Book of abstract-17th International symposium on Biomineralization, Saint Etienne, France, August 28th to September 1st

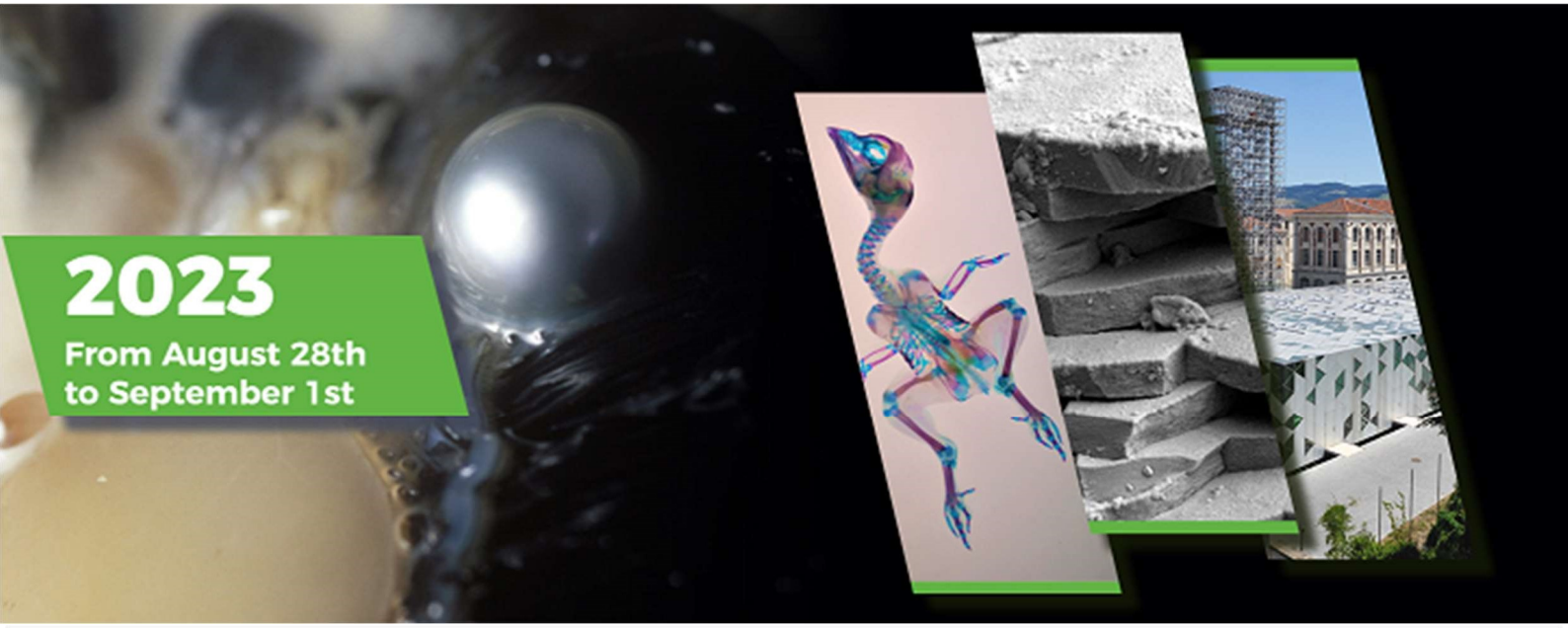
Joël Gautron, Marthe Rousseau

17th International Symposium on Biomineralization

BIOMIN XVII

Saint-Étienne, France

BOOK OF ABSTRACTS



Venue

Faculty of medicine
Jean Monnet University
Saint Etienne, France

Date

28th August
1st September
2023

Université Jean Monnet Saint-Étienne

Faculté de Médecine Jacques Lisfranc • Saint-Étienne

Welcome note of the conference chairs

It is our great pleasure to welcome you to Saint Etienne to join us at the 17th International Symposium on Biomineralization (BIOMIN XVII).

Since 1970, year of the first symposium on biomineralization organized in Germany, the biomineralization community has been making great advances in different aspects of biomineralization, including the hierarchical structures of biominerals and their development, the important genes and functional proteins of biomineralization, the mechanisms of biomineralization in living systems and biomimetic mineralization, the discovery of non-classical crystallization pathways, and the fabrication of advanced functional materials inspired by biomineralization.

The knowledge gained in biomineralization has been applied in hard tissue repair, pharmaceutical, agricultural, energy and environmental sciences, paleontology, and materials-assisted artificial life systems. Biomineralization becomes an important resource for developing new structured materials and solving health-care problems. We believe that we are standing on the historic stage to link the fundamental studies of biomineralization to the challenging global problems.

This biannual conference has been organized in many countries in the world, but only once in a French speaking place, Monaco in 1993. It is time to organize it in France.

Due to the pandemic the BIOMIN XVI edition has been organized virtually by our Chinese colleagues in august 2021.

It is a great pleasure to welcome you to Saint Etienne to have the opportunity to meet face-to-face again

Marthe Rouseau

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Joël Gautron

Organization

Venue Faculty of medicine, Jean Monnet University
10 Rue de la Marandière,
42270 Saint-Priest-en-Jarez
France

Conference Website https://www.biomin2023.com/en/page/welcome/

Conference chairs

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C. BLIN: Université de Nice
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Odile SABIDO, Saint Etienne

The detailed program

Monday 28 August 2023

11-00 - 14:00 **Registration and poster set-up**

13:45 - 14:45 **Opening Session**

13:45 Opening and welcome
Marthe Rousseau and Joël Gautron cochairs of the conference
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14:15 Opening keynote Lecture
Steve Weiner (Weizmann Institute of Science, Israël)
Future advances and perspectives in biomineralization research

14:45 - 18:00 **Topic 1 Fundamental of biomineralization (Chairs N. Kroger and T. Mass)**

14:45 **Topic 1 Keynote lecture**
Stephan Wolf (Friedrich-Alexander-University, Erlangen-Nuremberg, Germany)
Calcareous Mineral Formation — from complex solutes to graded structures

15:15 (T1 O1) A. I. Arns, D. Evans, R. Schiebel, A. Jantschke, S. E. Wolf, G. H. Haug
bridging biomineralisation fundamentals to paleoclimate reconstructions

15:30 (T1 O2) K. Benzerara, J. Gaëtan, G. Gaschignard,C. Mangin , M. A. Khan, N. Mehta
The biomineralization of intracellular amorphous carbonates by bacteria

15:45 (T1 O3) H. Ehrlich
Insights into Biosilicification: how does sophisticated biological glass grow

16:00 (T1 O4) S. Milita, T. Zaquin, S. Fermani, D. Montroni, I. Pinkas, L. Barba, G. Falini, T. Mass
Assembly of the intraskeletal coral organic matrix during calcium carbonate formation

16:15 - 17:00 **Coffee break and posters**

17:00 (T1 O5) C.A. Schmidt, A. Hopanchuk, E. Tambutté, S. Tambutté, P. Gilbert
Amorphous and crystalline precursors to coral skeleton formation in acidifying oceans

17:15 (T1 O6) N. Kroger, C. Heinze, I. Babenko, F. Benjamin
The molecular basis for pore pattern morphogenesis in diatom silica

17:30 (T1 O7) M.L. Lemloh
Diversity and function of biomineralized materials in ciliates

17:45 (T1 O8) J. Tyska, J. Golen, U. Bickmeyer, J. Bijma, K. Godos, Y. Nagai, T. Toyofuku
Organic architecture of chamber biomineralization in main foraminiferal calcifiers

18:00 - 20:00 **Welcome Cocktail**

Tuesday 29 August 2023

8:15 - 12:45 **Topic 2 (Session 1) Biomineral structures and crystallization mechanisms (Chairs S. Wolf and A. Checa)**

8:15 **Topic 2 Keynote lecture**
Helmut Cölfen (University of Konstanz, Germany)
Biomimetic Mineralization to investigate Biomineral structure formation and mineralization mechanisms

8:45 (T2 O1) I. Zlotnikov, D. Karpov
The role of lattice distortions in coccoliths morphogenesis

9:00 (T2 O2) O. Ben Joseph, L. Aram, D. de Haan, K. Rechav, A. Gal
Intracellular biomineralization of rhombohedral calcite by haploid life phases of coccolithophores

9:15 (T2 O3) K. Berent, K. Nalepka, P. Czaja, Ł. Maj, T. Machniewicz, M. Bieda-Niemiec, K. Sztwiertnia, A.G. Checa
Twin-based strengthening mechanism in a crossed-lamellar structure

9:30 (T2 O4) J. Huang, H. Liu
Calcification of the egg capsule in the apple snail: the organic matrix and the calcium-rich granules

9:45 (T2 O5) W. E. G. Müller, X. Wang
Biomimetic transformations of amorphous calcium phosphate to dental and ossifying crystalline hydroxyapatite

10:00 (T2 O6) K. Berent, A.G. Checa
Organization of a plywood biomaterial: the calcitic cross-foliated microstructure of limpets

10:15 - 11:00 **Coffee break and posters**

11:00 (T2 O7) F. Nudelman, A.L. Rossi, F. Laidlaw, G. Graziani, G. Falini
Structural and mechanical adaptation of Lingula anatina shells

11:15 (T2 O8) N. Oehlsen, J. von Döhren, S. Weiner, J. Ibrahim, L. Stegbauer
Stabilization of Amorphous Calcium Phosphate by Barium: A Novel Approach Inspired by a Marine Worm

11:30 (T2 O9) M. de Frutos, A.B. Rodríguez-Navarro, X. Li, A.G. Checa
Monochromated STEM-EELS analysis of the structure and composition of biogenic calcite reveals the biomineral growth pattern

11:45 (T2 O10) T. Okumura, M. Suzuki, T. Kogure
Anisotropic lattice parameters change in aragonite induced by Na+ substitution

