharpened LSPR band but also an additional amplification of electro-magnetic (EM) field at the interface. We demonstrate an enhance fluorescence photoemission on this sheet, where the resonance wavelength of the sheet is tuned by the interparticle distances according to the excitation wavelength of the dyes. This flexible, transferable nanosheet, which can trap and transport bulk light at nano-interface, promises new application in the field of bio-related devices.

Kaoru Tamada

34 - «SMART» SYNTHETIC HYBRID LIPIDS FOR BIOMEDICAL APPLICATIONS

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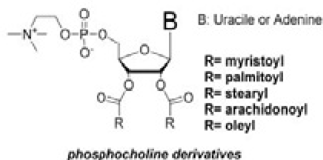
The combination of nucleic acids chemistry (e.g., nucleoside, nucleotides, oligonucleotides, fig. 1) with supramolecular principles provides an efficient and powerful approach to prepare well-defined systems with tunable physico-chemical properties and functions. We develop new nano-systems based on nucleic acids for i) drug delivery applications, ii) bioimaging purposes and iii) novel ligands specific to new biological targets. This communication will present the recent new molecular and supramolecular tools using the "smart" lipids (nucleolipids) developed in our lab.

Nucleoside lipids

Lipid Oligonucleotide Conjugates



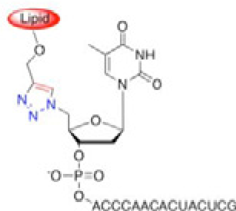


Fig. 1. Examples of bioinspired lipids (Nucleoside lipids and Lipid Oligonucléotide Conjugates) developed in our lab.

35 -DESIGN OF MULTIFUNCTIONAL NANOPARTICLES FOR IN VIVO APPLICATIONS

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The Institut de Chimie de la Matière Condensée de Bordeaux is an academic research laboratory dedicated to the solid state chemistry, materials sciences and molecular sciences (www.icmcb-bordeaux.cnrs.fr). The research group « Chemistry of Nano-materials » leaded by Prof Etienne Duguet is composed of 12 permanent staff and about 20 (non-permanent) young researchers. It aims to study (i) solid state chemistry at the nanoscale, (ii) shaping and surface functionalization of nanoparticles, and (iii) self-assembly. Currently, we investigate 3 application areas: optics, environment and medicine. In relation to biology and health, we combine competences of inorganic and polymer chemistry, colloidal stabilization in bio-related fluids and biofunctionalization. Our activity is divided in three actions: 1) Design of multifunctional nanoparticles for in vivo imaging: contrast agents for MRI and optical markers. All synthesis stages are controlled from the inorganic cores (iron oxide, gold, QDs, etc.) to functional ligands such as antibodies. We experienced different application fields as various as tumour diagnosis, monocyte tracking, diagnosis of the instability of atherosclerotic plaques, etc. 2) Design of functionalised magnetic nanoparticles for innovative therapeutic strategies: local hyperthermia for oncology, heat-triggered drug release, mechanical rupture of drug devices, etc. In particular, we develop hyperthermia nanomediators with a controlled Curie temperature for self-regulated heat release. An induction output working at different frequencies is available in the lab. 3) Preparation and thorough physico-chemical characterization of batches of well-calibrated nanoparticles of controlled chemical composition, shape and surface chemistry, for systematic toxicity studies on human and animals.

E. Duguet, S. Mornet, M.H. Delville, G. Goglio and M. Treguer

36 - ROMP IN DISPERSION: A POWERFUL APPROACH TO PRODUCE PLURIFUNCTIONAL AND ENVIRONMENT SENSITIVE PARTICLES FOR THERAPY

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In the past 10 years, our team designed a variety of norbornene-based nanoparticles with the aim at evaluating them in the biomedical field as drug delivery devices. Such nanoparticles (NPs) are obtained by Ring-Opening Metathesis (co)Polymerization ROMP in dispersed media of norbornene with poly(ethylene oxide) macromonomer fitted at one extremity by a norbornenyl polymerizable group and functional entity (D*) at the other end1. Macromonomers act as a steric stabilizer and functional agent. As stabilizers they allow the dispersion of the polymer as spherical particles and induce the formation of a poly(ethylene oxide) (PEO) shell on the particle surface giving them stealthy properties. The NPs obtained exhibit narrow size distribution and diameter ranges from 300 to 500 nm. Advantages provided by this strategy are multiple. First, it allows a plurifunctionalization with two or more drugs. Secondly, functional group such as carboxylic acid groups could be introduced onto the NPs surface and permit the anchoring of the NPs on biomaterial surface. Finally, it allows obtaining NPs bearing high drug densities (0.3 mmol of drug per gram of NP We developed new generations of therapeutic NPs sensitive to environmental changes such as pH2 and temperature3 variations. These NPs have essentially been designed to treat human tumors but could equally be employed to treat bacterial infections4 or to promote cell adhesion. References

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37 - FOLDING OLIGOMERS WITH PREDICTABLE PROPERTIES

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Our group has developed helical foldamers – oligomers that adopt stable helical folded conformations – derived from aromatic amino acids.[1] Some of these folded objects have shown unprecedented conformational stability,[2] and constitute convenient building blocks to elaborate synthetic, very large (protein-sized) folded architectures.[3] They possess a high propensity to assemble into double, triple and quadruple helices.[4] Cavities can be designed within such synthetic molecules that enable them to act as artificial receptors[5] including for chiral guests. Water soluble analogues of these foldamers show promise in nucleic acid recognition.[6]

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38 - DRUG DESIGN, VIRTUAL SCREENING, ALL-ATOM AND COARSE-GRAIN MOLECULAR DYNAMICS: A TOOLBOX FOR MEDICINAL CHEMISTRY, BIOCHEMISTRY AND BIOPHYSICS

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The Molecular Modeling team comprises 3 permanent CNRS staff, 4 post-Doc and 4 PhD students. The team is well equipped with 11 multicore blades and 1 IBM cluster for a total of 368 cores coming with a 80 To storage facility. The team has also an access to the Regional Calculation Mesocenter and the 2 National HPC Facilities (CINES & IDRISS). The team's research is developed along three axes : drug-design & virtual screening, all-atom & coarse-grain molecular dynamics and physical simulations around Nanotechnologies. The first activity lies at the frontier between biology and chemistry. Starting from a biological problematic, we are searching for -small molecules able to interact with protein targets, virtual screening is performed with pre-filtered chemical databases, or with in-house collections. This leads to the discrimination of the best putative ligands. Several collaborations are under course on the same kind of project aiming at identifying putative ligands for known proteins -structures in the field of cancer, neurodegenerative diseases, GPCR, to get a better understanding of the interactions between a hit molecule and its own receptor. The second axis encompass on one side molecular dynamics of complex lipidic assemblies using an all-atom representation : spherical or cylindrical micelles of various surfactants, Langmuir films and various bilayers of biologically relevant lipids but also fluorinated gemini derivatives or bicelles. On a second side, soluble proteins and membrane proteins within biomembrane models are studied, alone or in interaction with various compounds: natural or artificial (drugs) ligands, membranes or lipidic assemblies, polyphenols or sugars, the main objective being the dissection of various regulation pathways mainly in cancer. The last axis is split into 2 different approaches both involving Nanotechnologies. In the first one, the goal is to develop an optical biosensor based on SPR (Surface Plasmon Resonance) to monitor in real time biomolecular interactions and to recover kinetic rate constants. The activity is devoted to the modelling of the experimental data, mainly by solving the Maxwell equations in a (for the moment) 2-D layer system where the rugosity can be arbitrarily changed. The second approach concerns the the building of DNA Origamis: the aim is to fold single stranded DNA of a virus to form «arbitrary» 2D (3D) shapes and the basic idea is to hold together parts of the DNA virus using locally complementary strands (staples). The final goal will be to develop an origami platform for single molecule SERS.

