Innate immunity in a biomineralized context : trade-offs or synergies ?

Maxwell.T. Hincke, Sophie Réhault-Godbert

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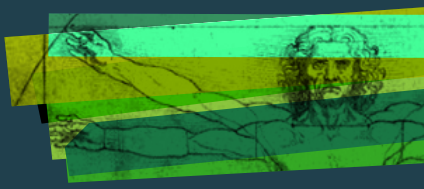
VIRTUAL MEETING | 2021



23 - 24 March 2021

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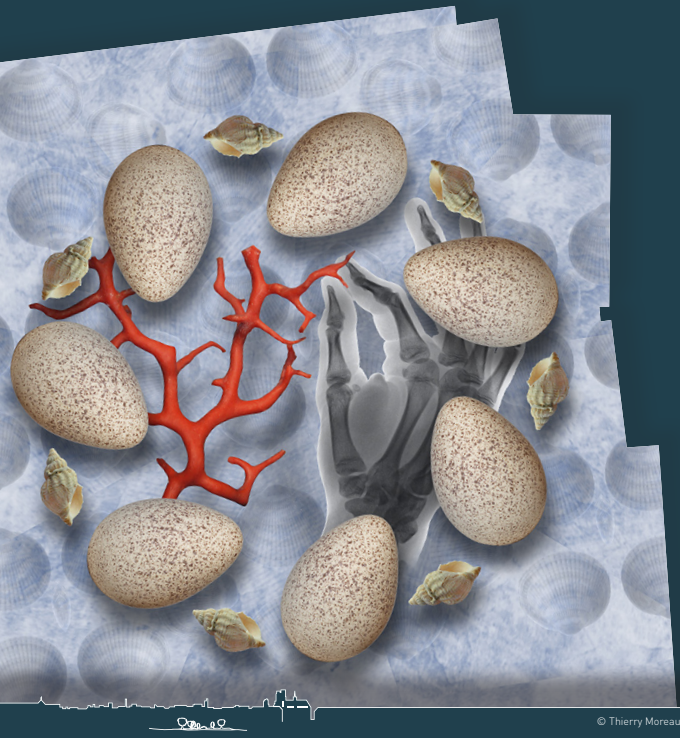

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LOCATION

VIRTUAL MEETING

CONVENORS

Prof. Maxwell Hincke

LE STUDIUM RESEARCH PROFESSOR

FROM Innovation in Medical Education & Cellular and Molecular Medicine, University of Ottawa - CA

IN RESIDENCE AT Avian Biology & Poultry Research (BOA) / Centre INRAE Val de Loire, University of Tours - FR

Dr Sophie Réhault-Godbert

Avian Biology & Poultry Research (BOA) / Centre INRAE Val de Loire, University of Tours - FR

LE STUDIUM Loire Valley Institute for Advanced Studies

INRAE université de TOURS

LE STUDIUM

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ABSTRACTS

Innate immunity in a biomineralized context: trade-offs or synergies?

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Prof. Maxwell Hincke

LE STUDIUM RESEARCH PROFESSOR
FROM: Innovation in Medical Education & Cellular and Molecular Medicine, University of Ottawa - CA
IN RESIDENCE AT: Avian Biology & Poultry Research (BOA) / Centre INRAE Val de Loire, University of Tours - FR

Dr Sophie Réhault-Godbert
Avian Biology & Poultry Research (BOA) / Centre INRAE Val de Loire, University of Tours - FR

ORGANIZING COMMITTEE

Sophie Gabillet, General Secretary

Dr Aurélien Montagu, Scientific Relations Manager

Maurine Villiers, Communication & Events Manager

LE STUDIUM Loire Valley Institute for Advanced Studies • Région Centre-Val de Loire • FR

EDITO

Created in 1996 on the CNRS campus in Orleans La Source, LE STUDIUM has evolved to become the multidisciplinary Loire Valley Institute for Advanced Studies (IAS), operating in the Centre-Val de Loire region of France. LE STUDIUM has its headquarters in the city centre of Orleans in a newly renovated 17th century building. The amazing facilities are shared with the University of Orleans. In 2014 new developments and programmes linked to the smart specialisation of the Centre-Val de Loire region came to strengthen existing IAS collaborative relationships with the local and the international community of researchers, developers and innovators.

LE STUDIUM IAS offers to internationally competitive senior research scientists the opportunity to discover and work in one of the IAS's affiliate laboratories from the University of Tours, the University of Orleans, National Institute of Applied Sciences (INSA) Centre Val de Loire and ESAD Orléans, as well as of nationally accredited research institutions located in the region Centre-Val de Loire (BRGM, CEA, CNRS, INSERM, INRAE). Our goal is to develop and nurture trans-disciplinary approaches as innovative tools for addressing some of the key scientific, socio-economic and cultural questions of the 21st century. We also encourage researchers' interactions with industry via the IAS's links with Poles of Competitiveness, Clusters, Technopoles, and Chambers of Commerce etc.

LE STUDIUM has attracted two hundred and thirty experienced researchers coming from 47 countries for long-term residencies. In addition to their contribution in their host laboratories, researchers participate in the scientific life of the IAS through attendance at monthly interdisciplinary meetings called LE STUDIUM THURSDAYS. Their presentations and debates enrich the regional scientific community at large (researchers of public and private laboratories, PhD students, research stakeholders' representatives, etc...)

For the period 2015-2021, LE STUDIUM operates with an additional award from the European Commission in the framework of the Marie Skłodowska-Curie Actions (MSCA)-COFUND programme for the mobility of researchers. Since 2013, LE STUDIUM is also an official partner of the Ambition Research and Development 2020 programmes initiated by the Centre-Val de Loire Regional Council to support the smart specialisation strategy (S3) around 5 main axes: biopharmaceuticals, renewable energies, cosmetics, environmental metrology and natural and cultural heritage. New programmes are currently designed to include all major societal challenges.Researchers are also invited and supported by the IAS to organise, during their residency and in collaboration with their host laboratory, a two-day LE STUDIUM CONFERENCE. It provides them with the opportunity to invite internationally renowned researchers to a cross-disciplinary conference, on a topical issue, to examine progress, discuss future studies and strategies to stimulate advances and practical applications in the chosen field. The invited participants are expected to attend for the duration of the conference and contribute to the intellectual exchange. Past experience has shown that these conditions facilitate the development or extension of existing collaborations and enable the creation of productive new research networks.