12:00 (T2 O11) V. Sardhalia, C. Do Reis Ferreira, S. Amini, P. Fratzl, M. De Frutos, D. Talbot, A. Vallée, A. Fitch, S. Checchia, N. Nassif, T. Azaïs, M. Albéric
Halochromic ACC crystallization in the presence of naphthoquinones unveils biomineral pigmentation in sea urchins

12:15 (T2 O12) C. Grenier, E. Griesshaber, W. Schmahl, B. Bernin, A.G. Checa
Microstructures and biomineralization patterns of gymnolaemate bryozoans (Bryozoa, Gymnolaemata)

12:30 (T2 O13) N. Shaked, S. Barinova, I. Pinkas, S. Addadi, S. Weiner, L. Addadi
Calcite and guanine formation by the freshwater green alga Phacotus lenticularis

12:45 - 14:15 Lunch and poster session

14:15 -15:30 Topic 5 immunity in biomineralized barriers (Chairs S. Réhault-Godbert and M. Mc Kee)

14:15 **Topic 5 Keynote lecture**
Sophie Réhault-Godbert (INRAE-Université de Tours, France)
Dynamics of the chicken chorioallantoic membrane and the eggshell during chicken embryonic development: a fine regulation between eggshell decalcification and maintenance of egg defences

14:45 (T5 O1) C.R. Ferreira, C. Djediat, X. Lekube, U. Izagirre, N. Garcia-Veslaco; Y. Politi, L. Bertinetti, N. Nassif, M. Albéric
Shedding light on the nature of sea urchin pigment-bearing vesicles

15:00 (T5 O2) L. Zorzetto, E. Scoppola, E. Raguin, P.Fratzl, C.M. Bidan
Induced mineralization in E. coli biofilms: the role of extracellular matrix

15:15 (T5 O3) P. Działak, M. Słowakiewicz, A. Borkowski
Morphology and size of mineral particles may be affected by bacteriophages

15:30 -18:00 Topic 8 biomineralization and environmental changes (Chair S. Fitzer)

15:30 **Topic 8 Keynote lecture**
Susan Fitzer (University of Stirling, UK)
Impacts of climate change on mollusc biomineralisation pathways

16:00 (T8 O1) I. Coronado1, J.A. Cruz1,, A. Owczarek, M.Sáenz-Navajas, J.R. Mateos-Carralafuente, P. Cózar, E. Fernández-Martínez, L. Fernández-Díaz, J. Stolarski
Secondary aragonite recrystallization during otoliths diagenesis: natural and experimental evidence

16:15 - 17:00 Coffee break and posters

17:00 (T8 O2) T.Mass, F. Scucchia, P. Zaslansky
Primary coral polyps responses to decreasing seawater pH: observations from cell to the complete organism

17:15 (T8 O3) A. Venn, M. Gilbert, E. Tambutté, S. Tambutté
Investigating the impact of ocean acidification on CaCO3 crystal growth rates in the reef coral Stylophora pistillata

17:30 (T8 O4) D.M. Chevrier, A. Juhin, N. Menguy, R. Bolzoni, P.E.D. Soto-Rodriguez, M. Kojadinovic-Sirinelli, G.A. Paterson, R. Belkhou, W. Williams, F. Skouri-Panet, A. Kosta, H. Le Guenno, E. Pereiro, D. Faivre, K. Benzerara, C.L. Monteil, C.T. Lefevre
Organization and magnetic properties of magnetic ectosymbiotic bacteria optimize a collective magnetotaxis behavior in a microbial holobiont

17:45 (T8 O5) M. Hu
Cellular pH regulatory systems underlying calcification in the sea urchin larva

Wednesday 30 August 2023

8:15 – 12:30 **Topic 4 Imaging methods for biomineralization researches (Chairs A. Guignandon and P. Zaslansky)**

8:15 **Topic 4 Keynote lecture**
Paul Zaslansky (Charité - Universitätsmedizin Berlin, Germany)
A darker side to X-ray brightness - assessing calcium mediated structural degradation during bone studies

8:45 (T4 O1) E. Raguin, R. Weinkamer, P.Fratzl
Dynamic interpretation of the bone mineralization process in the chick embryo based on Cryo FIB-SEM images

9:00 (T4 O2) V. Chamard, T.A. Grünewald, H. Dicko, J. Vidal-Dupiol, B. Petton, J. Legrand, A. Campos, E.Tambutté, A. Venn, S. Tambutté, J. Le Luyer, M. Sztucki, M. Burghammer, J. Duboisset
Towards in vivo imaging of the physico-chemistry processes involved in the biomineralization of mollusc shells

9:15 (T4 O3) C. Wendt, A.L. Rossi, J. Cypriano, C. Dilnei de Castro Oliveira, C. Arrouvel, J. Werckmann, M. Farina
Unique features of Caudofoveata (Mollusca) spicules revealed by combined light and electron microscopy techniques

9:30 (T4 O4) S. Amini, T. Zhu, E. Griesshaber, W.W. Schmahl, P. Werner, P. Fratzl
Uncovering the complex world of elastic deformations in biological ceramics

9:45 (T4 O5) R. Guo, A. Somogyi, D. Bazin, E. Letavernier, E. Tang, K. Medjoubi
Study of the role of trace elements on kidney stones pathogenesis with multi-scale and multimodal scanning X-ray tomography

10:00 (T4 O6) M. Hegedűs, V.K. Kis, Z. Kovács
Quantitative evaluation of enamel prism arrangement based on image processing technique

10:15 - 11:00 **Coffee break, posters and group picture**

11:00 – 12:30 **Topic 7 Biomineralization in aquaculture (Chairs S. Tambutté and M. Suzuki)**

11:00 **Topic 7 Keynote lecture**
Masahiko Awaji (The Graduate School of Agricultural and Life Sciences, the University of Tokyo)
Contribution of Biomineralization Research to the Advancement of Pearl Aquaculture

11:30 (T7 O1) C. Martinand-Mari, E. Potier, E. Gasset, G. Dutto, S. Lallement, C. Bourdy, M. Debiais-Thibaud, E. Farcy
Estradiol and bisphenol A effects on early skeletogenesis in the European sea bass D. labrax

11:45 (T7 O2) P. Ganot, G. Loentgen, F. Marin, L. Plasseraud, D. Allemand, S. Tambutté
An alternative method for extracting the organic matrix from the skeleton of the red coral Corallium rubrum

12:00 (T7 O3) M. Bott, F. Drescher, S. Machill, A. Jantschke
Biogenic organic crystals are solid solutions: HPLC-Quantification of purine-bases

12:15 (T7 O4) Y. Namikawa, M. Suzuki
Carbonic anhydrase activity identified in the powdered nacreous layer of Pinctada fucata

12:30 - 14:30 **Lunch and poster session**
Meeting of the scientific committee

14:30 – 17:45 Topic 6 Biomineralization and biomimetics (Chairs D. Eglin and Y. Ma)

14:30 **Topic 6 Keynote lecture**
David Kisailus (Materials Science and Engineering, University of California at Irvine)
Biological Adaptations and Blueprints for Extreme Environments

15:00 (T6 O1) R. Zarivach
Structure-function studies of intrinsically disordered proteins-iron oxide interaction

15:15 (T6 O2) N. Luo, B. Lu, X. Chen, F. Chen
The calcium phosphate-based biomineral clusters for rapid remineralization of tooth enamel

15:30 (T6 O3) M. Vitori, V. Srot, L. Korat; B. Bussman, F. Predel, Van Aken Peter A, J. Štrus
Structural and mechanical anisotropy in crustacean claws mineralized with amorphous calcium phosphate

15:45 (T6 O4) M. Burgos-Ruiz, K. Elert, C. Rodriguez-Navarro, E. Ruiz-Agudo
Bio-Inspired Fluorescent Consolidants for the Conservation of Gypsum Plasterwork

16:15 - 17:00 Coffee break and posters

17:00 (T6 O5) A. Kubiak, M. Pajewska-Szmyt, M. Kotula, B. Leśniewski, H. Ehrlich
Iron-based biominerals of marine sponge origin as inspiration for biomimetics

17:15 (T6 O6) Y. Ma
Controlled crystallization of guanine nanocrystals

17:30 (T6 O7) P. Biswas
Role of Polyamines in the condensation of silicic acid for efficient silicification and its study with pH

Thursday 31 August 2023

8:30 – 12:30 **Topic 3 Vertebrate mineralized tissues in health and disease (M.H. Lafage-Proust and E. Beniash)**

8:30 **Keynote lecture**
Marc D. McKee (McGill University, Faculty of Dental Medicine and Oral Health Sciences, and Faculty of Medicine and Health Sciences, Montreal, Quebec, Canada)
Enzymatic patterning of bone mineralization at the microscale for crossfibrillar mineral tessellation: The Stenciling Principle

9:00 (T3 O1) H. Yamazaki,C.A. Stifler, C. Gabe, A. Thu Bui, L. Lukashova, K. Verdelis, P.U.P.A. Gilbert, H.C. Margolis, E. Beniash
Biological control of enamel mineralization is dependent on phosphorylation of amelogenin

9:15 (T3 O2) R. Hugon, L. Hivert, N. Boutahar, A. Vanden-Bossche, N. Laroche, M. Thomas, M.T. Linossier, L. Vico, M.H. Lafage-Proust, L. Malaval
Low phosphate diet differentially affects bone mineralization in mice deficient in Bone Sialoprotein, Osteopontin or both

9:30 (T3 O3) M. Duclos, A. Gloux, A. Narcy, J. Gautron
Altered bone health in laying hens is associated with FGF23 overexpression in the medullary bone

9:45 (T3 O4) A. Cohen, L. Gotnayer, S. Gal, D. Aranovich, N. Vidavsky
Multicellular spheroids containing synthetic mineral particles to investigate breast precancer malignancy potential according to the mineral type