J. Dessolin, J. Elezgaray & M. Laguerre

39 - POLYMER NANOTECHNOLOGY FOR LIFE SCIENCE

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The Laboratory of Chemistry of Organic Polymers (LCPO, UMR 5629) is devoted to academic researches in Polymer Science that focus on the mechanisms of polymerization, macromolecular engineering and self-assembly, topics that have propelled its reputation to an international level. Polymer science as it is investigated at LCPO is at the interface of several sciences: molecular chemistry, soft matter physics and engineering processes. Three research axes are currently investigated: Sustainable Polymer Chemistry, Polymer Nanotechnology for Life Science and Polymers for Organic Electronics. The team «Polymer Nanotechnology for Life Science» leaded by Sébastien Lecommandoux focuses its research on new and innovative polymer nanoassemblies for medical applications such as polymersomes, stimuli-responsive nanocarriers, magnetic hybrid nano-assemblies for therapy and diagnosis, DNA and siRNA delivery systems. Our main competences concern the design of new polymer-based nano-carriers using original methodologies ranging from self-assembly, emulsion or microfluidic. During the last 10 years, we developed new generation of block copolymers based on polypeptides and polysaccharides. We mainly focus on the relationship between the macromolecular architecture and the morphology of the self-assemble structures. In addition, we used the ability of the polypeptide chains to undergo conformational changes due to environmental changes such as pH, temperature or ionic strength, in order to build unprecedented smart nanoparticles based on polypeptide and/or polysaccharide (Angewandte Chemie 2002, JACS 2005, JACS 2006, Angewandte Chemie 2009, Angewandte Chemie 2010). We also proved that such nanoparticles were able to incorporate either hydrophobic or hydrophilic small and large molecules, and that they were stable over very long time under different conditions. We now acquired a degree of control on the self-assembly that allows us in using such nanoparticles for diagnostic or therapeutic applications (Adv Mat 2005, Biomacromolecules 2009, Biomaterials 2010, Macromolecular Biosciences 2010, Journal of Controlled Release 2010, ACS Nano 2011). Our future challenge aims at using a biomimetic approach towards material design, allowing the formation of multifunctional and responsive polymer nanoconstructs. Learning and mimicking the mechanisms used in Nature to achieved active materials would allow a better “integration” or “coding” of materials properties from the molecular level. In that direction, peptide-polymer chimers and protein-like polymers are programmed and synthesized.

S. Lecommandoux, J.F. Le Meins, O. Sandre, C. Schatz, E. Garanger

40 - ENGINEERING MINIATURE MEMBRANE-LESS GLUCOSE/O2 BIO-FUEL CELLS

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The objective of our work is to design the smallest enzymatic glucose/O2 biofuel cell (BFC) ever built and the only in-vivo source of power that will use glucose and O2 as fuel. Such BFC may power a subcutaneously implanted continuous glucose monitor for diabetes management; the local temperature, indicative of infection of an internal wound after surgery or microsurgery; a local flow, indicative of blockage of a duct, such as the bile duct; or a pressure difference in the central nervous system, indicative of partial blockage of the flow of the cerebrospinal fluid. The operational power density of a biofuel cell depends mainly on: (I) the operating voltage; (II) the limiting turnover rates of the anodic and the cathodic bioelectrocatalysts, defining the current density when mass transport is not limiting, (III) the specific surface of the electrode, (IV) the stability of the biofuel cell. For these reasons, our research addresses each of these points from enzyme engineering to the elaboration of porous and hierarchical electrode materials.

N. Mano, S. Gounel

41 - SELF-ASSEMBLY AND BIOMOLECULES

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We focus on the study of self-organization of biological macromolecules with anisotropic shape. These macromolecules may be associated with amphiphilic molecules or polymers. These systems can then be used as model systems to probe, for example, the effect of confinement on the organization of biological molecules. They can also be proposed as nano carriers for the delivery of nucleic acids.

Self-organization of fd virus

These rod-shaped viruses are model system because of their uniformity of length. Experiments on suspensions of these viruses have recently led to the discovery of two phases of hexagonal symmetry (columnar and crystalline), allowing for the first time a validation of theoretical predictions. We also showed (in collaboration with the Research Center Jülich, Germany) that, contrary to what was expected, the rods arranged in lamellar phases don't change position within a given layer, but jump from layer to layer. Finally, while these viruses have an intrinsic right helicity , we could show that they are organized in a left helical phase. (collaboration with the University of Padua, Italy).

Nanoconfinement of anisotropic biomolecules

We are interested in complexes formed by the insertion of DNA molecules in a lipidic lamellar phase. We have recently shown (with the Institute of Physics, University of São Paulo, Brazil), the existence of neutral lipid-DNA complexes and we suggest that there is attractive effective interaction between bilayers and DNA molecules. The study of the dynamic properties of DNA - neutral lipids complex has allowed us to propose a new model to describe the anisotropy of diffusion in anisotropic systems in a fluid and confined regime at the nanoscale. This new method offers the prospect of studying a variety of experimental systems.

Bioinspired supramolecular systems

In collaboration with colleagues from INSERM (Bordeaux) and INRA (Nantes and Bordeaux) we explore different strategies of formulating supramolecular systems from bio inspired amphiphilic molecules. The originality of this approach is the use of natural molecules such as nucleotides or fatty acids. We seek to establish the mechanisms of formation of supramolecular systems by focusing on the structural characterization and the study of the nature and the specificity of interactions involved in the formation of complexes with nucleic acids. We focus our efforts on self-assembled systems preferentially from weak forces like specific interactions.

François DOLE, Annie FEVRIER, Eric GRELET, Frédéric. NALLET, Laurence NAVAILLES and Gilles SIGAUD

42 - COUNTERION BASED CONTROL OF NANO-ASSEMBLIES OF SURFACTANTS, TOWARDS ORGANIC-INORGANIC HYBRID MATERIALS

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We have demonstrated that cationic amphiphiles and chiral counteranions can lead to formations of chiral nanostructures. A subtle modification of these ionic interaction or hydrogen bonds can result in extremely rich chiral polymorphisms. Such self-assembled nanostructures can then be used as templates for the formation of inorganic nanostructures giving rise to hybrid nanomaterials with controlled chirality with promising optical, catalytic and molecular recognition properties.

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Reiko Oda

43 -SESSION DESIGNED GLUCOSE-RESPONSIVE MICROGELS FOR SENSING AND INSULIN DELIVERY

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Glucose-responsive hydrogels are attractive materials due to their potential to sense changes in blood glucose levels and respond to these changes by regulating insulin release. In this context, we develop glucose-responsive microgels with a controlled submicrometric size and a well-defined internal structure. These microgels are obtained from the copolymerization of a thermoresponsive monomer, from the N-alkylacrylamide family, with a phenylboronic acid (PBA) monomer. PBA is known to bind cis-diol functional groups on glucose. The complexation of glucose with the PBA group shifts the equilibrium in the direction of increasing the charged phenylboronates. When PBA is bound to a polymer chain, the addition of glucose increases the hydrophilicity of the polymer chain, resulting in the microgel swelling and its permeability increase. The microgels were shown to swell in the presence of glucose, with a swelling degree proportional to the glucose concentration. By varying the composition of the microgels, the nature of the alkylacrylamide monomer and the substituents of the PBA derivative, we obtained microgels that were able to sense glucose concentrations in the patho-physiological range, under physiological conditions. Moreover, we successfully designed glucose-selective sensitive microgels. More advanced multiresponsive microgels with a controlled internal structure are presented, such as those bearing core-shell morphology or Janus-type ones. Fluorescently labelled insulin was further loaded within the microgels and its release versus temperature and glucose concentration was studied. Both stimuli were found to trigger the microgel permeability and tune insulin release. The coupling between glucose concentration and insulin release kinetics represents a significant progress for diabetic patients as it opens the route towards closed-loop insulin delivery.

Valérie Ravaine, Bogdan Catargi, Véronique Lapeyre, Christophe Ancla, Léa Messager

44 - NANOCARRIERS FROM POLYMERS AND SIZE-SORTED MAGNETIC NANOPARTICLES

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Assemblies made from diblock copolymers and magnetic nanoparticles enable to mimic the vesicular morphology of lipid membranes. Apart from biomimetism, these magnetic polymersomes can be utilized as smarted nanocarriers of drugs. We present results regarding both their properties as MRI contrast agents for cancer diagnosis and as carriers of a therapeutic load that can be released by irradiation with an alternating magnetic field at 500 kHz.

Julie Thevenot, Hugo de Oliveira, Olivier Sandre, Sébastien Lecommandoux

56 - «IN VIVO» MULTI-PHOTON AND SUPER-RESOLUTION MICROSCOPY FOR ELUCIDATION OF NEURAL ACTIVITY

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Two-photon microscopy with near infrared femto second pulse laser is one of the most powerful techniques for functional analysis of living cells. In the field of neuroscience, this microscopy has clearly demonstrated morphological changes of synapses and movements of cell bodies in vivo or in vitro. Especially, only two-photon microscopy enables the observations of neuronal process and synapse in cortex layer2/3 in the mouse brain cortex. Recently, we have successively developed "in vivo" two-photon microscope, which visualized neurons in all the layers of the cortex. Noticeably, this microscopy enabled to observe pyramidal neurons in the hippocampal area CA1 below the cortex. On the other hands, we have also recently developed super-resolution microscopy. The spatial resolution of light microscope is limited to a finite by optical diffraction of light. To improve the spatial resolution, we have employed a newly developed laser beam, "vector beam", with distributions in the power, the polarization direction, and the phase. We first made a liquid crystal device to convert liner polarized Gaussian (LP) to a vector beam. One of such vector beams, a higher-order radially polarized beam with six concentric rings (HRP) was generated simply by inserting the device into the front of an objective lens. As the result, a confocal microscope equipped with the HRP beam enabled to distinguish each if aggregated 170 nm beads, those was not resolved in the conventional confocal microscopy. HRP beam also showed improvement in the biological specimens. In addition, this device was applied to two-photon microscopy to show similar improvements both in phantom samples and in biological specimens. Thus, we hope that such newly developed microscopy will give us important insights to elucidate physiological activities in living body. Here, we will discuss capability of these newly developed microscopies with newly obtained data.