The present LE STUDIUM CONFERENCE named is "*Innate immunity in a biomineralized context: trade-offs or synergies?*" the 109th in a series started at the end of 2010 listed at the end of this booklet.

We thank you for your participation and wish you an interesting and intellectually stimulating conference. Also, we hope that scientific exchanges and interactions taking place during this conference will bring opportunities to start a productive professional relationship with presenting research laboratories and LE STUDIUM Loire Valley Institute for Advanced Studies.

Yves-Michel GINOT

Chairman
LE STUDIUM

INTRODUCTION

Biomineralized structures can function as a barrier to the external environment, and as such are conceptually entwined with innate immune processes. Disentangling immune and biomineralization mechanisms represents a significant challenge for researchers trying to understand how organisms could integrate biomineral formation and plasticity with maintenance of critical innate immune protection. In fact, there is increasing evidence that immune proteins can serve genuine dual-functional roles, both in regulation of biomineralization, as well as resisting pathogens. This awareness is growing in models as diverse as the dual-functionning haemocytes of marine bivalves, and in mineralization / demineralization of the avian eggshell. Moreover, reef corals, in which calcification is coupled to the photosynthetic activity of their mutualistic symbiots, must adapt their innate immune system to achieve this tolerance. Cnidarian immune-related processes in response to abiotic stresses are increasingly implicated in loss of symbiosis and coral bleaching.

This conference aims to bring together scientists working with diverse models of biomineralization, for an exchange of perspectives on the innate immune function of the calcified barrier and the dual role played by specific genes / proteins in these two critical functions.

PROGRAMME

TUESDAY 23RD MARCH 2021 - 13:00 - 18:10 (GMT+1:00 - PARIS)

13:00 Official Opening
Sophie Gabillet (Le Studium Loire Valley Institute for Advanced Studies - France)

13:20 Prof. Maxwell Hincke & Dr Sophie Réhault-Godbert - Introduction: Dynamics of Structural Barriers and Innate Immune Components

SESSION 1: OVERVIEW OF INNATE IMMUNITY

13:30 Prof. Bernd Kaspers - The developing immune system of the chicken embryo

14:00 Prof. Marc McKee - The structure of avian (chicken) eggshell

14:30 Break (10 minutes)

SESSION 2: AVIAN EGGS: STRUCTURE, MICROBIOTA AND MOLECULES OF INNATE IMMUNITY

14:40 Prof. Alejandro Rodriguez Navarro - Mechanisms of eggshell biomineralization

15:00 Dr Liliana D'Alba - Eggshell mineralization in relation to nesting ecology in reptiles

15:20 Dr Sophie Réhault-Godbert - The eggshell microbiome

15:40 Dr Ian Dunn - The genetics and function of the cuticle, the eggs antimicrobial outer barrier

16:00 Prof. Maxwell Hincke - The chroriallantoic membrane: insight from proteomics.

16:20 Dr Nicolas Guyot - Phylogenetic and structural evolution of egg antimicrobial proteins and peptides

16:40 ***Flash posters presentations:***
Maeva Halgrain - Deciphering the role of the chorioallantoic membrane in eggshell decalcification during chicken embryonic development.

Dr Garima Kulshreshtha- Food safety of table eggs is modified by bird environment and behaviour as reflected by eggshell cuticle quality.

16:50 Break

SESSION 3: BONE

17:00 Dr Claudine Blin - The innate immune function and diversity of osteoclasts

17:20 Dr Natalie Reznikov - Application of deep learning for segmentation of 3D images in biomineralization research

SESSION 4: INTEGRATIVE WORKSHOP

17:40 Dr Joël Gautron - Avian eggshell biomineralization and innate immunity

18:10 End of the first day

WEDNESDAY 24TH MARCH 2021 - 13:30 - 16:45(GMT+1:00 - PARIS)

13:30 Prof. Maxwell Hincke & Dr Sophie Réhault-Godbert - Introduction to the second day

SESSION 5: THEME INVERTEBRATES (BIVALVES, SNAILS)

13:40 Prof Inna M. Sokolova - Multifunctionality of bivalve hemocytes: A potential source of trade-off between immunity and biomineralization?

14:00 Dr Frédéric Marin - Mollusk shell matrices: unexpected functions in biomineralization

14:20 Dr Sophie Berland - Probing the mechanical properties and biochemical defence offered by shell matrix proteins in bivalves

14:40 Dr Christine Paillard - The Brown Ring disease in clams, a double-edged defense mechanism for shell disease recovery!

15:00 Dr Robbie Rae - Biological armour used to kill parasites

15:20 *Flash posters presentations:* Dr Liu Chuang - Proteomics of shell matrix proteins from the cuttlefish bone reveals unique evolution for cephalopod biomineralization

15:25 Break (20 minutes)

SESSION 6: THEME CORALS

15:45 Dr Nikki Traylor-Knowles - Coral eco-immunity in a disease landscape of unknowns

16:05 Dr Jeroen Van de Water - Host-microbe interactions in octocoral holobionts

SESSION 7: INTEGRATIVE WORKSHOP

16:25 Prof. Marc McKee & Prof. Maxwell Hincke - Lessons learned and the path forward for innate immunity in biomineralization

16:45 End of the conference

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CONVENORS

SPEAKERS

CONVENORS

Prof. Maxwell Hincke

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Dr. Maxwell Hincke (Ph.D. Biochemistry, University of Alberta, Canada). Le Studium Research Professor with Centre INRAE Val de Loire, Université de Tours; Tenured Full Professor, Department of Cellular and Molecular Medicine and Department of Innovation in Medical Education, Faculty of Medicine, University of Ottawa, Canada. International authority on the proteins associated with the eggshell matrix / eggshell membranes and their function in eggshell mineralization and innate immune protection of the egg with over 110 peer-reviewed journal articles. Expert in biomedical applications of products derived from eggshell, eggshell membranes and chicken RBCs. Fundamental studies on eggshell strength and cuticle antimicrobial resistance are also underway.