10:00 (T3 O5) C. Wendt, A. Linhares, C. Santos, V. Zelaya, R. Lopes, M. Farina, A. Rossi
Ultrastructure of mineral ellipsoids at bone-calcium phosphate interface in bone healing process

10:15 - 11:15 **Coffee break and posters**

11:15 (T3 O6) A. Berdal, S. Renaud, D. Hotton, L. Laurencena, M. De la Dure-Molla, M. MacDougall
The dental and periodontal mineralization defects related to FAM20A gene mutations

11:30 (T3 O7) Y. Bertache-Djenadi, K. Nguyen, A. Vanden-Bossche, M. Thomas, S. Mundweiler, M.T. Linossier, S. Peyroche, D. Farlay, H. Marotte, L. Vico, N. Rochereau, M. Rousseau
Effect of MOP consumption on calcium metabolism and gut in ovariectomized rat model

11:45 (T3 O8) C.M. Gabe, A. Thu Bui, B.P. Vasquez, E. Beniash, H.C. Margolis
Lack of Amelogenin Phosphorylation Leads To Acidification Of Secretory Enamel

12:00 (T3 O9) C. Dittfeld, P. Metzner, M. Feilmeier, A. Jannasch, K. Matschke, S. Manthey, S. Rammelt, S.M. Tugtekin, G. Steiner
FT-IR spectroscopic imaging of human aortic valve biomineralization and protein secondary structure combining hard and freeze cutting technique

12:15 (T3 O10) I.L. Jaabar, B. Foley, A. Mezzetti, F. Pillier, F. Berenbaum, J. Landoulsi, X. Houard
Dynamics of extracellular matrix remodeling associated with mineralization during the hypertrophic differentiation of articular chondrocytes

12:30 - 14:00 **Lunch and poster session**

14:00-18:00 **Discovery cruise to Saint Victor sur Loire**

19:30-00:00 **Gala dinner at Dame d4 café**

Friday 1 september 2023

8:45 – 10:15 **Topic 2 (Session 2) Biomineral structures and crystallization mechanisms (S. Weiner and H. Coelfen)**

8:45 (T2 O14) A. John Samuel, B. Bellec, I. Zlotnikov
The origin and function of geometric frustration in spicule morphogenesis

9:00 (T2 O15) G. Takahashi, T. Okumura, T. Nagaya, M. Suzuki, Y. Takahashi, T. Kogure
Crystallographic Characteristics of Vaterite in Fish Otolith

9:15 (T2 O16) Zhuanfei Liu, Yunya Niu, Zeyao Fu, Mason Dean, Zhengyi Fu, Yongming Hu and Zhaoyong Zou
3D interrelationship between hierarchical canal network and the gradient mineralization of shark tooth osteodentin

9:30 (T2 O17) J. Yang, T. Willhammar, H. Zeng, J.D. Gale, N. Hedin,D. Gebauer, B.Q. Lu
Novel crystalline phases derived from amorphous calcium hydrogen phosphate

9:45 (T2 O18) A.B. Rodríguez Navarro, S. Madero, M. Greiner, P.A. Rodriguez-Jimenez, C. Jimenez-Lopez, W.W. Schmahl
Changes in bone chemistry, mineralogy and structure during heating

10:00 (T2 O19) F. Grosso Giordano, N. De Belie, N. Boon, C. Rodriguez-Navarro
Infrared and Raman spectroscopy for the fingerprinting of microbially-induced calcium carbonate precipitation

10:15 - 11:00 **Coffee break and posters**

11:00 – 11:45 **Topic 9 Evolution of molecular tool kits (Chair F. Marin)**

11:00 **Topic 9 Keynote lecture**
F. Marin (UMR CNRS-EPHE 6282 Biogeosciences, Université de Bourgogne, Dijon)
Few thoughts on the evolution of calcifying matrix proteins in metazoans

11:30 (T9 O1) L. Nicolas , C. Martinand-Mari, M Debiais-Thibaud
The vertebrate skeleton through the eye of a cartilaginous fish: Evolutionary perspectives raised by the study of the small spotted catshark Scyliorhinus canicular

11:45-12:30 **Closing remarks**
Presentation of BIOMIN XVIII

12:30 **Farewell and lunch box**

Abstract of presentations

Opening Session

Keynote lecture

Future advances and perspectives in biomineralization research.

Steve Weiner, Ron Shahar and Lia Addadi

Weizmann Institute of Science, Israël

Advances are almost impossible to predict, but we can present our perspectives on future trends. Technological developments have almost always resulted in major breakthroughs in this field. The huge technological progress in the last decade that enables correlating live optical 3D observations with high resolution 3D cryo-electron imaging, opens many opportunities. In this way cells, extracellular matrices and mineral can be visualized in, or almost in their in vivo state. Furthermore, new insights into the 3D structure of mineralized biological materials can improve understanding of their mechanical function, especially when reconsidering how isotropic or anisotropic the structure is. The structural variations in pathological mineralized tissues can be better related to the genetic mutation(s) causing the pathology and how the function of pathological mineralized tissue affects function.

Marine organisms producing abundant calcium carbonate shells are a crucial link in the reduction of atmospheric carbon dioxide that is in part driving climate change. We need therefore to better understand the basic mechanisms involved in mineral formation of Coccolithophoridae, foraminifera, pteropods and dinoflagellate cysts. This we think will lead to a firmer foundation for predicting how these organisms will respond to climate change. The recent discovery that most single-celled marine organisms tested are producing organic crystals such as guanine, raises the novel question whether these organic crystals are directly or indirectly also involved in calcium carbonate formation in some of these organisms? And in general what are the functions of these organic crystals, especially in light manipulation?

Many ions fulfill fundamental processes in cell metabolism. Much more can be learned about their roles by taking advantage of the ability to cryo-fix cells and tissues, thus preserving the ion distributions in their in vivo contexts. Furthermore by studying the cells and tissues that are concentrating, storing and translocating very large amounts of ions during mineralization, the biomineralization field can contribute significantly to this important topic in cell biology.

But one prediction that we can make with certainty is that the future breakthroughs in the field of biomineralization, will not be predicted!

Keywords: Mineralized collagen, marine carbonates, calcium

Topic 1 Fundamental of Biomineralization

Keynote lecture

Calcareous Mineral Formation — from complex solutes to graded structures

Stephan E. Wolf

Friedrich-Alexander-University Erlangen-Nuremberg (FAU), Department of Materials Science and Engineering (WW), Institute of Glass and Ceramics (WW3) Martensstrasse 5 · 91058 Erlangen · Germany

Regarding quantity, calcareous minerals formed by biological processes are the most abundant biominerals in the Tree of Life. However, the molecular and physicochemical machinery that drives calcareous mineral formation in organisms is not yet fully decoded. Even "simple" calcite prisms formed by the nacroprismatic bivalve *P. nobilis* require an intricate and diversified set of proteins. Remarkably, their prism morphology can be predicted by standard physical models — which allows for the speculation that significant parts of this "shellome" control mineral assembly processes on lower length scales or at earlier stages. In this contribution, we give an overview of nanoscale assembly processes that drive mineral deposition in calcareous shells and exemplify their ameliorating impact on biomineral properties via structural and functional gradation. On an even lower length scale, we further discuss recent advances in our molecular understanding of mineral solutes interacting with organic additives — be the latter peptidomimetics of biomineralization proteins or even additives of small-molecular weight. These molecular interactions operate beyond classical conceptions as they explicitly rely on the involvement of solute oligomers, often denoted as so-called prenucleation clusters. Our findings provide an explanatory basis for the unexplainedly strong impact of specific organic compounds, calling already for a re-evaluation of the putative role of small molecules in biomineralization processes. Moreover, our findings show that these solute oligomers act as gatekeepers for the chemical composition of amorphous calcium carbonate, the common transient mineral precursor in numerous biomineralization processes. Ultimately, the results point to solute oligomers as the binding partners of "calcareous" biomineralization proteins in solution, breaking new grounds for detailed assessment of protein-mineral interactions.

Keywords: mineral formation mechanisms, organo-mineral interactions

Oral presentations

T1 O1

Bridging biomineralisation fundamentals to paleoclimate reconstructions

A. I. Arns[1], D. Evans[2], R. Schiebel[1], A. Jantschke[3], S. E. Wolf[4], and G. H. Haug[1,5]

[1] Max Planck Institute for Chemistry, 55128 Mainz, Germany.[2] School of Ocean and Earth Science, University of Southampton, Southampton, UK, [3] Institute of Geosciences, Johannes Gutenberg University Mainz, 55128 Mainz, Germany. [4] Department of Materials Science and Engineering, Friedrich Alexander University, 91058 Erlangen, Germany. [5] Department of Earth Sciences, ETH Zurich 8092 Zurich, Switzerland.

The biomineralisation of organisms that serve as archives for environmental reconstruction, such as planktic foraminifers, is of particular interest to paleoclimate research. Confident reconstructions over millions of years rely on understanding the mechanistic connection of environmental parameters to trace element and isotopic proxy signals in the shell through biomineralisation, both from a biological and an inorganic perspective.