Ryosuke Kawakami, Terumasa Hibi, Tomomi Nemoto

57 - DEVELOPMENT OF NOVEL THERAPEUTIC STRATEGIES TO TACKLE MULTIDRUG-RESISTANT PATHOGENS

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Since the discovery of antibiotics, the battle between humans and drug resistant bacteria has never stopped. Bacteria have developed various ways to resist the toxic effects of antibiotics and other drugs. One of these mechanisms involves the production of enzymes that inactivate antibiotics by hydrolysis or lead to the formation of inactive derivatives. A second mechanism of resistance is target alteration. Cellular targets can be altered by mutation or enzymatic modification in such a way that the affinity of the antibiotic for the target is reduced. These mechanisms are all specific for a single drug or a single class of drugs. However, there are more general mechanisms of resistance in which access of the unaltered agent to the target is prevented by the barrier and active transport functions of biological membranes. The barrier cannot prevent the drugs from exerting their toxic action once they have entered the cell, and the active efflux of drugs is essential to ensure significant levels of drug resistance. Multidrug efflux transporters are integral membrane proteins that utilize cellular energy to extrude antibiotics or biocides actively out of the cell. In this presentation, we first introduce the post-genomic approach to analyze all putative drug efflux genes. Next, we discuss the regulation of drug efflux transporters responding to environmental signals. We also introduce the physiological roles of drug efflux transporters in virulence, which is an ongoing research area. Multidrug efflux transporters have greater clinical relevance than has previously been thought, because there is now accumulating evidence that certain classes of efflux transporters not only confer resistance to drugs used in therapy but also have a role in bacterial pathogenicity.

Mitsuko Hayashi-Nishino, Ryosuke Nakashima, Keisuke Sakurai, Akihito Yamaguchi, Kunihiko Nishino

58 - ORGANELLE-DERIVED SIGNAL CONTROLS PLANT CELL CYCLE

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Eukaryotic cells arose from an ancient endosymbiotic association of prokaryotes, with plant cells harboring three genomes as the remnants of such evolution. In plant cells, plastid and mitochondrial DNA replication (organelle DNA replication, or ODR) occurs in advance of the subsequent cell cycle(s) composed of nuclear DNA replication (NDR) and cell division. However, the mechanism by which replication of these genomes with different origins is coordinated is largely unknown. Recently in the primitive red alga Cyanidioschyzon merolae as well as in plant cells, we showed that NDR is positively regulated by a chloroplast-derived tetrapyrrole molecule, Mg-protoporphyrin IX (MgProto), which has been suggested as an organelle-to-nucleus retrograde signal (1). Subsequently, we have made clear that the receptor for this signal is an F-box protein of E3 ubiquitin ligase, which ubiquitinates a G1/S cyclin and results in the degradation. Binding of MgProto to the receptor prevents the ubuiquitination and degradation of the cyclin, thus activates the S phase initiation (2).

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Kan Tanaka, Mitsumasa Hanaoka & Yuki Kobayashi

Poster session - Day 2

9 - REDOX REGULATION OF ROTATION OF THE CHLOROPLAST-TYPE F1-ATP SYNTHASE

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ATP synthase occurs ubiquitously on energy transducing membranes such as bacterial plasma membranes, mitochondrial inner membranes and chloroplast thylakoid membranes. The basic architecture of this enzyme complex is highly conserved: the F1 portion which is powered by ATP, and the Fo portion which is powered by the proton electrochemical gradient across the membranes (proton motive force). On the membranes, these two motors are directly connected by protein-protein interaction, and their functions are coupled to each other. When a proton motive force forms across the membranes as a result of respiratory or photosynthetic electron transport, the Fo portion is forced to rotate by the proton motive force. Rotation of Fo induces rotation of the central axis subunits, gamma and epsilon, of F1, and finally ATP is formed at the catalytic sites on beta subunits. Vice versa, rotation of F1 forced by ATP hydrolysis induces rotation of Fo and consequently protons are transferred in the opposite direction. This enzyme may therefore hydrolyze ATP and transport protons in the opposite direction when there is insufficient proton motive force to drive ATP synthesis. However this reverse reaction catalyzed by the enzyme is highly restricted in vivo since the reaction is a wasteful ATP consuming process. To avoid such an unfavorable reaction in vivo, several regulatory systems are known to maintain ATP synthesis reaction. For example, chloroplast F1-ATPase is subject to redox regulation, whereby ATP hydrolysis activity is regulated by formation and reduction of the disulfide bond located on the gamma subunit. In order to understand the molecular mechanism of this regulation system, we constructed a chimeric F1 complex using cyanobacterial F1, which mimics the regulatory properties of the chloroplast F1-ATPase, and studied regulation at a single molecule level. Redox state of the gamma subunit did not affect the ATP binding rate to the catalytic site(s) and the torque for rotation. However, the long pauses caused by ADP inhibition were frequently observed in the oxidized state. In addition the duration of continuous rotation was relatively shorter in the oxidized complex. These findings lead us to conclude that redox regulation of CF1-ATPase is achieved by controlling the probability of ADP inhibition.

Toru Hisabori, Hiroki Konno, Kim Yusung

10 - THE CHEMOTACTIC RESPONSE AND CORRELATION OF THE MULTIPLE FLAGELLAR MOTORS IN A SINGLE BACTERIAL CELL

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An Escherichia coli cell transduces extracellular stimuli sensed by chemoreceptors to the state of an intracellular signal molecule, which regulates the switching of the rotational direction of the flagellar motors from counterclockwise (CCW) to clockwise (CW) and from CW back to CCW. Here, we performed high-speed imaging of flagellar motor rotation and show that the switching of two different motors on a cell is controlled coordinately by an intracellular signal protein, phosphorylated CheY (CheY-P). The switching is highly coordinated with a sub-second delay between motors in clear correlation with the distance of each motor from the chemoreceptor patch localized at a cell pole, which would be explained by the diffusive motion of CheY-P molecules in the cell. The coordinated switching becomes disordered by the expression of a constitutively active CheY mutant that mimics the CW-rotation stimulating function. The coordinated switching requires CheZ, which is the phosphatase for CheY-P. Our results suggest that a transient increase and decrease in the concentration of CheY-P caused by a spontaneous burst of its production by the chemoreceptor patch followed by its dephosphorylation by CheZ, which is probably a wave-like propagation in a sub-second time scale, triggers and regulates the coordinated switching of flagellar motors.

11 - POLYMER MATERIALS TO MANIPULATE BIOPOLYMER FOLDING AND FUNCTIONS

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Proper folding and assembling are essential for biopolymers to exhibit their activities. In living system, molecular chaperones support precise folding of proteins and nucleic acids. We have designed cationic comb-type copolymers consisting of a cationic backbone and hydrophilic graft chains to manipulate folding and assembling of anionic biopolymers such as DNA and acidic peptides. The copolymer formed soluble interpolylectrolyte complex with DNA and acidic peptides. The copolymers significantly facilitate DNA assembling including DNA duplex, triplex and quadruplex formations. The copolymers also accelerate strand exchange reaction between double stranded DNA and its homologous strand, indicating nucleic acid chaperone activity of the copolymer. The activity of the copolymer was applied to DNA analysis including genotyping method. Peptide folding was also chaperoned by the copolymer. The copolymers facilitated alpa-helix formation of membrane-disrupting peptides and enhanced their activity.

Atsushi Maruyama

12 - TOWARD UNDERSTANDING BIOLOGICAL PHENOMENA BY GENETICALLY-ENCODED MOLECULAR SPIES

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Our primary goal is to better understand how biological molecules function in space and time. To this end, we are developing several techniques to visualize physiological events at molecular level in living cells and whole body. One approach is the use of the green fluorescent protein and its derivatives (FPs) which are spontaneously fluorescent. To expand color palette of FPs, we recently invented a pH-insensitive ultramarine fluorescent protein, Sirius, with enhanced photostability and an emission peak at 424 nm, the shortest wavelength among fluorescent proteins reported to date. The pH-insensitivity of Sirius makes possible prolonged visualization of biological events in an acidic environment. Combination of FPs with fluorescence resonance energy transfer (FRET) technique allows us to develop functional indicator, thereby we can visualize localized molecular events in their natural environment in vivo. For example, we have developed an ultra-sensitive Ca2+ indicator by introducing some modification into Ca2+ sensing domain of YC3.60. Its small Kd value (20 nM) allows us to detect Ca2+ dynamics even at 10-150 nM ranges without affecting cellular viability. Large dynamic range (1400 %) also enables us to detect the signaling pattern in 100,000 cellular networks at single cell resolution, being the largest scales to be achieved so far. Furthermore, we applied the FRET technique to make a photoconvertible fluorescent protein, Phamret, which can be highlighted by UV stimulation inducing a change in fluorescence emission from cyan to green color. Phamret can be monitored by single-excitation-dual-emission mode allowing mobility analyses over a broad range of kinetics. In this symposium, I will introduce not only several kinds of FP-based indicators mentioned above but also autoluminescent indicators for combining use with optogenetic technology.