The chroriallantoic membrane: functional insight from proteomics.

Co-authors : Dr. Tamer Ahmed (University of Ottawa)

In oviparous animals such as birds, embryonic development occurs in the egg; after oviposition, there is no further possibility of material exchange from the hen to fulfill the physiological needs of the embryo. In such a context, the egg must contain all resources required for survival and proper development of a living organism. During embryonic development, the chorioallantoic membrane (CAM) is a placenta-like structure which is the nexus for many different physiological and metabolic processes including acid-base balance, respiration, and calcium solubilization from the eggshell that is re-allocated for bone and tissue formation. The highly vascularized CAM occupies a strategic position, as it forms a lining under the eggshell and surrounds the embryo from ED12 onwards. The cellular and genetic bases for its protective mechanisms remain to be fully elucidated. One approach to understanding the functions of the CAM is to identify its protein constituents and how they change during development. In this study, we have characterized the CAM proteome at two stages of development (ED12 and ED19), and assessed the embryonic blood serum proteome to determine its contribution. LC/MS/MS-based proteomics allowed the identification of 1470, 1445, and 791 proteins in CAM (ED12), CAM (ED19), and embryonic blood serum (EBS), respectively. In total, 1796 proteins were identified in the entire study. Of these, 175 (ED12), 177 (ED19), and 105 (EBS) were specific to these stages / compartments. These protein constituents have been interpreted in the context of CAM functions, including Ca2+ solubilization / transport and protection against invading pathogens.

Dr Sophie Réhault-Godbert

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Sophie Réhault-Godbert is a protein biochemist working as a scientist in the team "Défenses de l'Oeuf, Valorisation, Evolution" at the "Institut National de la Recherche pour l'Agriculture, l'Alimentation et l'Environnement", France. The aim of her study is to explore the physiological functions of egg proteins and more specifically their contribution to egg defenses. She possesses an expertise in the physiology of chicken egg formation, in egg innate immunity and in the functional and structural characterization of egg proteins including proteases, antiproteases and antimicrobials. She is currently conducting researches on the role and the regulation of extraembryonic structures in egg defenses during chicken embryonic development. She authored 30 peer-reviewed articles, 8 book chapters, and holds two patents in the field of Egg Science.

The eggshell microbiome

Avian eggs possess very efficient and orchestrated systems to protect the embryo during incubation, until hatch. Although the internal components of the egg are assumed to be sterile, the surface of the eggshell is covered by microbes (essentially bacteria) that may contribute to prevent eggshell colonization by pathogenic bacteria, through direct inhibition and/or competitive exclusion. The composition of the eggshell microbiome is a heritage from both maternal microbiota (caeca/faeces) where the egg meets caecal secretions in the cloacal segment during oviposition, and from the nesting environment (contaminated litter/feathers and air environment including dust). At laying time, the egg surface is still moisturized, but will progressively dehydrate during incubation. The surface characteristics of the egg, the loss of the moisture layer and the presence of antimicrobial molecules composing the cuticle are likely to dictate the bacterial communities that will survive on the surface of the eggshell. Although the literature on the composition of the eggshell microbiome of eggs originating from current commercial chicken hens is quite sporadic, this talk aims to provide an overview of the bacterial communities that colonize the chicken eggshell surface, and will discuss the role of the eggshell microbiota as the first barrier against pathogenic bacteria.

SPEAKERS

Dr Liliana D'Alba

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Liliana D'Alba holds a M. Res. in Ecology and Environmental Biology and a Ph.D. in Evolutionary Biology from Glasgow University, UK. She currently works as a senior research scientist at the Evolution and Optics of Nanostructures research group at the University of Gent. Dr. D'Alba is an Integrative Biologist whose main research foci include: the production mechanisms and evolution of animal coloration and the functional and evolutionary morphology of vertebrate eggs. Her research integrates field and laboratory methods to answer a broad range of ecological, physiological, behavioral and evolutionary questions about birds and reptiles. She has authored 50 peer-reviewed articles, she has been invited over 15 times to give special talks at international conferences and symposiums and her work has been funded by more than five international research agencies including US-NSF, FWO-Belgium, The European Commission, National Geographic and CONACYT-Mexico.

Eggshell mineralization in relation to nesting ecology in reptiles

Co-authors : Prof. Matthew Shawkey
Department of Biology, EON-unit, Universiteit Gent.

Egg morphology is essential for animal survival, mediating the interactions between embryos and their environment, and a result have evolved into an enormous diversity of forms and functions in modern vertebrates. Reptiles show considerable variation in the degree eggshell calcification, which through evolution shows a tendency to increase. Several hypotheses have been proposed to explain the advantages of intensified mineralization, for example that calcified eggshells increase protection of embryos from mechanical and biological stressors, modulate gas exchange and water physiology or that calcification might be a detoxification mechanism. However, these hypotheses still await experimental validation. Moreover, a vast proportion of reptiles successfully reproduce based on eggshells with incipient or no calcification. Thus, the questions of why the process of increasing calcification of eggshells occurred or about the advantages of shell calcification still remain. In this study I characterized eggshell structure and chemical composition of eggshells of 96 species, and performed comparative phylogenetic analyses to investigate the relationship between life-history, nesting ecology and functional properties on the evolution of calcification of reptile eggshells. The findings show that reptile eggs display larger diversity in egg phenotypes than previously thought and that this diversity is coupled with a large range of functional properties. Calcium content is correlated with adult body size but it seems a poor predictor of its functional performance. At a large scale, the nesting environment seems to play a minor role in determining the level of mineralization of eggshells but specific nesting microclimates might lead to particular calcification patterns. Inferences generated in this project will contribute to increase our understanding about the importance of reptile eggs on vertebrate evolution and diversification.

Dr Sophie Berland

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I took my first degree in Life Sciences and my PhD, on the evolution of Sensory nervous system at the University of Pierre et Marie Curie (Paris). I then joined the National Museum of Natural History for the rest of my career where I am based in the department "Adaptations du Vivant" as a research engineer. Today in the scope of the team "Biodiversity, plasticity, adaptation and conservation, I attempt to unravel features of functional ecology and evolution of biological systems by highlighting adaptive and environmental signatures in biomineralized systems using new spectroscopic or imaging methods and proteomics.