To explore fundamental abiotic mechanisms, we investigated nucleation and crystallisation processes along with element and isotope fractionation in an in-vitro inorganic experimental model for marine biomineralisation. In this model system, we assume calcification from modified seawater at elevated pH and [CO_3^{2-}], with and without the involvement of metastable phases such as amorphous $CaCO_3$, thereby mimicking processes implied and observed in-vivo.[1-4] We observe that in a complex seawater matrix, the dependencies of prenucleation, nucleation and postnucleation regimes in potentiometric titration experiments on the ionic composition of the solutions, as well as the carbonate phases present, differ compared to previous descriptions in compositionally simpler matrices.[5] Hence, the application of models developed for compositionally simple solutions to carbonate crystallisation processes in seawater is unlikely to yield an accurate description, for example for the presence of transient amorphous phases.[5]

[1] D. Evans, W. Müller, J. Erez, *Geochim. Cosmochim. Acta* **2018**, *236*, 198–217.
[2] J. Erez, *Rev. Mineral. Geochemistry* **2003**, *54*, 115–149.
[3] A. I. Arns, D. Evans, R. Schiebel, L. Fink, M. Mezger, E. Alig, J. Linckens, K. P. Jochum, M. U. Schmidt, A. Jantschke, G. H. Haug, *Geochemistry, Geophys. Geosystems* **2022**, *23*, 1–18.
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Keywords : Carbonate crystallisation, Seawater, Amorphous CaCO3

T1 O2

The biomineralization of intracellular amorphous carbonates by bacteria

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Bacteria forming intracellular amorphous carbonates (iAC) have been increasingly discovered, including diverse Proteobacteria, among which some magnetotactic bacteria, as well as Cyanobacteria [1-2]. The iAC grains can be massive and fill a large proportion of the cell volume. They are found in very diverse environments from marine to freshwater, anoxic sediments, soils or thermal water. Bacteria can form iAC even in aqueous solutions significantly undersaturated with all $CaCO_3$ phases. Sometimes, these bacteria can be locally abundant such as in the case of *Microcystis*, a cosmopolitan bloom-forming cyanobacterium [3]. This suggests a potentially important environmental impact, although efforts to better quantify this are in progress. These biocarbonates can have major and/or trace element composition at odd with what is expected for abiotic carbonates precipitated in the same environment. In particular, one iAC-forming cyanobacterium efficiently sequesters the radioactive ^{226}Ra isotope intracellularly, even in Ra-diluted solutions and in the presence of competing cations such as Ca, Ba and Sr [4]. This may benefit the future development of efficient ^{226}Ra bioremediation strategies.

The mechanisms involved in the formation of bacterial intracellular carbonates are under study. They likely differ among the diverse bacteria. Interestingly, a gene with no homologue of known function was discovered, which serves as a good marker of the capability of cyanobacteria to form iAC [5]. Phylogenetic reconstructions suggest that this gene was present in ancestral cyanobacteria with losses in various lineages along the evolution of Cyanobacteria, indicating that this biomineralization pathway is ancient.

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Keywords: cyanobacteria, ACC, intracellular, gene

T1 O3

Insights into Biosilicification: how does sophisticated biological glass grow

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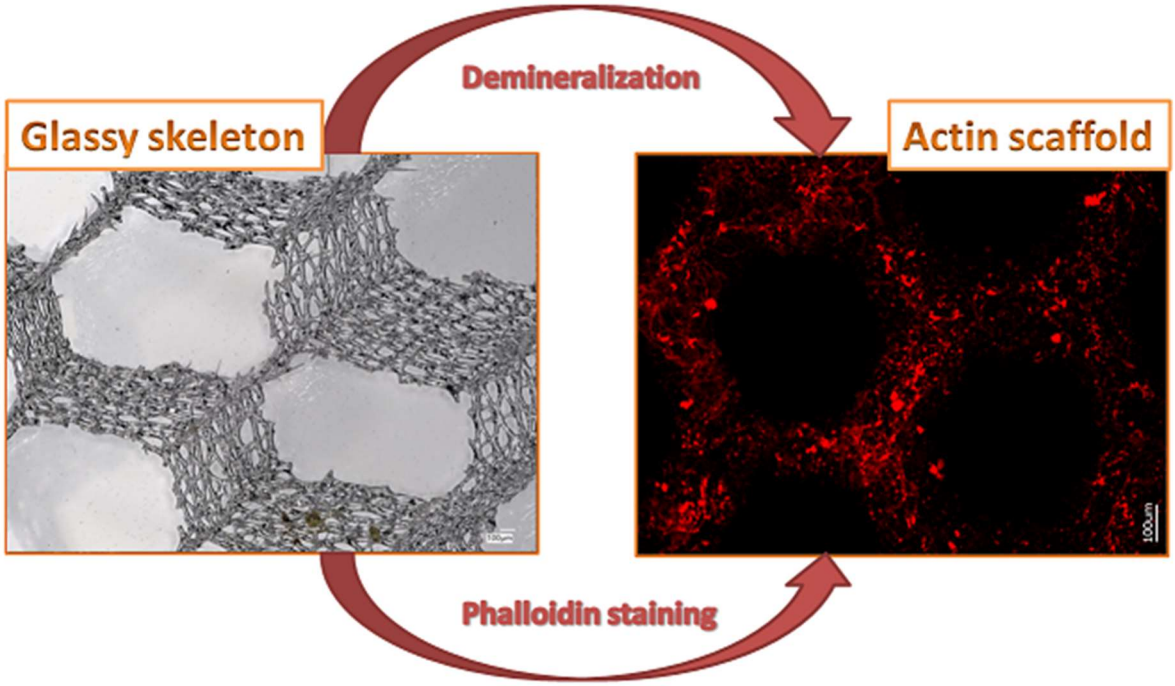
Our work provides an answer to one of the fundamental questions of science, namely, why biological glass grows past at ambient temperatures and reaches dimensions in the meter range on example of glass sponges.These first metazoans represent an evolutionarily ancient and simultaneously preserved archive of unique nanostructural biosilica. The diversity of shape (more than 80 morphotypes), size (up to 3 meter-long), highly sophisticated network connectivity and ornamentation in skeletal formations of sponges are well recognized. That there is proteinaceous axial, sometimes branched, filament in the extracellular spicules of sponges is known, and we have now shown that this first skeletal pattern generator is in fact actin. Since actin has been perceived as a strictly intracellular protein, no one has ever looked for it and has not been predicted it in such extracellular structures as glassy spicules.

We used a battery of bioanalytical techniques to show that axial filaments in 22, 31 and 2 representatives of poriferan classes Hexactinellida, Demospongiae and Homoscleromorpha, respectively, are made of F-actin.The conserved biomaterials morphology of sponges suggests this is an ancient role that actin has played for at least 545 million years. We suggest that F-actin was localized in an ancestral, intracellular siliceous construct, and as spiculogenesis moved to extracellular spaces the actin continued to play its pattern forming role, leading to a release on a size constraint on fibrous actin complexes. The branching capability of the actin filament bundle was an exaptation that led to the diverse, sometimes very complex composite-based skeletal geometries at the macro level in sponges. Thus, the “epitaxy” of biosilica structures is due to the presence and growth of actin filaments. Being arrested in glass, actin shows new function in sophisticated architecture of biosilica in sponges.

Keywords: biosilicification, biomaterials, actin, marine sponges



T1 O4

Assembly of the intraskeletal coral organic matrix during calcium carbonate formation

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Scleractinia coral skeleton formation occurs by a heterogeneous process of nucleation and growth of aragonite in which intra-skeletal soluble organic matrix molecules, usually referred to as SOM, play a key role.[1] Several studies have demonstrated that they influence the polymorphic precipitation of calcium carbonate.[2] However, the structural aspects that occur during the growth of aragonite have received less attention. In this research, we study the deposition of calcium carbonate on a model substrate, silicon, in the presence of SOM extracted from the skeleton of two coral species representative, which we previously characterized.[3] The study is performed by grazing incidence X-ray diffraction with the support of Raman spectroscopy and electron and optical microscopies. The results show that SOM macromolecules once adsorbed on the substrate self-assembled in a layered structure and induced the oriented growth of calcite inhibiting the formation of vaterite. Differently, when SOM macromolecules were dispersed in solution they induced the deposition of amorphous calcium carbonate, ACC, still preserving a layered structure. The entity of these effects was species dependent. In conclusion, we discovered for the first time that the SOM from corals can have a 2D lamellar structure. This structure is preserved when the SOM interacts with ACC but is lost when the interaction occurs with calcite.

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Keywords: Coral skeleton, calcium carbonate, grazing incidence X-ray diffraction, soluble organic matrix, Sty-lophora pistillata, Oculina patagonica

T1 O5

Amorphous and crystalline precursors to coral skeleton formation in acidifying oceans

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We compared the transient precursor phases on the forming surface of fresh corals grown at different pH values: pH 7.2, 7.4, 7.8, and 8.0 [1]. Using PhotoEmission Electron Microscopy [2, 3] at the calcium L-edge we identified the transient metastable precursor phases [4-6] as a function of distance from the surface. We expected to find the most and least metastable precursors at the outermost and innermost layers of the surface, but that is not what we found. One of the intermediate phases penetrates deepest into the skeleton.

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Keywords: coral, amorphous, precursor, acidification

T1 O6

The molecular basis for pore pattern morphogenesis in diatom silica

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Diatoms are unicellular, eukaryotic algae characterized by silica-abased cell walls that display complex, species-specific nano- and micropatterns. A hallmark of diatom silica are the hierarchically arranged patterns of pores that endow this material with favorable properties regarding interaction with light, materials transport, and mechanical stability. The porous cell walls are thought to be critical to the evolutionary success of diatoms, and in the materials science community are increasingly utilized to develop new silica-based nanotechnologies. Despite the importance of diatom silica, very little is known about the molecular machinery that enables morphogenesis of this extraordinary material. Recently, we have performed in the model diatom *Thalassiosira pseudonana* the first proteomics analysis of the organelle for silica biogenesis, the silica deposition vesicle (SDV). This led to the identification of 39 proteins that we regard as components of the machinery for silica biogenesis. We have focused on functional characterization of three of these SDV proteins, dAnk1-3, that are widely distributed among the diatoms and contain common protein-protein interaction domains (ankyrin repeats). We demonstrate through gene knockout and rescue experiments together with semi-autonomous, quantitative analysis of electron microscopy images that dAnks control the formation and the patterned arrangement of the pores in the silica. We propose a model that can explain how dAnks control pore formation and provides novel, experimentally testable hypotheses for future studies on silica morphogenesis.