Takeharu Nagai

13 - DEVELOPMENT OF THE REACTIVE OLIGONUCLEOTIDES WITH HIGH SELECTIVE REACTIVITY TO A TARGET BASE

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A vast number of genomic studies have disclosed new aspects of the functions and structure of nucleic acids. Recently, microRNAs (miRNA) endogenously expressing small regulatory non-coding RNAs (ncRNA), are recognized as playing a critical role in regulating the gene expression and to be attractive targets for artificial control of the gene expression. Synthetic oligonucleotides (ONs) have been widely used to artificially inhibit gene expression at the translation step by the antisense, short interfering RNA (siRNA), and so on, and their targets are increasing in number. Functions of natural-type ONs arise mainly from non-covalent hybridization. By contrast, unnatural oligonucleotides with an attached accessory molecule may give rise to an irreversible chemical change in the target nucleic acids depending on chemical properties of the accessory molecules. We have previously reported that 2-amino-6-vinylpurine (2-AVP) nucleotide exhibited efficient and selective cross-linking to cytosine at the target site of DNA. The proximity effect within DNA hybrids between 2-AVP and cytosine at the target is responsible for both high efficiency and high selectivity of the cross-linking reaction. In this study, we have designed two novel cross-linking agents which have the unique structural features with both the hydrogen bond donor-acceptor sites as recognition sites and the vinyl group as a reactive moiety in a single molecule. 2'-O-Methoxy (2'-OMe) analogue (1) of 2- AVP was expected to enhance metabolic stability in cells. 4-Amino-6-oxo-2-vinylpyrimidine derivative (2) was designed in anticipation of highly selective reactivity to uracil by forming a complex with the two hydrogen bonds. We have synthesized 2'-OMe RNA bearing 1 and ONs bearing 2, and evaluated the cross-linking reactivity to target RNA. Both reactive ONs reacted to a uracil base on the target position of RNA with highly selective under neutral conditions. Each cross-linked nucleoside was isolated by enzymatic hydrolysis, and structure of the cross-linked products was examined by measuring NMR and HR ESI MS spectrum. From these results, we have anticipated that 1 would form the cross-linking with 4-oxygen of a uracil base and the 2 would form the cross-linking with 2-oxygen of a uracil base. The new cross-linking motifs will be generally useful in the antisense strategy as well as for inhibition of miRNA and investigation on cell experiments using our cross-linking agents is now ongoing.

Fumi Nagatsugi

14 - LIGAND-ASSISTED COMPLEX OF TWO DNA HAIRPIN LOOPS

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In contrast to the loop–loop interactions in RNA, there are only a limited number of studies on DNA loop interactions. We here describe the interaction of a series of mismatch-binding molecules with hairpin DNA containing a d(CGG)3 sequence in the loop. Native polyacrylamide gel electrophoresis of hairpin DNA showed that the newly synthesized mismatch-binding molecule N-methoxycarbonyl-1,8-naphthyridine assisted the formation of a loop–loop complex of two DNA hairpin loops. We here report our attempt to induce the connection of two DNA hairpin loops with the assistance of small molecular ligands (Chart 1). A tetrameric form of N-methoxycarbonyl-1,8-naphthyridine (NCT) (Figure 1a) brought two hairpin loops consisting of a d(CGG)3 sequence together to produce a ligand-assisted complex of two DNA hairpin loops, of which formation was firmly confirmed by double-labeling experiments using native polyacrylamide gel electrophoresis (PAGE). The data described here provide new insights into the ligand-assisted construction of higher-order structures of DNA.

Kazuhiko Nakatani, Changfeng Hong, Masaki Hagihara

15 - MOLECULAR MECHANISM OF BIOLOGICAL HEME DEGRADATION

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Heme oxygenase (HO), a central enzyme in heme catabolism, converts heme to biliverdin, CO, and a Fe ion through three monooxygenase. Electronic states, reactivities, and the crystal structures of eight intermediates have been examined with a help of cryo-reduction and annealing to trap unstable intermediates, such as hydroperoxo and α-meso-hydroxyheme intermediates. HO forms an enzyme-substrate complex with heme, which serves both as the substrate and the active center. The O2 binds to the ferrous heme iron with an acute Fe–O–O angle of ~110° with its terminal oxygen atom close to the α-meso-carbon. An extended distal pocket hydrogen bonding network functions as a conduit for transferring protons required for the formation of hydroperoxo, generated by one-electron reduction of the oxy form, and also for the activation of hydroperoxo, leading to the hydroxylation of the heme α-meso-carbon. Hydroperoxo cannot be formed upon loss of the nearby H2O, indicating a critical role of this H2O in the HO catalysis. Ferrous verdoheme formation proceeds by reaction of the ferrous porphyrin neutral radical of ferric α-meso-hydroxyheme with O2 and one electron. Conversion of verdoheme to biliverdin is realized in a manner very similar to that for the hydroxylation of the heme α-meso-carbon, first step of oxygenation step.

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M. Ikeda-Saito, T. Matsui, and M. Unno

16 - NOVEL STRATEGY FOR EXTERNAL STIMULUS RESPONSIBLE ARTIFICIAL NUCLEIC ACIDS - TOWARD THE CREATION OF CANCER CELL SPECIFIC GENE THERAPEUTIC OLIGONUCLEOTIDES

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We have recently proposed a new strategy and a practical tool for cancer cell specific gene therapeutic artificial nucleic acids, named Peptide Ribonucleic Acids (PRNAs) with active on-off control of miRNA function corresponding the cancer cell specific intracellular environmental condition. The PRNAs can be actively switching the target miRNA complexation behavior by a low oxygen concentration of the cancer cellular cytoplasm. This strategy utilizes a new category of artificial nucleic acid that carries a ribonucleoside unit tethered to a peptide backbone as a recognition and stimulus-sensitive module. In this artificial nucleic acid called peptide ribonucleic acid (PRNA), the 5'-amino-5'-deoxypyrimidine ribonucleoside unit, which is in the anti conformation in normal cellular cytoplasm condition in the presence of borate, but functions as a built-in switch to be triggered by a low oxygen concentration of the cancer cellular cytoplasm, is attached to the alpha-glutamine backbone as a pendant. Under normal cellular cytoplasm condition, the cis-2',3'-diol of ribose forms a cyclic borate ester with phenyl boric acid moiety of the PRNA to switch the nucleobase orientation from anti to syn through the change in sugar puckering to 2',3'-planar-O4'-exo synchronized with the hydrogen-bond formation between the 5'-amide proton of ribose and the 2-carbonyl oxygen of pyrimidine nucleobase. The results obtained in these studies are promising, validating that the original alpha-PRNAs with anti-oriented nucleobases form stable complexes with the target miRNA under low cytoplasm pH (pH = ca. 6.5) of a low oxygen concentration of the cancer cell, which are readily dissociated under normal cellular cytoplasm pH (pH = ca. 7.2). This means that the PRNA strategy can be used as a powerful tool for on-off switching the miRNA complexation behavior, which is potentially applicable to the cancer cell specific oligonucleotide-based gene therapeutics of the next generation.

Takehiko Wada

17 - PERISTALTIC MECHANISM OF MULTIDRUG EFFLUX TRANSPORT

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Multidrug resistance of pathogenic bacteria has become a serious problem of modern chemotherapy. One of the major causes of bacterial multidrug resistance is a multidrug efflux transporter. Multidrug efflux transporter exports an extraordinarily wide variety of drugs and toxic compounds. The biochemical basis of such a broad substrate specificity of multidrug transporters has not been unknown. We succeeded to determine the crystal structure of the major multidrug efflux transporter AcrB in 2002 (1) and revealed the structural basis of multidrug recognition at the drug binding site in 2006 (2). AcrB is a cytoplasmic membrane protein and acts as a tripartite complex with an outer membrane channel TolC and a membrane fusion protein AcrA. The complex exports drugs directly out of the cells driven by a proton motive force. The crystal structure of AcrB is a homo trimer. Drugs are exported by the functionally rotation mechanism through the ordered conformational change of access, binding and extrusion monomers. Multidrug recognition is based on the multisite binding mechanism in which there are partially-overlapped multi-binding sites in the voluminous binding pocket and the combinations of the pockets recognize a wide variety of substrates. Recently, we have determined the AcrB structure bound with large-molecular-weight drugs such as rifampicin and erythromycin. As a result, one other drug binding pocket (proximal binding pocket) was found. Two binding pockets are lined along the drug translocation channel in the AcrB molecule. Drugs first bind to the proximal pocket at the access stage and they are then transferred to the distal pocket at the binding stage and finally released from the exit at the extrusion stage by a peristaltic mechanism.