Probing the mechanical properties and biochemical defence offered by shell matrix proteins in bivalves

Co-authors : Dr Arul Marie[1], Dr Jaison Arivalagan[2]

[1]UMR 7245 CNRS/MNHN, Sorbonne Universités, Muséum national d'Histoire naturelle Paris, France

[2]Proteomics Center of Excellence, Northwestern University, Chicago, IL-60611, USA

Calcium carbonate is paired up with shell matrix proteins in the suited organo-mineral outer shell in molluscs. Advanced knowledge in the shell proteome is achieved with the development of proteomics providing insights in the biological control of biomineralization at the shell side. Four bivalve species of distant kinship were subjected to proteomics for sequence and function pattern analysis of their retrieved shell matrix proteins. Domains ruling for calcification mechanisms, e.g. carbonic anhydrase, chitin binding and tyrosinase remained common in all the species signing sustained calcifying control proteins. Other shell proteins were fitted with functions beyond mineralization of which with relationship to immunity and especially the phenoloxidase pathway.

Some populations of M. edulis, have adapted to the low saline Baltic Sea conditions, resulting in peculiar phenotype towards shell dawrfism with oversize periostracum, increased organic content and disorganized mineralized layers. The shell proteome was analysed with focus on the modulation of the shell proteins as a meaning of adaptive response to unbalanced conditions for shell calcification. Interestingly, proteins with immunity-related domains appeared modulated.

Provided the hypothesis that the shells have embraced evenly aspects of inheritance and adaptive response, decrypting this molecular information is critical to understand the biomineralization adaptation demands and to identify calcifyers populations resilience as well.

Dr Claudine Blin

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Claudine Blin is research director and head of the team "Osteoimmunology, Niches and Inflammation" at the Laboratory of Molecular Physiomedicine, Nice, France. She is also secretary of the French Society for Mineral Tissue Biology (SFBTM). After a PhD in molecular endocrinology and a postdoc on homeogenes and craniofacial development, she moved to Nice in 2000 for a second postdoc on osteopetrosis. She developed her research group on osteoimmunology that allows her nomination as research associate in 2005 and research director in 2012. Her projects are at the interface between osteology, immunology and stem cell biology and focus more specifically on the in-depth characterization of the heterogeneity of osteoclasts in terms of origin, phenotype and innate immune function, on understanding their role in inflammation and targeting specific osteoclast subsets for therapeutic strategies for inflammatory bone destruction.

The innate immune function and diversity of osteoclasts

Osteoclasts are the cells responsible for bone resorption in steady state and bone destruction in chronic inflammatory diseases and osteoporosis. Up to recently, they have been considered only as a single population of bone-resorbing cells whose differentiation and activity are increasing in pathologies associated with bone destruction. However, recent data demonstrated that besides bone resorption, osteoclasts are innate immune cells. In particular, they present antigens and activate T cell responses towards tolerance in steady state. Moreover, they are also able to stimulate inflammatory T cells in the context of chronic inflammation. Using RNAseq on purified mature osteoclasts, we showed that these divergent immune effects are related to functionally and transcriptionally distinct subsets of osteoclasts. Therefore, bone destruction not only relies on an increased number of osteoclasts but also on the emergence of different osteoclast subsets having opposite immune outcomes. Taking advantage of the immune characteristics of these different subsets and in particular their different capacity to respond to danger signals arising from gut dysbiosis, we could specifically block the differentiation of inflammatory osteoclasts and reduced bone destruction in ovariectomized mice. These new data on the diversity and innate immune function of osteoclasts open very novel perspectives for fighting against inflammatory bone destruction.

Prof. Ian Dunn

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I have worked in poultry science for over 40 years, starting on laying hen urolithiasis and deep pectoral myopathy. With the growth of molecular biology I moved into the genetic and genomic age, understanding the neuroendocrine control of reproduction and later moving into more classical quantitative genetics to locate chromosomal regions controlling traits including egg quality, broodiness and bone strength. This has continued and I have applied these skills to reproduction but particularly egg quality and osteoporosis in close collaboration with poultry breeders. This has led to new selection tools such as dynamic stiffness and a greater understanding of egg biology. I have authored around 96 publications and I lead a group of researchers on aspects of poultry research.

The genetics and function of the cuticle, the eggs antimicrobial outer barrier.

Avian eggs have a proteinaceous cuticle covering the outside of the eggshell forming a barrier to the transmission of microorganisms. Although the cuticle is similar to the organic matrix, its secretion is separate from the organic matrix and occurs just prior to oviposition. Using NGS sequence it was possible to investigate genes that might be involved in cuticle deposition, which suggested that an endogenous clock may control events in the uterus, including cuticle deposition. There is considerable variation within breeds of chicken and indeed between species in the quantity of cuticle deposition. Around 40% of the variation within chicken breeds is genetically determined. Environmental stressors explain some of the variation but the remainder is not known. Within the range of natural variation there are considerable differences in the antimicrobial protection that the cuticle affords the egg. The eggs with the best cuticle are not penetrated when challenged by E.coli and Salmonella. Interestingly, although the cuticle protein had intrinsic antimicrobial activity, we could not prove that the glycosylation level was important for this activity. The cuticle is a fascinating structure with antimicrobial effects but also on the shell, which has been rarely studied.

Dr Joël Gautron

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I'm research director and co-leader of the team "Egg defenses, enhancement, evolution". My research focuses on biochemistry and molecular biology related to the formation of eggs and their qualities. I worked on the formation of shells and the identification of proteins from the organic matrix of the eggshell, involved in the calcification process and coordinated several national and international research programs. My research also focuses on the high throughput methodologies used to identify and characterize the biological activities linked to the egg's natural defenses. I have published more than 70 publications in international scientific journals, 12 book chapters, and have been invited to present my results at more than 30 congresses and I count about 120 papers at conferences.