Keywords: Silica, Morphogenesis, Proteomics

T1 O7

Diversity and function of biomineralized materials in ciliates

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Eukaryotic unicellular organisms such as ciliates (Protist) represent an excellent model system to discover the principles of biomineralization with respect to intracellular mechanisms involved in ion accumulation, vesicular transport, and biomineral formation. Ciliates occur naturally in freshwater, brackish, and marine habitats as well as in extreme environments. Examples of mineral precipitates with known functions include calcium carbonate or barium sulfate biominerals. For other, intracellular mineral-rich inclusions, the function is mostly unknown. To identify mechanisms with specific adaptations involved in mineral formation, we combine dynamic in vivo studies of mineral-forming ciliates together with high-resolution methods such as analytical electron microscopy. A recent example of mineralization that functions in detoxification is the intracellular bioaccumulation of the rare earth element gadolinium in ciliate cells, resulting in the formation and excretion of biogenic particles.

Keywords: protist, ciliate, intracellular mineralization, detoxification

T1 O8

Organic architecture of chamber biomineralization in main foraminiferal calcifiers

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Majority of marine foraminifera construct very diverse shells (tests). These unicellular eukaryotes are characterized by anastomosing granular pseudopodial networks (granuloreticulopodia), mostly used for sensing, feeding, attachment and locomotion. However, such networks are not the only pseudopodial structures used by foraminifera to survive in highly competitive and harsh conditions. There are several other pseudopodial structures, such as globopodium and lamellipodia, involved in specific physiological functions. Some of these cytoplasmic structures control formation, shaping and biomineralization of organic or mineral-organic skeletons supported by extracellular organic linings. Simple, monothalamous foraminifera build a single chamber. Much more complex skeletons are constructed by polythalamous foraminifera that form shells by highly algorithmic, iterative additions of chambers. Two main classes of the phylum Foraminifera, i.e. Globothalamea and Tubothalamea, identified on the basis of small subunit rDNA sequences developed distinct morphogenetic strategies (Pawlowski et al., 2013 Mar. Micropal.). The most intriguing feature of foraminiferal shells is their astonishing variety of biomineralizing material. Foraminifera employ synthesis of organic compounds, agglutination of foreign sediment grains, as well as secretion of calcium carbonate mesocrystals. It is worthwhile to stress that foraminifera, constructing calcareous shells, represent one of the main carbonate producers in the oceans with almost 25% of the global oceanic $CaCO^3$ production. Foraminifera provide archives of the past climates and the patterns of evolution. This contribution presents organic architecture and dynamics of subcellular structures employed by the prevalent group of benthic and all planktic foraminifera (Rotaliida, Globothalamea) to secrete their calcareous hyaline tests. Our research is supported by the Polish National Science Center (Grant 2020/37/B/ST10/01953).

Keywords: calcification, calcium pathway, mophogenesis, foraminifera, fluorescence staining

Poster presentations

Poster 1

On identification of Proline-alanine Rich Phospho-Proteins (PARPs) in the sea urchin tooth and their localization relative to the very high magnesian calcite microstructural elements.

Keith Alvares, Guanying Li, Wenle Xu and Derk Joester,

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The sea urchin tooth is a fascinating example of the exceptional level of biological control over microstructure of mineralized tissues and displays a highly hierarchical and functionally graded architecture. Optimized for grazing on hard substrates, its structure comprises both fibrous and platy elements that convey wear resistance, toughness, and self-sharpening to the continuously growing tooth. A unique aspect is that distinct microstructural elements in the tooth differ dramatically in the amount of magnesium incorporated into the calcite ($CaCO_3$) lattice (1). In fact, concentrations of Mg in the columns (up to % of Ca lattice positions) far exceed the solubility limit of Mg in calcite. Such highly Mg-substituted calcite currently cannot be synthesized in the laboratory. Our working hypothesis is that biomacromolecules occluded in the tooth are involved in the formation of the high magnesium calcite.

Using transcriptomics and proteomics, we have identified a set of proline-alanine rich phospho-proteins (PARPs) that are unique to the *L. variegatus* tooth and do not occur in other sea urchin biominerals, where Mg levels are lower (2). Based on sequence homologies, the 4 PARPs identified could be classified into 2 subgroups. We raised polyclonal antibodies to a 20 amino acid peptide sequence that were unique to two of the PARPS, one in each subgroup. (3) These antibodies were used to localize the PARPs across (a) immature, poorly mineralized and (b) mature, highly mineralized regions of the sea urchin tooth. We will report on our ongoing effort to correlate immuno-histochemical localization of PARPS with microstructural characterization and elemental mapping of the mineralized tissues using SEM-EDS. It appears that the PARPs localize to regions of very high magnesium calcite,

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This work was in part supported by NSF DMR-2104759

Keywords: Sea urchin tooth

Poster 2

Periostracum formation in *Sepia officinalis* (Sepiidae, Cephalopoda)

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The morphology, composition and mechanical properties of the *Sepia* cuttlebone have been intensively studied. Since the first detailed descriptions by Appellöf (1893), much work has been conducted on the adult shell structure and microstructure. Evolution of the shell from the embryo to the adult was thoroughly detailed by Bandel and Boletzky (1979) and more recent contributions were added by Le Pabic et al. (2016, 2019) and Dauphin et al. (2020). Nevertheless, the shell sac epithelium and his involvement in shell formation have been little studied. At present, there is no clear evidence of how the cells of shell sac epithelium participate in biomineralization. Our goal is to characterize the shell-forming tissues and structures in *Sepia officinalis*, elucidate their role in biomineralization and search possible homologies with other molluscs. Embryos of *S. officinalis* collected from the coast of Málaga were anesthetized and fixed in glutaraldehyde. Histochemical and TEM analyses of the shell sac were conducted in paraffin- and resin-embedded specimens, respectively. Four types of epithelia have been differentiated: simple squamous (dorsal), transitional (lateral bending zones), columnar (lateral fold tips) and simple cuboidal (ventral). The dorsal shield originates at the lateral fold tips where columnar cells secrete numerous vesicles, possibly containing chitin, as indicated by calcofluor dyes. These folds seem deeper than previously depicted and resemble a periostracal groove, from which a periostracum emerges. This periostracum coarsens due to the addition of laminae secreted by the upper and lower epithelia. Thus, the dorsal shield is homologous to the periostracum, which constitutes a support for further mineralization.

Keywords: Sepia officinalis, embryo, periostracum, shell sac epithelium, vesicles, chitin

Poster 3

Electron-dense compartments role in calcium transport during coccolithophore biomineralization

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Coccoliths are mineralized scales made of an organized assembly of calcite single crystals grown by ocean dwelling phytoplankton. These structures are a model example of how biological systems control crystallization to produce complex and functional materials. In addition, coccolith production sequesters carbon in ocean sediments, in turn influencing the carbon distribution in the ocean. Given their importance, it is surprising that the mechanisms of coccolith mineralization are still not fully understood, and it is unclear how this process will respond to rising ocean acidity caused by climate change.

One area of uncertainty is how large amounts of calcium are trafficked across the cell during mineralization without raising the free concentration in the cytosol to toxic levels. To avoid this, calcium is stored in electron-dense compartments, although there is no evidence these are involved in coccolith production. It is also unclear how calcium moves in and out of these compartments. A more dynamic imaging technique is therefore needed to quantify how compartments, and the calcium concentrations within, change over the course of calcification.

We imaged electron-dense compartments within the coccolithophore *Chrysotila carterae* using cryo-ptychographic X-ray computed tomography and laser-scanning confocal microscopy. We show compartments changing in both morphology and electron density across calcification, directly linking them to coccolith mineralization. Small compartments are observed at early stages of calcification. During active calcification, compartments become larger and increase in electron density, indicating calcium is being accumulated during coccolith production. Upon completion of calcification, compartments decrease both in size and electron density. These findings improve our understanding on the cellular pathways involved in coccolith production, which will be important in predicting the fate of coccolith mineralization in future acidified oceans.

Keywords: coccolithophores, coccoliths, calcium, ptychography

Topic 2 Biomineral Structures and crystallization mechanisms

Keynote lecture

Biomimetic Mineralization to investigate Biomineral structure formation and mineralization mechanisms

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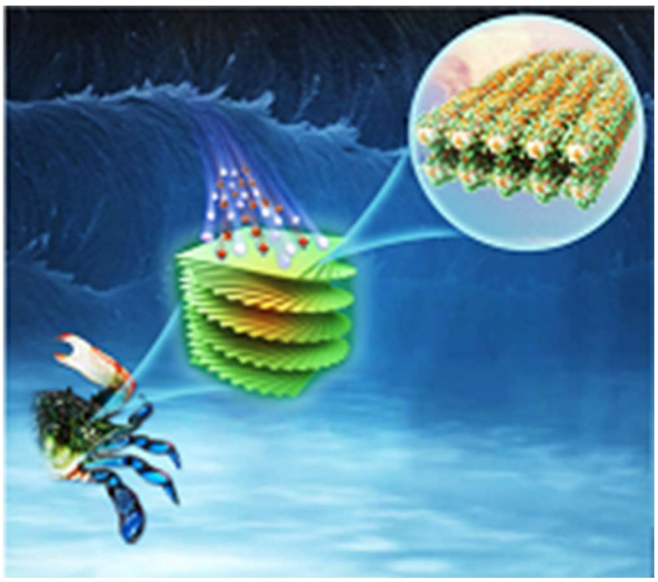
The structures of Biominerals are often difficult to investigate. It is even more difficult to reveal the structure formation and mineralization mechanisms. In this presentation, 2 biomimetic examples will be discussed, which target to reveal structure formation and mineralization in Biominerals.