Akihito YAMAGUCHI, Ryosuke NAKASHIMA, Keisuke SAKURAI, Seiji YAMASAKI and Kunihiko NISHINO

18 - CARBON NANOTUBE PROBES FOR AFM : SYNTHESIS AND IN-TAKES

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With their size, long aspect ratio, exceptional mechanical properties, carbon nanotubes are ideal probes for Atomic Force Microscopy (AFM). The probes require to obtain one unique nanotube, with a specific orientation, and a strong mechanical attach to the cantilever. In CBMN, we are installing a dedicated room for carbon nanotube probes fabrication. We describe two routes of fabrication: • direct growth of single-walled nanotubes on commercial AFM probes by hot filament chemical vapor deposition • indirect fabrication for multi-walled nanotube probes by chemical vapor deposition followed by manual welding of the nanotube on commercial AFM probes. Examples of imaging with nanotubes on phospholipids, proteins, DNA origamis are displayed. We compare nanotube versus commercial probe imaging on graphen. Future developments for electrochemistry and biological functionalization with carbon nanotubes will follow.

S Marsaudon, J Buchoux, T Douar, JP Aimé

19 - ELECTROCHEMICAL AND OPTICAL NANO-SENSORS FOR STUDIES OF BIOLOGICAL ACTIVITIES

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Our main activities are focused on the development of analytical tools and techniques with a special accent on miniaturized systems (micro to nano-scale) applied to the study of biological systems and biomedical issues. Our research addresses problems resulting from the ever increasing demands of our modern society concerning various aspects like public health, security and environmental issues. In order to tackle these problems, the discipline of Analytical Chemistry has to evolve constantly and improve its performance. Distinguishing molecules with almost identical physico-chemical properties, tracking their evolution with a high spatio-temporal resolution, detecting them at the cell or subcellular level in complex environments, are some of the challenges that we work on. With this respect, our current projects focus on : the development of sensors (molecular probes for detection of bio-relevant species, micro/nanogels for drug delivery, electrochemical biosensors), micro/nanoscale imaging (structured optical fiber bundles for imaging and enhanced coupled methodologies, scanning electrochemical microscopy), and on the electrochemistry of biosytems (cell & sub-cellular analysis of oxygen and reactive chemical species, protein/peptide electrochemistry, electron transfer in DNA).

Dr. Stéphane Arbault, Dr. Laurent Bouffier, Dr. Isabelle Gosse, Pr. Alexander Kuhn, Dr. Sandra Pinet, Dr. Valérie Ravaine, Pr. Neso Sojic, Dr. Dodzi Zigah

20 - VISCOELASTIC PROPERTIES OF A SINGLE CELL NUCLEUS PROBED BY LASER-GENERATED GHZ ACOUSTIC WAVES

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Cells behave in vivo in response to the biological signals they receive from their surrounding environment. Materials used in repairing the human body have thus to reproduce the correct signals that guide the cells towards a desirable behavior. To ensure cell colonization of these biomaterials, one suitable approach is to functionalize the biomaterials by using growth factors likely to improve cell adhesion and cell differentiation. These growth factors act directly upon the cell nucleus rigidity, and thereby dictate the organization of the cytoskeleton to modify the cell structure. To identify the intricate signalization processes at work inside the cell, it is therefore crucial to probe the cell nucleus rigidity separately from the other cell compartments. The recent use of the picosecond ultrasonics technique (PU) on animal cells [1] has allowed probing the cell compressibility, density [2] and thickness. In the present work, we use PU for the remote optical generation and detection of GHz acoustic frequencies in single nuclei extracted from bone cells. A bone morphogenetic protein 2 (BMP2), a typical growth factor used to stimulate the adhesion on biomaterials, is introduced for several hours in the cell environment. The nuclei are then extracted from the cells by dissolving the cell membrane. The nuclei are covalently bonded to a Ti6Al4V alloy for ease of manipulation. Both pump and probe beams are focused at the nucleus/transducer interface. The pump absorption in the transducer yields a temperature rise and a picosecond acoustic pulse is generated in the transducer through the thermoelastic effect. Owing to the contact at the nucleus/transducer interface, an acoustic pulse is transmitted to the nucleus. The probe beam is partially reflected from the metallic interface and partially scattered by the acoustic wavefront propagating in the nucleus. Interferences arise from these two probe contributions causing the so-called Brillouin oscillations in time. Optical phase variations due to acoustically induced changes in nucleus thickness are simultaneously measured. The result of a semi-analytical calculation is fitted to the data. We thereby determine the sound velocity at ~25 GHz from the frequency of the Brillouin oscillations and the thickness of single nuclei from the optical phase variations. Comparison between the sound velocity in the extracted nuclei and in complete cells should enlighten cell signalization processes on a subcell scale.

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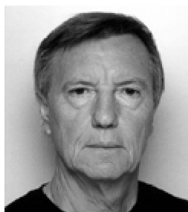
T. Dehoux, B. Audoin, O. Zouani, C. Chanseau, MC Durrieu

21 - COUPLED BIOIMAGING VIBRATIONAL SPECTROSCOPIES WITH MICROFLUIDIC AND MICROSCOPIC TECHNIQUES



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Vibrational spectroscopies have made great progress in recent years with the advent of fast imaging Raman and infrared instruments. Coupled with optical microscopy, they are able to give information in 2D/3D dimensions with micrometric lateral resolution or better. Taking advantage of the potential of these methods, our group develops in one hand advances solutions to monitor the kinetics of biochemical systems and on the other hand multi-techniques analysis (Ellipsometry, Raman, AFM, SNOM, Fluorescence, Infrared, X-Rays) on the same biosamples (membranes, cells, tissues, etc).

B.Desbat, S. Lecomte, C.Petibois, S.Castano, E.Harte

22 - BIOPHYSICS OF MEMBRANES ASSEMBLIES: MAGNETIC RESONANCE OF NANOBJECTS MADE OF LIPIDS, ANTIMICROBIAL PEPTIDES, WINE TANNINS AND PROTEINS

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Major biological processes occur at the biological membrane. Understanding the function of chemical or biological molecules or their passage through membranes is tightly linked to their in situ aggregated structure and dynamics as well as to their topology and orientation in the membrane lipid bilayer. The group "Biophysics of Membrane Assemblies" develops at the Institute of Chemistry & Biology of Membranes & Nanoobjects innovative self-assembling systems made of lipids, proteins and tannins that fall into the generic term of "Membrane and Colloid Science". Fields of interest include i) lipid biodistribution and assembly in nuclear membrane; ii) role of sterols in plant membranes; iii) construction of magnetically sensitive lipid nanodiscs (field orientation/deformation) for imaging or structural biology; iv) wine tannins interacting with saliva proteins or membrane lipids; v) action/design of new peptide antibiotics on membranes and vi) structure of membrane proteins in lipid environment. In addition, the group is a scientific resource for the high-field NMR equipment that belongs to the French Large Scale facility (TGIR-RMN-THC). This NMR facility is spread over six locations in France and welcomes scientists and projects whose proposal has been selected through a referring process.

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23 - VALIDATION OF IMPLANTABLE DEVICES: FROM CYTOCOMPATIBILITY TO PILOT CLINICAL TRIALS

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The Clinical Research Centre for Innovative Technology in Biomaterials and Implantable Medical Devices is an interface for medical research valorization. Its abilities take place between fundamental and clinical research in Biomaterials, Medical Devices and Tissue Engineering fields, to make the proof of concept of innovative technologies, and then conduct a pilot clinical study. The CRC IT associates manufacturers to hospital and university environment into the Technological Platform for Biomedical Innovation in Xavier Arnozan. It brings together clinicians, recognized research departments, and local industries, with access to performing equipments. The CRC IT assumes first-quality R&D skills, since the preparation phase of the project until its realization and its follow up. It offers methodological support and technical assistance during the whole process, in compliance with ISO NF EN 10993-6, ISO 9001 and ethical and law international regulations. Its team performs biocompatibility studies (In vitro: cell biology, biochemistry, In vivo: on little and big animals (implantation, imaging, histology)), pilot clinical studies and post market clinical follow up (protocol writing, regular steps, access to clinical departments (Clinical Research Centers), GCP).