Avian eggshell biomineralization and innate immunity

Dr Nicolas Guyot

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Nicolas Guyot is a research scientist at the French Research Institute for Agriculture, Food and Environment (INRAe) and conducts his research in the UMR Avian Biology and Poultry Research in Nouzilly, France. He completed his PhD in 2005 at the University of Tours after working on the biochemical characterization of recombinant protein inhibitors targeting the neutrophil serine proteases. From 2006 to 2010, he worked as a postdoc fellow on topics related to proteases and antiproteases in lung inflammation, successively at the Royal College of Surgeons in Ireland in Dublin and at the University of Reims Champagne-Ardenne. Since his recruitment at INRAe in 2010, he is developing research projects on the functional and structural characterization of egg antimicrobial peptides and proteins, and on the regulation of egg antibacterial activities induced by the hen physiology or by the egg environment.

Phylogenetic and structural evolution of egg antimicrobial proteins and peptides

The avian egg is designed to support the autonomous development of the chick embryo in the outside environment. It initially contains all the nutrients required for embryonic growth and provides efficient physical and immune protections primarily via the calcified eggshell and the numerous antimicrobial proteins and peptides. The antimicrobial content of eggs has been shaped during evolution to cope with the microbial pressure and potential contaminations. These antimicrobials are present within all parts of the egg, although they are mostly abundant and active in the egg white and in the perivitelline layer. Egg antimicrobial molecules show high structural and functional diversity, displaying bactericidal or bacteriostatic activities via different but complementary mechanisms (e.g. interaction with bacterial cells which further triggers permeabilization and bacterial death, decrease in the bioavailability of nutrients required for microbial growth, inhibition of microbial proteases involved in virulence). Interestingly, some of these antimicrobials lack orthologs in mammals and are specifically deposited in eggs by the hen during egg formation, which suggests that they have a specific role in the innate immune protection of the avian egg, or even other biological functions related to avian reproduction. The presentation will mainly focus on a selected panel of relevant egg antimicrobial proteins and peptides with a particular emphasis on their structural and phylogenetic features.

Prof. Bernd Kaspers

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Received his doctoral degree in 1989. In 1991 and 1992, he worked as a postdoctoral fellow at the USDA, Beltsville, MD, where he focused on the immune response to coccidosis. Since 1997 he is a Professor for Animal Physiology at the University of Munich. He has more than 30 years of research experience in avian immunology and published more than 100 peer-reviewed publications. He is co-editor of "Avian Immunology" published in a 2nd edition in 2014 and hosted the 2004 and 2016 Avian Immunology Research Group meetings. His work involves research on the innate and adaptive arms of the avian immune system with a particular focus on host-pathogen interaction, immune system maturation and mucosal immunity.

The developing immune system of the chicken embryo

Development of hematopoietic cells has been investigated intensively in the chicken embryo. Early hematopoietic stem cells develop in the aortic region and seed to primary lymphoid organs where they mature into T- and B-lymphocytes. Subsequently, they start to colonize the periphery at embryonic day 15/19 and around hatch, respectively. Monoclonal antibodies, recombinant cytokines and new in vivo technologies were instrumental to dissect these pathways. For example, bursal development is dependent on B cell activating factor of the TNF family (BAFF) as shown by retroviral mediated overexpression of BAFF or its decoy receptor (BCMA). This technology may be useful to study other regulatory systems including chemokines which media targeted migration during haematopoiesis. Using small soluble inhibitors, we recently could show that the chemokine receptor CXCR4 and its ligand CXCL12 are essential for the colonization of the bursa anlage by B-cell precursors. Microarray studies of the developing bursa identified a range of additional cytokines and chemokines, which may play an important role at distinct developmental stages. Amongst them, we found OPG and RANKL, which have been cloned in the chicken and would be available for future studies not only in B-cell but also in bone biology. Further progress came with the first gene knockout in chickens now enabling phenotypic studies and rescue experiments to better understand the functional role of such factors.

Dr Frédéric Marin

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I received a BSc in fundamental geology (1987), a Master (DEA) in palaeontology (1989) and a PhD in palaeontology in 1992 (Univ. Paris XI). After a 1st post-doc (1993), I spent 6½ years at the Royal University of Leiden, the Netherlands (1994-2000) and 2 years in a private biotech company (Isotis, Bilhoven, The Netherlands), before being recruited by CNRS in Dijon (2003). I obtained my ability to conduct researches in 2009 and became research director at CNRS in 2012. I have piloted different research projects (national, international) and set up from the scratch a laboratory devoted to the study of organic matrices associated to CaCO3 biominerals in metazoans (mollusks, corals, sea urchins...). I have authored/co-authored more than 130 papers in periodicals, proceedings and book chapters.

Mollusk shell matrices: unexpected functions in biomineralization

To construct their skeletons, all metazoans secrete a complex array of macromolecules that are supposed to display key-functions in biomineralization, such as crystal nucleation and crystal growth orientation. These macromolecules - generally less than 1% of the skeletal weight - are occluded during skeletal growth and can be retrieved and analyzed by dissolving the mineral phase. They comprise proteins, glycoproteins, peptides, polysaccharides, and sometimes, lipids, pigments and metabolites. They constitute collectively the 'calcifying matrix', from which proteins and glycoproteins are the most studied.

In the last decade, the coupling of high-throughput screening techniques (transcriptomics + proteomics) has allowed the identification of a large number of proteins of the "skeletal repertoires", in diverse metazoan phyla. To give an idea, in mollusks, more than 1000 proteins are now listed as putative shell proteins, in about 30 different genera.

Proteomic data underline the diversity of these proteins, which goes along with the diversity of functions required for calcifying a skeleton. Beside expected members (acidic proteins, proteins with hydrophobic domains), shell proteomes (aka 'shellomes') reveal a large variety of proteins with very different low complexity domains. Above all, the surprise comes from the discovery of a huge set of proteins involved in immunity and defense mechanisms in general. This last aspect will be particularly discussed in my talk.

Prof. Marc McKee

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Marc D. McKee is a full professor at McGill University in Montreal, Canada Research Chair in Biomineralization, with a joint appointment in Dentistry and Medicine. He received his Ph.D. from McGill University, followed by postdoctoral training at Harvard / The Children's Hospital Boston, and then held appointments at the Forsyth Institute in Boston and the University of Montreal. His research focuses on biomineralization in bones and teeth, otoconia and eggshells, and in pathologic calcification. With over 238 publications and more than 28,500 citations, and for his accomplishments in biomineralization research, he has received two Distinguished Scientist Awards from the International Association for Dental Research (1996 Young Investigator Award, 2003 Biological Mineralization Award), and an Esteemed Award (2019 Adele L. Boskey Award) from the American Society for Bone and Mineral Research. He is also the recipient of the 2018 C.P. Leblond Award honoring a Quebec scientist for exceptional involvement in bone research.