The first example is inspired by crab shells with photonic properties. These can be mimicked by crystalline nanocellulose, which self assembles into a chiral structure. This can then be used for infiltration with $CaCO_3$ for the formation of a hybrid material. Depending on the mineralization time, a strong and flexible material can be formed as well as a hard and stiff material, which leads to outstanding mechanical properties, simultaneously showing photonic effects.

The second example is cellulose nanofibers, which are functionalized with carboxyl groups and subsequently mineralized by $CaCO_3$. The cellulose nanofiber network mineralized by extremely small amorphous $CaCO_3$ nanoparticles exhibits outstanding mechanical properties based on multiple toughening mechanisms, which will be discussed.

These toughening mechanisms are also used in Biominerals



Oral presentations

T2 O1

The role of lattice distortions in coccoliths morphogenesis

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Coccoliths—micrometer-sized calcitic scales formed by unicellular algae called coccolithophores—are a prime example of biological regulation over crystal morphogenesis. They have the unique capacity to induce anisotropic growth of calcite single-crystals and thus, to control their final morphology. Whereas calcite is expected to grow as a perfect rhombohedral crystal having identical symmetry-related {104} facets, coccolithophores are able to break the thermodynamically induced symmetry and morph calcite single-crystals into the most intricate shapes in 3D. Our previous work suggests that lattice defects and internal residual strains play an important role in the process of biogenic crystal growth. Therefore, in this work, state-of-the-art X-Ray-based coherent diffraction analysis and imaging methods were used to characterize local lattice properties in single calcitic units in two coccoliths species in 3D. For comparison, two model systems were studied: holococcoliths composed of symmetric calcitic rhombohedral crystals produced by *C. braarudii*; and elaborately shaped heterococcoliths produced by *C. leptoporus*. Combination of Bragg Coherent Diffraction Imaging (Bragg-CDI), which allowed us to visualize local lattice distortions with a spatial resolution of below 10 nm, with high-resolution electron microscopy, provided new mechanistic insights into how coccolithophores break the expected symmetry of singe-crystalline calcite and, thus, manipulate its growth.

T2 O2

Intracellular biomineralization of rhombohedral calcite by haploid life phases of coccolithophores

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Organisms can execute tight control over crystal morphology using a cellular toolkit that is not well understood. A prominent example of biological control over crystal morphology is the calcite crystals of coccolith scales produced by marine algae. Interestingly, crystal morphology fundamentally differs in coccoliths produced by the same species' diploid and haploid life-cycle phases, called heterococcoliths and holococcoliths. While calcite crystals of heterococcoliths are highly complex and species-specific, holococcolith crystals are simple rhombohedra, the most common morphology of calcite. In this study, we focus on the growth environment of the simple holococcolith crystals utilizing advanced sample preparation techniques for TEM and FIB-SEM volume imaging. Our results indicate that rhombohedral crystals nucleate and grow intracellularly within voluminous vesicles while experiencing an unconfined, isotropic environment; this environment facilitates the simple morphology of holococcolith crystals. Comparing these findings with the known heterococcolith formation environment suggests that confinement of the crystallization process is a critical factor in shaping biomineral morphology.

Keywords: calcite, crystal growth, nucleation, coccolith

T2 O3

Twin-based strengthening mechanism in a crossed-lamellar structure

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Biological shells typically possess hierarchical structures and carry out multiple functional roles thanks to their remarkable strength and toughness. An example of such a complex hierarchical structure is the crossed-lamellar structure, which is made of the 1st-order lamellae comprising the laths (the 2nd-order lamellae) composed of parallel rods (the 3rd-order lamellae), with relatively low organic content (0.1-1 wt.%). Such biological structures offer a wealth of inspiration for addressing the classic challenge in material design where strength and toughness, two crucial structural properties, are often perceived as mutually exclusive.

Our research focused on understanding the microstructure of the smallest building unit, which forms the crossed lamellar structure. Thin foils were prepared from the *Sinustrombus sinatus* shell by a focused ion beam (FIB) technique in a scanning electron microscope (SEM). High-resolution transmission electron microscopy (HRTEM) was performed to gain more information about the nanoscale structure of the individual rod. The results revealed that the examined lamellas are not single aragonite grains but are divided by densely spaced twin plates. The energetically favorable twin disorientation is formed with an accuracy of 1º. The geometrical phase analysis (GPA) was used to measure strain from HRTEM images. By implementing the original GPA, the recorded crystal lattice was correlated with a reference lattice that includes an ideal twin boundary, allowing for identifying deformation fields within the 3rd-order structural units. It was found that the twin plates experienced strong compression in the direction perpendicular to the longest dimension, resulting in a strain of -0.1%. The high frequency of twin placement in the internal structure of the tertiary lamellae induces prestressing of the basic structural units. This revealed mechanism significantly improves the fracture toughness and the strength of the protective exoskeleton.

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Keywords: strengthening mechanism, crossed-lamellar structure, geometrical phase analysis (GPA), twins

T2 O4

Calcification of the egg capsule in the apple snail: the organic matrix and the calcium-rich granules

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The apple snail Pomacea canaliculata is an amphibian mollusk that lays aerial eggs above the water level. The hatching larvae is protected from sunlight, desiccation and other damages by a calcified capsule which is composed of vaterite. Vaterite is a metastable calcium carbonate seldom found in inorganic sediment, and the only found stable forms in nature are biominerals in molluscs and corals. Moreover, the egg capsule represents another evolved biomineral after the spiral shell in gastropod. Therefore, the calcified egg capsule provides an interesting biomineralization model to study the crystal polymorph and evolution of calcium carbonate biomineral. We first explored the calcification process of the eggs. It was found that numerous calcium-rich granules were present inside the newly deposited eggs and transported to the outer surface for capsule mineralization, indicating a unique mineralization pathway compared with the exoskeleton shell formation. We then analyzed the organic matrix extracted from the egg capsules and found that the organic matrix could stabilize vaterite and modify the crystal morphology. FTIR revealed that the matrix was composed of proteins and polysaccharides, similar to most molluscan shell matrices. Proteomics analysis showed that the shell proteins contain chitin-binding and calcium binding proteins, and several sulfatases. The sulfatase is unique and may be involved in vaterite deposition via generating sulfate ions from sulfonic polysaccharide. Our work suggests that the calcification of the P. canaliculata egg capsule may be accomplished via a unique pathway which calls for further studies.

Keywords: Pomacea canaliculata; egg capsule; organic matrix; shell protein; calcium-rich granule

T2 O5

Biomimetic transformations of amorphous calcium phosphate to dental and ossifying crystalline hydroxyapatite

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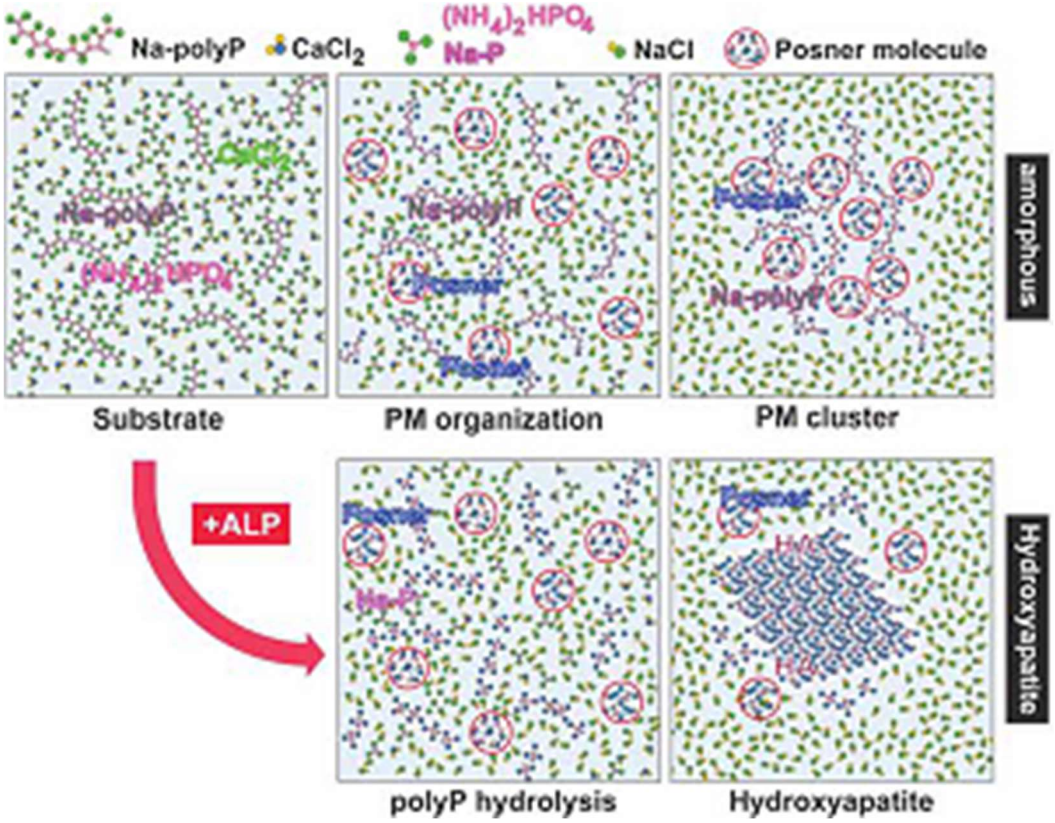
Mineral deposition in living organisms is not a passive deposition of the minerals but a highly controlled process starting from the amorphous phases and processed onto organic template(s), into crystalline mature product(s), like enamel or bone. We found that amorphous calcium phosphate (CaP) deposition during bone and teeth mineralization starts with the aggregation of Posner's clusters $Ca_9(PO_4)_6$ into amorphous Ca-phosphate (ACP), which then transforms into crystalline CaP and finally maturates to hydroxyapatite (HA). Using dentin/enamel of human teeth as a model system, we show that the physiological inorganic polymer polyphosphate (polyP), a phosphate donor in mineralization, prevents the transition from amorphous to crystalline CaP at concentrations > 15 wt%. Stabilization of the amorphous phase of CaP by polyP is reversed by hydrolysis of the polymer by alkaline phosphatase (ALP), an enzyme that releases phosphate for mineralization. The ALP is present in calcified enamel and dentin, as shown by immunostaining and enzyme activity measurements. The phase transfer into crystalline CaP can be prevented by ALP inhibitors. The modulating effects of polyP and ALP on the kinetics of the phase transition from amorphous to crystalline CaP are demonstrated. Molecular modeling studies show that the polyP chains are able to penetrate into the channels between the Posner molecules, preventing cluster association to ACP and impairing HA crystal formation.