M Durand, L Bordenave

24 - ENGINEERING OF BIOMATERIAL INTERFACES THROUGH MICRO- AND NANO-PATTERNING

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Tissue engineering is generating increasing interest as results obtained in cells associated with engineered materials have shown promise for the restoration of function to damaged tissues. Engineered tissue can be achieved by the combination of living cells, biomaterial, proteins or growth factors which will favour cell adhesion, proliferation, differentiation. Now it is important to answer several questions: which cells, which materials, which factors, which quantities, which organization used with the engineered tissue? Cells are likely to be able to respond to micro- and nano-structures, since in vivo they live inside an extracellular matrix and since their own surface is structured on the nanoscale level. Micro- and nano-technologies can be used to fabricate biomimetic materials to control the cellular microenvironment in a reproducible manner with high temporal and spatial resolution. Our researches focus on the synthesis of biomimetic materials (i.e.. grafting of biomolecules or peptides onto materials surfaces (homogeneous or micro-, nano-structured materials), materials presenting various stiffnesses) able to regulate and control cellular interactions (adhesion, migration and differentiation of mesenchymal stem cells and adhesion, migration, and morphogenesis of endothelial cells). In connection with these experimental studies, we focus on modeling of endothelial cell migration in the aim at correlating this simulation with experimental results using materials presenting various micropatternings. Today, cell stiffness is emerging as an important property. In medicine, researchers have shown that cancerous cells are less rigid than their healthy ones (70% softer). Defining the physical features of cells is essential to understanding how they function. In our project, we want to understand how cells respond to forces, to materials presenting various bioactivities, rigidities, micro-, nano-features distributions. We propose in this study to use an innovating technique which is picosecond acoustic technique which allows no strain on cell, a possible cell cartography and rigidity measurements. Right now, delivery systems of active molecules, called “Drug delivery system (DDS)” are under development. The goal of our project is to develop biomaterials allowing the controlled release, at the level of the site of implantation of these biomaterials (over an adjustable period of time), of an active molecule fixed covalently on the surface of the latter thanks to chemical anchoring of nanoparticles functionalised by the active molecule.

MC.Durrieu , O.Zouani, Y.Lei, L.Pichavant,C.Chollet, Z. Cheng, A.Cunha, C.Chanseau, J.Kalisky, M.Rémy, C. Bourget, T.Colin, P.Barberet, R.Oda, V.Héroguez, T.Dehoux, B. Audoin

25 - PHOTOCONTROLLED BIOCOMPATIBLE MOLECULAR SYSTEMS: CHEMICAL TRANSFER AND ACTIVATION

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Despite intense research, no single strategy has been shown to satisfactorily connect artificial molecular components in networks. This is perhaps the greatest hurdle to overcome if implementation of artificial molecular devices and sophisticated molecule-based arrays are to become a reality. One major goal of our work at the Institut des Sciences Moléculaires is to establish a strategy whereby functional molecular devices (e.g. photo- / electroactive) can communicate with one another in solution and in organized, self-assembled media (biotic and abiotic). Natural systems use chemical communication with small molecules and ions to promote transfer of information in different processes, including vision and neural transmission. In the current case artificial biomimetic systems are being developed integrating photonic and ionic processes, where remote control of ion release from synthetic molecular receptors, and thus the information transfer, is governed by a photonic stimulus in a bottom-up strategy. Ultimately this is anticipated to lead to coded information transfer through ion movement, which when combined with suitable receptors is signalled by fluorescent reporter groups and induced by photomodulated receptor groups in small photoactive molecules. A range of artificial nanoobjects have been synthesized where different ion and molecule messengers are considered and are under study using ultrarapid transient absorption spectroscopy and dynamic fluorescence. Fast processes of photoejection and migration of ions are particularly well-suited to studies in real-time (using time-resolved photophysical techniques) with high spatial resolution (using fluorescence confocal microscopy techniques) allowing evaluation of the versatility of this strategy in the treatment and transfer of information and incorporation into devices. Some hydrosoluble variants are well-adapted to address biomolecules and bio-architectures. As well as studies in solution, communication between distant sites / molecules considers the use of photoejected ions in nanocapsules and organized media including membranes, thin films, nanostructured hosts. Proof-of-principle of compartimental effects in dynamic micellar nanodomains has recently been demonstrated.

N.D. McClenaghan, G. Jonusauskas, R. Oda, S. Arbault

26 - 3D TRUEFISP MRI : AN UNIQUE TOOL FOR SMALL ANIMAL ANATOMICAL IMAGING

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The goal of this work was to show that fully balanced SSFP (bSSFP or TrueFISP or FIESTA) NMR imaging can be used at high magnetic field for anatomical small animals imaging. At such field strengths we failed to optimize field homogeneity over the entire imaging volume. Therefore, a multiple acquisition SSFP method was performed and followed by sum-of- squares (SOS) reconstruction processing to suppress classical TrueFISP banding artefacts. Images with high signal-to-noise ratio and high contrast were obtained in 3D with unparallel to date spatial resolution. Total acquisition time is reduced by a factor 4 compared to classical T2w imaging (RARE sequence). Method can be used for anatomical and volumetric studies of numerous small animal model (cancer, biomaterials) and has shown pertinent results on functional mouse cardiac imaging.

Sylvain Miraux, Jean-Michel Franconi, Line Pourtau, Emeline J Ribot, Emilie Bled, Lionel Chiron, Pierre Voisin, Joelle Amédée, Philippe Massot, Eric Thiaudière

27 - MR IMAGING OF NEUROINFLAMMATORY BLOOD-BRAIN BARRIER FUNCTION IN MULTIPLE SCLEROSIS

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In Multiple Sclerosis, inflammatory brain lesions involve cellular and molecular alterations of the blood brain barrier (BBB). Magnetic resonance imaging (MRI) distinguishes infiltration of blood monocytes into the brain parenchyma and BBB leakage. To identify inflammation induced molecular alterations of the microvascular endothelial cells we have performed an in vivo phage displayed 7 amino acids peptide ligand screening in the experimental acute rat model of MS (EAE) in comparison to healthy rats. Both in vitro and in vivo binding studies with selected phage peptide ligands have revealed specificity to vascular endothelial cells in neuroinflammation sites in EAE and in induced focal brain lesions. We have further tested the capacity of selected peptide ligands as potential in vivo drug carrier. For this proof of concept study, we have focused on one single peptide ligand. The synthesized peptide was covalently linked to the surface of a nanocargo in which were further incorporated a fluorescent dye and/or a MRI contrast agent. In a focal brain lesion, the i.v. administered peptide-nanocargo construct specifically labeled the neuroinflammation site in vivo as visualized by MRI and confirmed by histology. For some of the identified proteins that are individually mimicked by several (up to 6) peptide ligands, the physiological function in neuroinflammation is known. For many others, the genes of encoding proteins have been identified, but their functions remain unknown. Highly conserved between mammals the mimicked proteins provide potential new tools to study neuroinflammation.

BOIZIAU C, VEKRIS A, MIRAUX S, FRANCONI JM, DUGUET E, LECOMMANDOUX S, PETRY KG

45 - "MECHANOBIO-MATERIALS": DESIGN OF MICROPATTERNED ELASTIC GELS TO CONTROL CELL MECHANOTAXIS AND MOTILITY-RELATED FUNCTIONS

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Recently, mechanobiology-based cell manipulation technology has drawn much attention in bioengineering filed. As the advanced trends in the biomaterial researches that intend to control the mechanobiology of cells based on the design of micro-mechanical extracellular milieu, mechanical cell manipulation employing the elastic substrates for cell culture is focused in this paper. Control of cell motility on the well-designed elasticity gradient materials, i.e., control of mechanotaxis, and correlated stem cell lineage specification on the elasticity-micropatterned gels were investigated. For the first, we are focusing on the understanding and control of cell mechanotaxis, which might provide a basis for designing mechanobio-materials to manipulate cell motility. To establish the condition to induce cell mechanotaxis, we have developed the photolithographic surface microelasticity patterning method for fabricating a cell-adhesive hydrogel with a microelasticity-gradient (MEG) surface using photocurable styrenated gelatin. To investigate the condition to induce and control mechanotaxis, patterned MEG gels with different absolute surface elasticities in soft and hard regions, elasticity jumps between them, and sharpness of elasticity boundary. Three critical criteria of the elasticity jump and the absolute elasticity to induce mechanotaxis have been identified: 1) a high elasticity ratio between the hard region and the soft one, 2) elasticity of the softer region to provide medium motility, and 3) sharpness of the elasticity boundary. Especially, concerning the characterization of the effect of sharpness of elastic boundary on the induction efficiency of mechanotaxis, MEG gels with the different sharpness of elastic boundary and the same magnitude of elasticity jump between softer and stiffer regions were fabricated. While on the diffuse elastic boundary cells did not exhibit any directional movements, marked enhancement of mechanotaxis was observed on the discrete elastic boundary of ca .

Satoru Kidoaki

46 - MULTIBLOCK AMPHIPHILIC MOLECULE AS A MIMIC OF MULTI-PASS TRANSMEMBRANE PROTEIN

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Synthetic molecules mimicking the structure and function of proteins attract increasing interests for development of molecular devices and medicines. Membrane proteins are important targets, where several synthetic ion channels, including tubes, ribbons, and barrels as mimics of β-barrel structures have been developed so far. On the other hand, multipass transmembrane (MTM) proteins remain mostly unexplored targets, although they provide one of the major structural motifs of the membrane proteins. MTM proteins usually consist of α-helices connected by hydrophilic residues, where their ternary structures are stabilized by the helix-helix interaction. As simple structural mimics of MTM proteins, we developed alternating amphiphilic multiblock molecules, consisting of linearly connected hydrophilic oligoethylene glycol unit and hydrophobic 1,4-bis(4-phenylethynyl)benzene unit. The luminescent property of the hydrophobic unit allows us to monitor assembly of these units in liposomal membranes. Actually, we found that the hydrophobic units of these molecules tend to form face-to-face stacking in the membrane, to give folded structures like MTM proteins.