The structure of avian (chicken) eggshell

The functional properties of biomineralized structures found in Nature result from interactions between their hybrid components – both organic (mostly proteins) and inorganic (mineral) phases – to generate hierarchical organization across different length scales. In its dual function, the calcitic avian eggshell provides a protective barrier for the enclosed developing chick embryo while also serving to provide calcium for the growing chick skeleton by the process of shell dissolution. Here, a detailed structural analysis (including a description of nanostructure) is given for the eggshell produced by the domesticated chicken, along with changes that occur following eggshell dissolution – a shell-thinning and weakening process leading to hatching of the chick (pipping) which occurs after egg fertilization and incubation. Eggshell contains abundant proteins, and the localization of some of these (particularly osteopontin) will be described at the ultrastructural level, and correlated with generating nanostructure and shell hardness. X-ray and electron imaging and diffraction data, together with atomic force microscopy observations, describe an aligned nanostructure of mineral within the shell. A similar nanostructure could be reproduced in synthetic calcite crystals by the simple addition of osteopontin, which becomes occluded within the calcite to generate this mineralization pattern. Taken together, these findings are consistent with this protein's mineral-binding and regulatory role in biomineralization in a large number of biological systems, and point to a highly conserved, mineral nanostructure-regulating activity of osteopontin conserved over at least 300 million years of evolution.

Dr Christine Paillard

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Christine Paillard, CNRS Research Director, coordinates a "Environment-Host-Pathogen-Microbiota Interactions" research group within the LEMAR at IUEM-UBO. She is interested in how environmental factors modulate host-host-pathogen interactions, with clams and abalones interacting with vibrios as the main model organisms; her interests include ecophysiology, host-pathogen coevolutionary arm race, immunology and microbiology. C. Paillard discovered Vibrio tapetis as the bacterial agent responsible for the shell disease Brown Ring Disease in clams. She is studying the adaptations of molluscs to temperature and acidification by combining integrative studies. In the clam model, she is currently studying how the microbiota in relation to immune defence could interfere with shell repair processes.

The Brown Ring disease in clams, a double-edged defense mechanism for shell disease recovery!

Brown Ring disease is caused by Vibrio tapetis, which, by adhering to newly secreted shell matrices, interferes with the biomineralization process. Colonization and alteration of the matrices induce the deposit of conchiolin on the inner surface of the valves, forming a characteristic brown deposit instead of contributing to shell growth. This defense mechanism consists firstly in coating the bacteria within the organic layers of melanized matrices, and secondly in covering the brown deposit with shell layers to achieve complete healing of the shell. This defense phenotype varies greatly depending on, (1) the host physiology, its immune response and its microbiota, (2) the pathogen virulence and its capacity to modulate shell fluid pH, and (3) the environmental conditions. The first barriers V. tapetis faces, the mantle epithelium and the extrapallial fluids, react rapidly and hemocytosis and the activation of enzymes. This early immune response does not induce a decrease of host physiological parameters, suggesting a commensal relationship. On the other hand, in advanced stages of disease or incomplete recovery, the microparasite induces immunodepression and interrupted shell growth, clearly showing trade-offs between immune defense and biomineralization processes. Thus, in the context of Brown Ring Disease, immunity and shell repair processes interact closely either in synergy or as trade-offs depending on their position along the mutualism-parasitism interaction gradient.

Dr Robbie Rae

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I studied at the University of Aberdeen for my PhD, then moved to the Max Planck Institute for Developmental Biology in Tuebingen for my postdoc. I began at LJMU in 2013 and recently became Reader in Ecological Genetics in 2020. My research is focused on studying parasitic nematodes (Phasmarhabditis hermaphrodita) and their interactions with pestiferous gastropods. This nematode has been formulated as a biological control agent (called Nemaslug®) available across northern Europe. I am interested in how it evolved to be the only nematode out the entire phylum Nematoda able to kill slugs and snails. To do this I study natural variation in virulence as well as their host finding behaviour and we have begun using genomics and transcriptomics to elucidate the associated molecular mechanisms.

Biological armour used to kill parasites

The co-evolution of parasites and hosts has shaped the immune system. One such 'arms race' is between parasitic nematodes and their molluscan hosts. One such species (Phasmarhabditis hermaphrodita) can infect and kill several gastropod species and has been formulated into a biological control agent (Nemaslug®) for farmers and gardeners. In order to defend themselves from P. hermaphrodita infection we have recently found that several snail species encapsulate and kill nematodes by producing unknown cells which fuse them to the inner part of the shell. This is a rapid process occurring over 48 hours and can encapsulate hundreds of nematodes at once. Field studies have shown this is a common occurrence in snail shells collected across the U.K. and in northern Europe. By viewing conchology collections at Liverpool and Manchester museums we have shown this immune response is highly evolutionary conserved and the nematodes encapsulated and fused to the inner shell and will remain there for hundreds of years. This could allow for identification and understanding of the diversity of nematode species encased in the shells from hundreds, even thousands of years. We are pursing this idea as well as investigating the utility of micro CT scanning to be used to observe nematodes in snail shells. Also we have been using genetics and genomics to understand the diversity of nematodes fixed in shells. Our ultimate aim is to unravel how the snails' shell has been co-opted to kill parasites.

Dr Natalie Reznikov

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Dr. Natalie Reznikov is an Assistant Professor in the Department of Bioengineering at McGill University, Canada. She holds a PhD from The Weizmann Institute of Science in Israel. She has studied the architecture of bone using 3D tomographic imaging methods that span size scales ranging from atoms to entire skeletons. Currently, her research focuses on biomineralization, structure-function relationships in diverse biological materials, 3D imaging and image analysis. She closely collaborates with Object Research Systems Inc. (Montréal) whose product is DragonflyTM software for multimodal tomography and advanced image analysis.