It is concluded that during HA development this dynamic interrelation processes, amorphous ↔ crystalline CaP depositions, also run *in vivo* during hard tissue dental organ formation. The ALP exists there and the phosphate deposits, either in phosphorylated proteins or (presumably) also in polyP, are present stage-dependently in enamel-dentine regions.

Acknowledgements

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T2 O6

Organization of a plywood biomaterial: the calcitic cross-foliated microstructure of limpets

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To construct their shells, molluscs are able to produce a vast array of calcified materials, including granular, prismatic, lamellar, fibrous, foliated and plywood-like. The latter include an aragonitic (the crossed-lamellar) and a calcitic (the crossed foliated) variety, whose modes of formation are particularly enigmatic. We studied the crossed foliated calcitic layers, formed solely by members of the limpet family Patellidae, using scanning electron microscopy and electron backscatter diffraction. In the external layer, the distribution of 1st order lamellae is irregular; toward the interior, the material becomes progressively organized into commarginal 1st order lamellae, with 2nd and 3rd order lamellae dipping in opposite directions in alternating lamellae (the so-called concentric crossed foliated layer). At the same time, the crystallographic texture becomes stronger because each set of 1st order lamellae develops a single orientation for the c-axis, while both sets maintain common orientations for one {104} face (parallel to the growth surface) and one *a*-axis (perpendicular to the planes of the 1st order lamellae). Each 1st order lamella shows a progressive migration of its crystallographic axes with growth, toward the maxima corresponding to the set to which it belongs. Thus, all 1st order lamellae of the same set show similar orientations in the mature concentric crossed foliated layer. To explain the progressive organization of the material, we hypothesize that a secretional zebra pattern, similar to the one created by the 1st order lamellae on the shell growth surface, is developed on the shell-secreting mantle surface. Cells belonging to alternating stripes behave differently to determine the growth orientation of the laths composing the 1st order lamellae. In this way, we provide an explanation as to how plywood-like materials can be fabricated, which is based mainly on the activity of the mantle cells.

Keywords: molluscs, limpets, microstructure, crossed foliated, biogenic calcite, plywood material

T2 O7

Structural and mechanical adaptation of *Lingula anatina* shells

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Biominerals are a class of inorganic-organic composite materials that exhibit function-optimized properties. These properties arise from a hierarchical organization of primary building blocks. While some biomineral producing organisms can alter these properties in response to environmental stresses, this generally involves a time-intensive process of resorption and reprecipitation. Here, we report that the load-bearing shells of the phosphatic brachiopod *Lingula anatina* are an exception to this process. We observed that these shells are able to dynamically modulate their mechanical properties in response to a change in environment, i.e. they switch from hard and stiff when dry to malleable when hydrated within minutes. A passive and stimuli-responsive adaptation sought-after in structural materials. To understand the mechanisms that underpin these changes in mechanical properties, we first characterised the structure of the shell at the nano-scale. We used focused-ion beam scanning electron microscopy (FIB-SEM) to cut 70 nm-thin lamella from the shell for analysis using transmission electron microscopy, scanning-transmission electron microscopy, electron diffraction and electron energy loss spectroscopy. We show that the shell, at the nanoscale, is composed of 2-5 nm thick crystals of fluorapatite, interwoven with chitin and organized into 200 nm rod-like structures. As hydration is known to increase the mobility of chitin and to weaken its association with the mineral, it is conceivable that the observed organization of these two major components constitute a mechanism, at the nanoscale, that underpins the changes in mechanical properties that the shell undergoes as a response to hydration.

Keywords: Biomineralization, brachiopod shells, fluorapatite, transmission electron microscopy

T2 O8

Stabilization of Amorphous Calcium Phosphate by Barium: A Novel Approach Inspired by a Marine Worm

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Amorphous calcium phosphate (ACP) is a transient precursor phase in bone and teeth, transforming into crystalline carbonated hydroxyapatite. Some rare organisms like the Nemertean worm *Amphiporus lactifloreus* [1] produce ACP that is stable throughout the organism's lifetime in its stylet. The stylet is a 100-micron long nail-shaped "tooth" at the end of the proboscis and is used to capture and pierce prey. ACP, although transient under aqueous conditions, could be a valuable mineral phase for tissue-engineering of bone due to its similar elemental composition to carbonated apatite in bone. We aim to study the worm's ACP stability mechanisms to develop methods to stabilize synthetic ACP. We study the 3D structure using nano-CT and FIB SEM, as well as the elemental composition and distribution of stylets extracted from *A. lactifloreus.* The stylet consists of an inner core region, surrounded by an outer lamellar structured layer. In both regions, Ca and P are the main elements. Surprisingly, the concentrations of Ba and Sr are high with Ca/Ba 5:1 by atoms and Ca/Sr 10:1. The outermost layer of the stylet exhibits elemental contrast of heavier elements in nano-CT and is enriched in S and Ba according to EDS. Raman detected SO_4^{2-} . Nanoindentation results showed an elastic modulus of 10–28 GPa and a Vickers hardness of 30-160.

We carry out in vitro syntheses with different elemental compositions and monitor the ACP stability by FTIR. The addition of Ba at atomic ratio Ca/Ba = 9:10 is capable of stabilizing ACP and with Ca/Ba = 3:1 and 19:1 can delay crystallization. By substituting Ca in the ACP synthesis with different ratios of Ba and Sr, the crystalline transformation of ACP can be slowed down for weeks. This result shows that Ba can be used to modulate ACP stability. I future this might lead to ACP that can be tailored to various industrial and medical applications in the field of bone repair and replacements.

T2 O9

Monochromated STEM-EELS analysis of the structure and composition of biogenic calcite reveals the biomineral growth pattern

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The vast majority of calcium carbonate biocrystals differ from inorganic crystals in that they display a patent nanoroughness consisting of lumps of crystalline material (calcite/aragonite) surrounded by amorphous pellicles. Understanding this organization and its formation mechanisms implies to unveil the chemical composition and structure at the nanoscale. Compared to other spectroscopic approaches, STEM-EELS (scanning transmission electron microscopy couppled with Electron Energy Loss Spectroscopy) offers the advantage of an outstanding spatial resolution (at the nanometer scale) in both chemical analysis and imaging. Moreover the use of a latest-generation STEM microscope equipped with an electron monochromator and a direct-detection camera offers the possibility to detect very weak signals with a spectral resolution down to 7 meV, close to those obtained from X-ray based approaches This high spectral resolution gives access to the crystallinity of calcium carbonate phases through the measure of the crystal field splitting (CFS) on Calcium L_{23}-edge.

In the present study (1), high resolution STEM-EELS was used to map compositionally and structurally the calcite secreted by the giant acorn barnacle *Austromegabalanus psittacus*. Our data reveal that the material is composed of irregular lumps (up to two hundred nm in diameter) of crystalline phase (calcite) surrounded by relatively continuous cortexes (up to 20 nm thick) of an amorphous phase composed of calcium carbonate (ACC) and/or nanocalcite plus biomolecules, with a surplus of calcium relative to carbonate. Based on EELS results, we develop a model by which the separation of the crystalline and amorphous phases takes place upon crystallization of the calcite from a precursor ACC. The organic biomolecules are expelled from the crystal lattice and concentrate in the form of pellicles, where they stabilize minor amounts of ACC/nanocalcite. In this way, we change the previously established conception of biomineral structure and growth.

(1) Nanoscale Analysis of the Structure and Composition of Biogenic Calcite Reveals the Biomineral Growth Pattern. Marta de Frutos, Alejandro B. Rodríguez-Navarro, Xiaoyan Li, and Antonio G. Checa ACS Nano 2023 17 (3), 2829-2839; DOI: 10.1021/acsnano.2c11169