Kazushi Kinbara, Takahiro Muraoka, Tatsuya Shima

47 - NOVEL BIOMIMETIC POLYMER MATERIALS PREPARED BY SELF-ORGANIZATION

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Patterns generate functions. In the world of insects, plants, and animals, simply repeated structures sometimes generate functions. For example, the moth-eye, whose surface is covered by regularly arranged sub-cellar size dots pattern, is a "micro device" that enables high-speed night flight of its owner. "Biomimetic surface materials" have now attracted worldwide attentions because of their unique surface properties. In this report, anti-reflective silicon surfaces have been simply prepared by using self-organized honeycomb-patterned polymer films as masks for dry etching process. Simple casting of polymer solutions under highly humid condition can provide "honeycomb patterned" polymer films. Self-packed surface monolayer of mono-dispersed water droplets formed by evaporation cooling on the solution surface acts as a temporary template of micro-pores. The "honeycomb-patterned" film has a double-layered structure with pillars supporting the two porous layers on each vertex of the hexagons. Regular arrayed pillar-structure, like a "pincushion", was formed when the "honeycomb-patterned" film was cleaved into halves by peeling with an adhesive tape After UV-ozone treatment of the polystyrene honeycomb-patterned film, the film was fixed up side down on silicon substrate with poly vinyl alcohol as adhesive. After peeling of the bottom layer of the honeycomb-patterned film, pincushion-structured porous mask was formed on the silicon substrate. After reactive ion etching of the silicon substrate through the mask, the nano-structured silicon surface was obtained. Pincushion-like silicon structures with hierarchic spike structures from nano meter to micrometer were formed. The surface showed very small light reflectance and super-hydrophobic nature because a small amount of fluorinated compounds were remained on the silicon surface. The super-hydrophobic surface was turned to a super-hydrophilic surface after UV-ozone treatment for removing fluorinated compounds. "Ligia exotica" is one species of isopods living at waterfront of sea. Hariyama have found that Ligia exotica uptakes water from wet ground to its abdomen by using two pairs of legs for branchial respiration. Long-distance water transport was achieved by using open capillary structures formed at the outer surface of the legs. Biomimetic water transport surface is designed and prepared by using the patterned Si substrate having both super-hydrophobic and super-hydrophilic properties.

Y.Hirai, D.Ishii, H.Yabu, Y.Matsuo, K.Ijiro, T.Hariyama, K.Tsujii, T.Shiomozawa, M.Shimomura

48 - CELL DEATH-CONTROLLING MOLECULES

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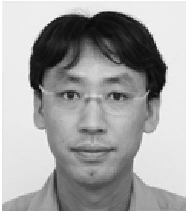
Bongkrekic acid (BKA) is a poisonous antibiotic produced by Burkholderia Cocovenenans that inhibits the mitochondrial permeability transition pore (mPTP) via binding to the adenine nucleotide translocator (ANT) in mitochondria and fixes its conformation in a state opening toward matrix. These particular effects on mitochondria have been shown to delay the programed cell death called apoptosis. As a result, BKA has been used as a pharmacological tool to modulate the properties of the mPTP or ANT in mitochondria. BKA prevented a number of phenomena linked to apoptosis, such as reduction of mitochondrial membrane potentials, chromatin condensation, oligonucleosomal DNA fragmentation, depletion of reduced glutathione, generation of reactive oxygen species, and translocation of NFκB. However, due to its limited availability from fermentation or chemical synthesis, the bioactivity of BKA has not been extensively investigated, especially its in vivo activity. In order to assess its use and potential contribution to apoptotic science, BKA and its analogues will have to be synthesized on a large scale in pure form. Herein, the efficient total synthesis of BKA and molecular design and synthesis of its potential analogues will be presented. Total synthesis of BKA was achieved via convergent strategy which contains the successive three fragment coupling including the Kocienski-Julia olefination and the Suzuki-Miyaura coupling. During our the SAR study, we have found that three carboxylic acid moieties of BKA are essential for exhibiting an apoptosis-preventing activity by using BKA analogues. Based on this result, we designed and synthesized simplified BKA analogues having different length of saturated carbon chain. The results of biological activity tests will also be presented.

Mitsuru Shindo

49 - TISSUE AND CELL SPECIFIC DELIVERY OF STRONG ANTI-INFLAMMATORY PROTEIN USING BIONANOCAPSULE



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Most, if not all, drugs exhibit not only beneficial effects but also undesired side effects when administered in excess doses to the whole body.
Thus, to maximize the beneficial effects while minimizing the side effects, pinpoint drug delivery systems (DDS) have recently attracted much attention in the field of chemotherapy. The bio-nanocapsule (BNC) developed by us is a virus-like empty nanoparticle composed of envelope L proteins derived from hepatitis B virus (HBV) and phospholipids derived from yeast. BNC can be easily obtained by overexpression of L proteins in the recombinant yeast cells. Since BNC does not contain the viral genome, it is nontoxic to the cultured cells used in in vitro experiments and is also assumed to be safe for the use in in vivo experiments. The envelope L protein of HBV contains an N-terminal region designated preS1, which bears the specific infectivity to human hepatocytes. Therefore, BNC is also recognized specifically by human hepatogenic cells. We previously developed a novel pinpoint DDS to hepatocytes using BNC incorporated with genes and drugs. Furthermore, the specificity to hepatocytes of BNC was altered by replacing the preS1 region of L protein with other bio-recognition segments. For example, an engineered IgG-binding domain (Z domain) of protein A derived from Staphylococcus aureus was fused in tandem to the N-terminus of L protein without preS1. The resultant BNC composed of ZZ-L proteins (ZZ-BNC) was shown to be useful for pinpoint DDS other than hepatocytes by attaching specific antibodies on the surface of ZZ-BNC.
Steroid agents are used widely as effective drugs for the treatment of intractable autoimmune failures accompanying inflammations. However, they often show serious side effects. Therefore, a new drug that can substitute for steroids is needed. Various cytokines and cell adhesion molecules are known to induce inflammatory responses to the cells through activation of multiple phosphorylation cascades, by binding to their specific receptors.
The cytosolic phosphorylation cascades ultimately converge into the activation of a nuclear transcription factor NFκB that enhances transcriptions of most proteins involved in the inflammation within the cells. Thus, the regulation of NFκB could be an effective way of depressing inflammations. We have identified a novel macromolecular translocation inhibitor II (MTI-II) that directly suppresses the transcriptional activity of NFκB. In the study reported here, we are aiming at developing a new system for the immunocyte-specific delivery of the strong anti-inflammatory protein MTI-II using BNC, which may be a clinically useful substitute for the treatment with steroid agents having severe side effects.

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50 - THE FATE OF HUMAN MESENCHYMAL STEM CELLS IN 3D MATRICES FOR TISSUE ENGINEERING

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It is now admitted that stem cells in 2D cultures can differ considerably in their morphology, cell adhesion, cell proliferation and differentiation from those growing in a 3D scaffold. Recently, it has been demonstrated that cell shape in a 3D scaffold also controls stem cell fate. In this context, it has been shown that 3D culture could support osteogenic differentiation of primary human osteoblasts. However, few papers described the cell behavior of mesenchymal stem cells in a 3D microenvironment and their orientation towards the osteogenic lineage within the scaffold without biochemical stimuli.
Regarding the scaffold, to mimic a 3D microenvironment, biomaterials are designed to create physical and chemical structures capable of promoting cell differentiation and the production of a specific mineralized extracellular matrix. Today, scaffolds for bone tissue engineering are bioactive ceramics such as calcium phosphate, bioactive glasses, or metallic implants, mainly used for maxillo-facial surgery and orthopaedic applications. Besides these bone substitutes, polymers allow a great design flexibility in terms of composition and their structure can be tailored to fulfill a specific need.
The main objectives of our study was to evaluate the potential of new hydrogel matrices, consisting of either polymers or thermosensitive self-assembling monomers, as a 3D microenvironment, to drive osteogenic differentiation of human MSCs arising from adipose tissue or bone marrow under static and/or dynamic cultures, in a culture medium deprived of osteogenic differentiation factors. Since many years, cell spreading of MSCs into the scaffold and cell-matrix interactions were a prerequisite to drive intracellular signals through integrin activities, in order to induce osteoblast differentiation. However, spheroids or multicellular aggregates of stem cells also appears favorable for osteoblast differentiation, probably as the result of cell-cell communications within the aggregates, which promote cell survival and differentiation, and extracellular matrix deposition.
Here, we give some examples of 3D hydrogel-based scaffolds that offer a suitable architecture, allowing the formation of muticellular aggregates, promoting cell interactions, stimulating osteoblastic differentiation of human MSCs, in the absence of osteogenic medium. The capability of these new tissue-engineered constructs are further evaluated using experimental models in mice and/or rat after their implantation in ectopic and heterotopic sites.