Application of deep learning for segmentation of 3D images in biomineralization research

Co-authors : Dr. Nicolas Piché

Object Research Systems Inc., Montréal, Québec, Canada

Modern 3D imaging methods in biomineralization – such as X-ray tomography and dual-beam electron tomography – produce datasets that are rich in fine detail and enormous in size, often containing inevitable artifacts. Rendering segmentations of such datasets is a daunting task. The recent introduction of artificial neural network-based deep learning into bioimaging has made 3D segmentation reliable, accurate and fast. A highlight of convolutional neural networks (CNNs) is that artificial "neurons" are interlinked hierarchically, similarly to how feature-forming patterns of an image are related. Accordingly, when a raw image is presented to a deep net, the neurons of different layers perceive the patterns of different complexity. Upper-level neurons detect small patterns within their local context, and the local context itself forms patterns for deeper neuronal layers, and within a larger context, and so on. Thus, identification of features based on overt (e.g. contrast, gradient) and covert patterns (e.g. level of noise, wavelet frequency) becomes not only accurate, but also generalizable. Once image patterns can be accurately enough identified as being features of interest – and thus the CNN is "trained" – such patterns can be segmented automatically on any similar image. In machine learning, as in biological learning, the accuracy of pattern detection and classification improves with experience. Once trained, a CNN can be treated like an image filter – easy to preview, fast to apply, simple to share, and handy to reuse. In this presentation, I will explain the essence of deep learning and CNN operation for non-computer scientists, and will illustrate this with examples of "difficult" 3D images (a chick embryo inside a fertilized egg, and coral).

Prof. Alejandro Rodriguez Navarro

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Dr. Alejandro Rodríguez Navarro is Full Professor at Departamento de Mineralogía y Petrología from Universidad de Granada, Spain. He has an expertise in X-ray diffraction and other analytical techniques (electron microscopy, infrared and Raman spectroscopies). His research interests are focused on understanding how organisms control the mineralization of tissues (eggshell, bone and mollusk shell) and how genetics and environmental factors (climate change, pollution) affect tissue calcification, structural organization and chemistry. Other interests are egg and bone quality in laying hens. He has over 100 publications on peer review journals and has developed an analytical software for 2D X-ray diffraction (XRD2DScan software, Malvern-PANalytical).

Mechanisms of eggshell biomineralization

The avian eggshell is a thin mineral layer (350 µm thick in chicken) that protects the egg content against mechanical impacts, dehydration and microorganism contamination. Eggshell formation is a highly controlled and rapid mineralization process occurring while the egg is residing in the uterus during the night. The composition of the uterine fluid changes at each stage of eggshell formation (initiation, linear growth, termination) with the expression of specific proteins that actively regulate calcium carbonate precipitation, selecting the mineral phase to form (calcite), inducing the nucleation of crystals and controlling their growth, morphology and size. Eggshell formation is terminated with the deposition of the cuticle about 2 hours before oviposition. Egg laying is highly demanding and female birds have developed specific physiological adaptations for it. They need a large and continuous supply of calcium. A stimulated production of vitamin D leads to an increase in calcium absorption by intestinal and uterine tissues. Also, hens develop a new type of bone within the marrow cavities of their long bones (medullary bone) that serves as a calcium reservoir for eggshell calcification during the night when hens are not eating and the intestinal calcium supply is exhausted. The formation and resorption of medullary bone is synchronized with the egg daily cycle. Laying hens also provide enough respiratory CO2 to form carbonate ions needed for eggshell calcium carbonate deposition. We will revise in this presentation the new developments in the study of eggshell structure, formation and quality.

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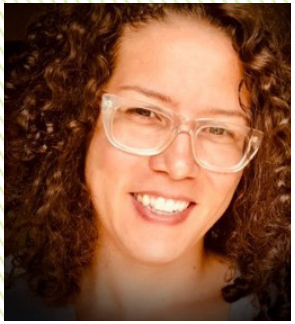
Inna Sokolova is professor and Chair of Marine Biology at the University of Rostock in Germany. Her research focuses on understanding of the physiological mechanisms underlying adaptive and stress responses of marine organisms to climate change drivers, ocean acidification, hypoxia and pollution. She uses bioenergetics to understand the impacts of multiple stressors and link the stress-induced shifts in physiological and molecular phenotypes to the ecologically relevant outcomes affecting survival and persistence of marine populations. She has published over 130 papers in peer-reviewed journals and serves as an editor-in-chief for Marine Environmental Research and as a managing editor for Marine Ecology Progress Series, Frontiers in Marine Sciences, and Nature Scientific Reports.

Multifunctionality of bivalve hemocytes: A potential source of trade-off between immunity and biomineralization ?

Molluscan blood cells (hemocytes) are morphologically and functionally cells critically involved in immune defense, wound healing, and biomineralization. The multifunctionality of the hemocytes allows for flexible allocation of this cellular resource towards the immune defense and biomineralization and can lead to trade-off between these functions. Investigation of the molecular specialization of hemocytes from two closely related molluscan species (Crassostrea gigas and Crassostrea virginica) showed that hemocytes of C. virginica (a species that is less pathogen resistant but shows superior mechanical properties of the shell) had higher levels of expression of multiple biomineralization-related genes and contained more intracellular calcium carbonate mineral compared with the more pathogen-resistant C. gigas that builds weaker shells. In contrast, the expression of immune genes was higher in the hemocytes of C. gigas. Taken together, our results show that the species-specific differences in physiology (such as disease resistance and exoskeleton properties) are reflected at the cellular and molecular levels in the differential specialization of hemocytes on potentially competing functions (immunity and biomineralization). Further studies should ascertain whether such trade-off is observed on intraspecific level, e.g. under conditions that require higher investment into biomineralization (such as ocean acidification) or immunity (such as pathogen exposure).



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Dr. Nikki Traylor-Knowles is an Assistant Professor in Marine Biology and Ecology at University of Miami, Rosenstiel School of Marine and Atmospheric Sciences. She received her B.S. and M.S. in Cell and Molecular Biology from Johns Hopkins University, and her Ph.D. in Biology from Boston University. Dr. Traylor-Knowles is passionate about innovative ocean conservation solutions and mentorship of BIPOC. She leads the Cnidarian Immunity Laboratory which investigates the mechanisms of immune function in corals. Her lab is particularly focused on developing innovative actions for saving coral reefs. She also founded the Black Women in Ecology, Evolution and Marine Science and has become an advocate for Black women in science and academia.