Sophia Ziane, Charlotte Lalande, Jean-Christophe Fricain, Olivier Chassande, Reine Bareille, Chantal Bourget, Julien Guerrero, Joëlle Amédée.

51 - A HIGH-THROUGHPUT PLATFORM FOR QUANTITATIVE ANALYSIS OF RNAI PHENOTYPES IN C. ELEGANS

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Genome scale RNAi studies are generating a wealth of information on the phenotypic effects of gene depletion in C. elegans. However, for about 90% of genes no physiological effect could be observed in the initial visual screens. The use of RNAi sensitised mutant strains and fluorescent reporters allowed to uncover phenotypes for an additional 5% of the gene tested. Provided we used the appropriate fluorescent reporter construct, it is likely that it would be possible to observe a modifying phenotype for the vast majority of genes with otherwise undetectable physiological effect on the animal. However, the number of RNAi screens to be performed to cover would be unpracticable using traditional microscopy and are extremely challenging for automated image analysis software. Here we present a high throughput platform for screening of fluorescent modulation in C. elegans. We use the COPAS-Profiler to perform multidimensional quantitative analysis on the knocked-down worm populations. This enables us to detect not only variation in growth and fertility but also any modification of the relative expression of up to three fluorescent reporters. We modified the Reflx plate handling system to increase the processing speed. We are currently able to collect quantitative information for up to 1000 worms from each well of a 96-plate in 25 minutes. In this set up we are able to measure, for each individual RNAi experiment, the total number of worms per well, their sizes distribution, as well as collect longitudinal fluorescence expression profiles. Importantly, we are able to compare expression patterns within individual animals, thus providing an endogenous control for stochastic variations in overall expression levels.

Ilyass ZNIBER, Karine REBORA, Léo GUIGNARD and Denis DUPUY

52 - LASER-ASSISTED BIOPRINTING FOR DEALING WITH TISSUE COMPLEXITY

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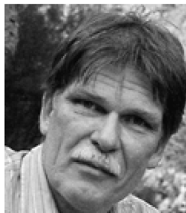
Parallel to scaffold-based approaches, technological advances in the fields of automation, miniaturization and computer-aided design and machining have led to the development of Bioprinting. This later concept has been defined recently as the "the use of computer-aided transfer processes for patterning and assembling living and non-living materials with a prescribed 2D or 3D organization in order to produce bio-engineered structures serving in regenerative medicine, pharmacokinetic and basic cell biology studies". As compared to traditional approaches in Tissue Engineering, bioprinting represents a paradigm shift. Indeed, its principle is not more to seed cells onto a biodegradable scaffold but rather to organize the individual elements of the tissue during its fabrication step (before its maturation) through the layer-by-layer deposit (bottom-up) of biologically relevant components. Besides ink-jet printing and bioplotting by means of pressure-operated mechanical extruders, the Laser-Assisted Bioprinting (LAB) technology has emerged as an alternative method, thereby overcoming some of the limitations of ink-jet and micropen printing devices, namely, the clogging (viscosity, cell agglomeration, ink drying, etc...) of print heads or capillaries used by these printers to achieve micron-scale resolution. In this context, after describing physical parameters involved in Laser-Assisted Bioprinting, we present its applications for printing nanomaterials and cells, both in vitro and in vivo and we discuss on how this high-throughput, high resolution technique may help in reproducing local cell micro-environment and dealing with tissue complexity and heterogeneity, and hence creating functional tissue engineered 3D constructs.

Fabien Guillemot

53 - HYBRID BIOELECTRONIC SENSOR DEVELOPMENT FOR LONG-TERM FUNCTIONAL SCREENING ON ISLETS AND INSULIN THERAPY IMPROVEMENT



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Background and aims: Sensor technology for insulin therapy has made considerable progress but hormonal regulation, detection of hypoglycaemia and real-time as well as closed-loop functioning are still open questions. Innovative sensors should also considerably advance long-term functional screening of healthy or diseased β-cells/islets. We report here the development of a hybrid biosensor based on the combination of electrophysiological recording of islet cells with multielectrode arrays (MEAs) and microelectronics devices. Results: We succeeded for the first time in the culture and the long-term recording of electrical signals of both clonal and primary β-cells on MEAs containing 60 extracellular microelectrodes. Spike frequencies increased in response to glucose dose-dependently in reversible and reproducible manner. The intestinal hypoglycaemic incretin hormone GLP-1 as well as the incretin-mimetic agent forskolin increased the firing rate. On the other hand, the hyperglycaemic hormone adrenalin, released during high-glucose-consuming situations, decreased electrical signals generated by primary β-cells and increased those from α-cells. The device is thus well-suited for simultaneous electrophysiological investigations on different islet cell types from the same biological sample. Functional monitoring over several days was feasible and we demonstrated the feasibility of acute pharmaco-toxicological screenings on β and non β-cells with several compounds targeting islet ion channels. Finally, algorithms were designed to improve the detection of very small amplitude spikes using adaptive thresholds after wavelet transform. Conclusion: Our results demonstrate the feasibility of long-term functional screening on islet cells with MEAs, the improvement of real-time spike detection by online microelectronics, and the interest of hybrid biosensor developments for high-throughput screening and for the treatment of diabetes.

Raoux, M., Bornat, Y., Quotb, A., Catargi, B., Renaud, S., Lang, J.

54 - FIBRE FABRICATION IN MICROFLUIDICS FOR TISSUE ENGINEERING

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Tissue engineering consists in assembling cells in order to reconstruct tissues; a key issue is to place the cells in a three-dimensional environment where they can interact, develop, and multiply specifically. It is shown in literature that for endothelial cells for instance, the cells can merge in order to fabricate cell capillaries. Among several techniques devoted to tissue engineering, hydrogels are often used in order to provide a 3D scaffold and mimic the extra-cellular matrix; a strong challenge consists in reaching sufficient cell concentration. Here, we use microfluidics to produce alginate fibres loaded with cells with a good control of the fibre geometry and high concentration of cells. Our microfluidic device consists of a PDMS chip which generates a cylindrical stratified flow: the core is a solution of alginate and the annulus contains calcium ions (gelling agent of the alginate). In our device, the shaping up of the fibre is done by hydrodynamic focalization in a coflow (ratio of flow rates will fix the radius of the fibre) and the gelation is achieved by diffusion of the gelling agent in the internal jet. We rationalized the parameters (flow, calcium concentration, etc.) that permit a good control of the fibre fabrication, and also identified specific regimes where the fibre can be loaded with a high concentration of cells. We demonstrated the viability of our process by a live/dead test on cells in the gel and actually work on the cell culture within the alginate fibre.

Oriane Bonhomme, Jacques Leng, and Annie Colin

55 - LIVE IMAGING APPROACHES TO STUDY THE MECHANISMS THAT ENSURE FAITHFUL CHROMOSOME TRANSMISSION

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We are studying the dynamics of chromosomes segregation and cell division in living cells by time-lapse video-microscopy. Mitosis is the final stage of the cell cycle where the duplicated genome has to be faithfully partitioned in each daughter cell. Failure to do so will produce daughter cells with an inappropriate genome content also called aneuploidy. The mechanisms that safeguard cells against aneuploidy are of great interest as aneuploidy contributes to tumorigenesis. To gain insight into these mechanisms, we studied the behaviour of cells entering mitosis with damaged chromosomes. We used the endonuclease I-CreI to generate DNA double strand beaks on chromosome 1 in the Drosophila larvae. Expression of I-CreI produces two distinct chromosome 1 fragments: a fragment lacking the centromere (acentric fragment) and a fragment containing the centromere (centric fragment), which is essential for proper segregation. While I-CreI expression produces acentric chromosome fragments in the majority of dividing cells, remarkably, it has no effect on adult survival. Live studies reveal that acentric fragments segregate efficiently to opposite poles. This faithful segregation to the poles is mediated through DNA tethers that connect the acentric fragment to its centric partner. The conserved kinases BubR1, Polo and Aurora B localize on the tether and facilitate the accurate segregation of acentric chromatids by maintaining the integrity of the tether. Chromosome segregation must be tightly coordinated with cell division to ensure that cells do not divide before the chromosomes have cleared the cleavage plane. To identify mechanisms coordinating these events, we analyzed the segregation of abnormally long chromosome arms during drosophila neuroblast asymmetric division. We found that cells adjusted their length to the length of the longest chromosome arms during anaphase. This increase in cell length was concomitant with a spread of concentric myosin ring around the trailing chromatid, which did not affect the rate of cytokinesis. This response was mediated by the Rho Guanine-nucleotide exchange factor, an activator of myosin. Finally, cell elongation was not triggered by the presence of dicentric chromosomes. These results suggest an adaptive mechanism where cells increase their length to clear long chromatid arms from the cleavage site via myosin contractile activity and, thus, ensure proper cell division.

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