Coral eco-immunity in a disease landscape of unknowns

Coral reefs are some of the most economically valuable tropical ecosystems on Earth. Yet they are critically endangered due to anthropogenic climate change and locally induced human stressors such as pollution and fishing damage. Corals, the animals which produce coral reefs, have a diverse, yet poorly understood immune system that responds to a many different stressors. In this presentation, we will discuss what is understood about the coral immune system from a gene to cell level, and present hypothesizes for the interaction of the coral immune system with the biomineralization system.

Dr Jeroen Van de Water

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Jeroen van de Water is a Research Scientist at the Monaco Scientific Centre. His research aims to understand better the interactions between microbes and corals, and can be divided in two main areas: (1) investigating the structure and regulation of microbial communities associated with corals and elucidating the roles of these symbioses in coral holobiont health and resilience; and (2) examining how environmental change could disrupt these interactions leading to disease. Jeroen obtained his PhD in 2015 from James Cook University and the Australian Institute of Marine Science, with his research addressing the responses employed by corals to environmental, biological and anthropogenic stress. He is currently also involved in projects linking ocean and human health, with a focus on the impacts of coastal urbanization and pollution on marine ecosystems, the associated health risk for humans, and strategies to mitigate diseases.

Host – Microbe Interactions in Octocoral Holobionts

Co-authors : Denis Allemand [1], Christine Ferrier-Pagès [2]

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Octocorals are one of the most ubiquitous benthic organisms in marine ecosystems from the shallow tropics to the Antarctic deep sea, providing habitat for numerous organisms as well as ecosystem services for humans. In contrast to reef-building corals, octocorals generally do not produce calcium carbonate skeletons. Instead, they rely on a hydrostatic or proteinaceous skeleton for support and produce only small fragments of CaCO3, called spicules or sclerites. These spicules have generally been considered to have a deterring effect on predators, but there may also be a role for them in antimicrobial defence. Recent advances have shown that octocorals possess remarkably stable bacterial communities on geographical and temporal scales as well as under environmental stress. This may be the result of their high capacity to regulate their microbiome through the production of antimicrobial and quorum-sensing interfering compounds. Despite these capacities, some octocoral populations have been severely impacted by disease outbreaks. In this talk, I will provide an overview of the current knowledge on octocorals and their interactions with microbes, with an emphasis on one of the few octocorals that actually do make biomineralized CaCO3 skeletons, the precious coral Corallium rubrum. In particular, I will discuss how a few microbial species dominate the octocoral microbiota, the stability of these associations and the co-evolutionary patterns between octocorals and their microbial symbionts that highlight intricate relationships. In addition, I will touch on the subjects of how octocorals use their immune system to fight pathogens, and how not only the octocoral but also its associated microbes may be involved in antimicrobial defence.

POSTERS

• Proteomics of shell matrix proteins from the cuttlefish bone reveals unique evolution for cephalopod biomineralization

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• Deciphering the role of the chorioallantoic membrane in eggshell decalcification during chicken embryonic development

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[4] LE STUDIUM Research Consortium, Loire Valley Institute for Advanced Studies, Orléans-Tours, Nouzilly, France.
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• Food safety of table eggs is modified by bird environment and behaviour as reflected by eggshell cuticle quality

Garima Kulshreshtha[1], Cristina Benavides Reyes[1], Alejandro Rodriguez Navarro [1], Maxwell Hincke[2]
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2021

Dr Rebecca Tharme & Prof. Karl Matthias Wantzen
Managing riverscapes and flow regimes for biocultural diversity
20-21 January 2021

2020

Dr Magdalena Malinowska & Dr Arnaud Lanoue
Exploring the molecular diversity of grape, a source of natural ingredients
3 December 2020

Dr Jean-François Deluchey & Prof. Nathalie Champroux
What are our lives worth to a neoliberal government?
Capitalism, War and Biopolitics in the Pandemic Era
18 - 19 November 2020

Prof. Pieter Hiemstra & Dr Mustapha Si-Tahar
Novel host- and microbiota-directed strategies for treating respiratory infections
24 - 25 September 2020

Dr Emilio Maria Sanfilippo & Xavier Rodier
FAIR Heritage: Digital Methods, Scholarly Editing and Tools for Cultural and Natural Heritage
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Towards Futuristic Energy Storage; paving its way through Supercapacitors, Li-ion batteries and beyond
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2019 International Conference on Fractional Calculus Theory and Applications (ICFCTA 2019)
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On the Ruins and Margins of European Identity in Cinema: European Identity in the Era of Mass Migration
2-3 April 2019

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Time and Science in the Liber Floridus of Lambert of Saint-Omer
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Adapting forest ecosystems and wood products to biotic and abiotic stress
12 - 15 March 2019

Dr Vincent Courdavault & Prof. Nathalie Guivarc'h
Refactoring Monoterpenoid Indole Alkaloid Biosynthesis in Microbial Cell Factories (MIAMi)
5-6 February 2019

Dr Denis Reis de Assis & Prof. Hélène Blasco
Induced Pluripotent Stem Cells (iPSCs): From Disease Models to Mini-Organs
28-30 January 2019

2018

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Water micropollutants: from detection to removal
26-28 November 2018

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Balance laws in fluid mechanics, geophysics, biology (theory, computation, and application)
19-21 November 2018

Dr Volodymyr Sukach & Prof. Isabelle Gillaizeau
Progress in Organofluorine Chemistry
15-17 October 2018

Jens Christian Moesgaard, Prof. Marc Bompaire, Bruno Foucray & Dr Guillaume Sarah
Coins and currency in the 10th and 11th centuries: issuing authorities, political powers, economic influences
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Dr Renaud Adam & Prof. Chiara Lastraioli
Lost in Renaissance
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Advancing Chemistry through Bilateral Collaboration
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12 April 2015

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Loire Valley Workshop on Conformal Methods in Analysis, Random Structures & Dynamics
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