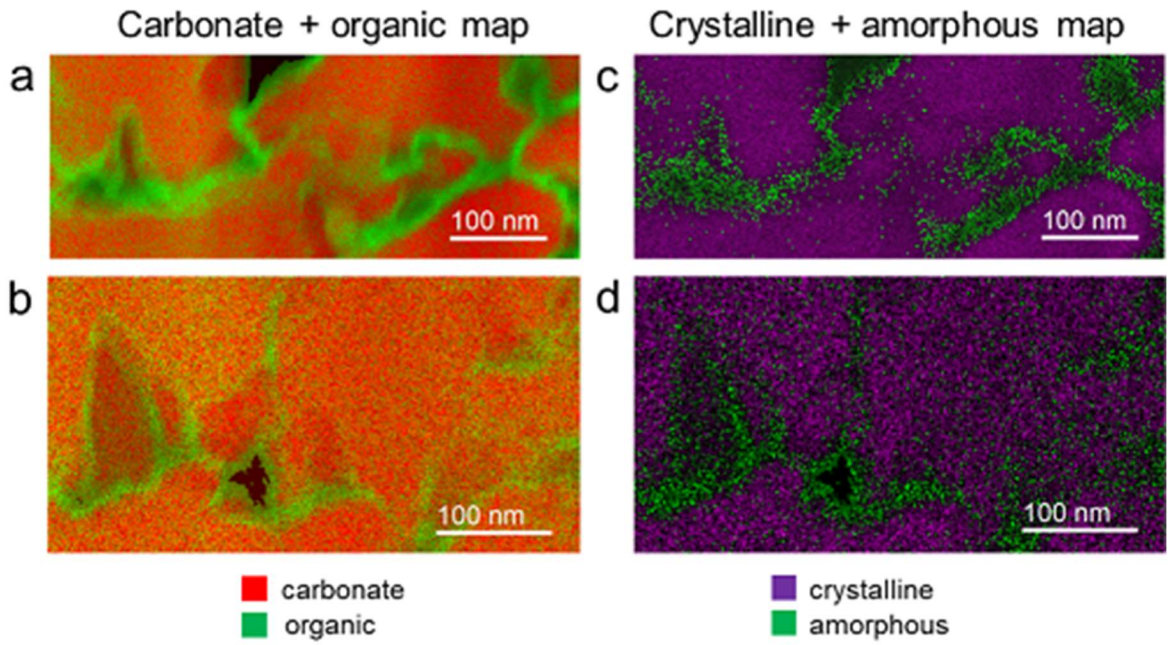

Figure 1: (a,b) Distributions of carbonate (in red) and organic compounds (in green) for two different areas. (c,d) Distributions of crystalline vs amorphous material. The amorphous areas (CFS values <1.1 eV) are represented in green and crystalline ones (CFS values >1.1 eV) in purple.

T2 O10

Anisotropic lattice parameters change in aragonite induced by Na^+ substitution

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The university of Tokyo, Jpan

Some biogenic aragonites possess larger axial ratios (*a*/*b* and *c*/*b* in orthorhombic *Pmcn*) than abiotic counterparts. Our previous research suggested that the anisotropic lattice parameters change is caused by substitution of Na^+ for Ca^{2+} because the axial ratios correlate with Na content. To verify this hypothesis, we synthesized aragonites *in vitro* in the presence of Na^+ in the present study. The synthetic crystals were obtained by mixing 50 mM $MgCl_2$, 10 mM $CaCl_2$, and 10 mM Na_2CO_3 with different concentrations of NaCl. After stirring the solutions at room temperature for 24 h, precipitates recovered by centrifugation were washed three times with Milli-Q water and once with ethanol, and dried at room temperature. The precipitates were investigated using X-ray diffraction and scanning electron microscopy, confirming that they were all composed of aragonites. When the NaCl concentration was lower, the synthetic aragonites exhibited euhedral columnar shapes and their axial ratios were comparable with those of abiotic ones. However, in the case of higher NaCl concentrations, the aragonites became granular and showed higher axial ratios. The increase in axial ratios was correlated with the Na content in the crystals. The axial ratios decreased by heating at 250 °C probably due to diffusion and segregation of Na^+ from the crystal lattice of aragonite. Thus, these synthetic experiments indicated that the hypothesis is plausible. Considering relatively lower Cl content in the crystals, the charge compensation for Na substitution can be attributed to the introduction of bicarbonate ions and/or vacancies.

Keywords : aragonite, sodium, anisotropy, lattice parameter, axial ratio, synthetic experiment

T2 O11

Halochromic ACC crystallization in the presence of naphthoquinones unveils biomineral pigmentation in sea urchins

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Adult sea urchin biominerals owe their different colors to the presence of 40 different polyhydroxylated naphthoquinone molecules (PHNQs: spinochrome A, B, C, *etc.*) across species.[1] PHNQs are incorporated within calcite likely during crystal growth,[2] occurring through amorphous calcium carbonate (ACC) precursors.[4] Interestingly, spinochrome A displays red color once extracted from purple $CaCO_3$ *P. lividus* spines in acidic conditions,[5] suggesting that pH plays a role in color variations as it does in $CaCO_3$ formation.[6,7] To understand the color variations in biominerals formed through ACC precursor as well as the role of PHNQs in ACC crystallization, we performed ACC precipitation in the presence of synthetic naphthazarin and study its crystallization. Then, the effect of biogenic spinochrome A, B, and E on calcite lattice distortions was investigated. pH was monitored during ACC precipitation and crystallization, *e.i.* after the addition of $CaCl_2$ to a Na_2CO_3 solution[8] in the presence of PHNQs. DSC/TGA, XPS, PDF analysis, UV-Vis, STEM-EELS, and ss-NMR spectroscopies were performed to characterize the amorphous and crystalline hybrid pigments. Here, we show that halochromic ACC crystallization may occur in biomineral pigmentation in sea urchins. Indeed, naphthazarin, which is red at acidic pH, turns blue before ACC precipitation, leading to lavender blue calcite due to successive OH deprotonation/protonation during ACC crystallization. However, naphthazarin does not significantly affect ACC structure and stability but does get incorporated into calcite most probably in the form of aggregates. Finally, spinochrome B and E lead to yellowish calcite and spinochrome A to purple calcite whereas the formers induce larger calcite lattice distortions than the latter. The preferential occlusion of spinochrome A in purple *P. lividus* spines may thus be explained by the combination of unaffected atomic structure and obtained intense color.

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T2 O12

Microstructures and biomineralization patterns of gymnolaemate bryozoans (Bryozoa, Gymnolaemata)

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Gymnolaemata is currently the most diverse and widely distributed class among bryozoans, and is mainly comprised of the order Cheilostomata. All cheilostomes are found worldwide and exclusively inhabit marine waters. They produce $CaCO_3$ skeletons that can be made of either calcite, aragonite, or both. Despite extensive previous research regarding their microstructures and mineralogy, no suitable crystallographic techniques had been applied and their biomineralization processes still remain unclear. We present a detailed study of the microstructures, mineralogy and crystallography of eight extant species of cheilostomes using SEM-EBSD, AFM and micro-CT. We focus on their main microstructures: granular and platy calcite, and fibrous aragonite. The calcitic microstructures are formed by aggregates of crystals that easily change from platy to granular or viceversa. Fibrous aragonite consists of spherulites made of long, thin needles. In both the calcitic and aragonitic microstructures, the crystallographic axes display an axial texture, with the c-axis as the fiber axis, which is stronger in aragonite. The calcite grains sometimes cluster in domains with internal misorientations. Crystal competition is widespread. We reconstruct the biomineralization process in the different species by taking into account the distribution and morphology of the growth fronts of crystals and the theoretical location of the soft tissue. In bimineralic species, calcite formation always predates aragonite formation. In internal walls, growth is bidirectional from the center. In external walls, however, growth proceeds from the cuticle, which acts as a nucleation template, extending outward toward the secretory epithelium. We conclude that biomineralization is seemingly remote in all cases and proceeds in a wide space formed between the cuticle and the soft tissue. The invoked process of crystal growth far from the soft tissue correlates with the inorganic-like appearance of the crystallites.

Keywords: Bryozoans, Gymnolaemata, microstructures, biogenic calcite, biogenic aragonite, crystallography, EBSD

T2 O13

Calcite and guanine formation by the freshwater green alga *Phacotus lenticularis*

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Many unicellular algae produce $CaCO_3$ minerals in marine and freshwater ecosystems [1]. One freshwater species, *Phacotus lenticularis,* is abundant in lakes and ponds. *P. lenticularis* produces a complex shell composed of aligned crystal plates of calcite. These shells may constitute a significant fraction of basin sediments especially during the bloom period [2].

We investigate the shell formation mechanism in *P. lenticularis* cells collected from a local natural habitat using *in-vivo* fluorescence assays combined with cryo-SEM, which enables the observation of complexes under fully hydrated conditions. During reproduction, 1-3 cell divisions occur to form up to 8 daughter cells that subsequently form a shell within a confined reproduction volume. We observed Ca ion trafficking into the daughter cells, and the formation of small, vesicle- contained intra-cellular crystal. Shell assembly occurs on the cell outermost layer, where biomineralization remains under control by virtue of the protected environment within the reproduction complex. Furthermore, we have identified intriguing indications of signals from the environment that may trigger calcification both in the cell cultures and in natural environments at the onset of the bloom period.

We also observed that cell cultures grown under phosphate stress conditions, produce birefringent crystals that were identified as □□guanine using micro-Raman spectroscopy. Cryo-SEM examination showed that the crystals are enclosed within vehicles, which are dispersed in the cell, as observed using cryo-soft X-ray tomography and cryo-FIB-SEM. Interestingly, guanine crystal deposition was observed in many unicellular species under nitrogen starvation. The occurrence of guanine crystal formation under different triggers may indicate a common reaction to stress response, resulting in nutrient storage.

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T2 O14

The origin and function of geometric frustration in spicule morphogenesis

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Sponges produce complex skeletal structures made out of individual elements called spicules. A subclass of Sponges named Demospongiae consist of spicules made of amorphous silica having various morphologies, ranging from needle-like megascleres to symmetric tripod-like dichotriaenes with multiple branches. All spicules have a central canal consisting of a hybrid crystalline mineral/organic axial filament composed of a protein, silicatein. The filament templates silica deposition and, therefore, plays a crucial role in spicule formation. However, the mechanisms underlying the morphogenesis of the filament, including its branching, have remained elusive.

In this study, we employed advanced X-ray-based techniques to investigate the internal structure of the axial filament in unprecedented resolution. Specifically, we used scanning X-ray Diffraction, Bragg Coherent Diffraction Imaging and nano-Holotomography to visualize the evolution of the crystal habit and to determine the local lattice distortions during filament formation. We demonstrate that during protein crystallization an internal strain is developed as a result of geometric frustration generated by fitting the silicatein units into the crystalline assembly. In turn, at a critical point, the accumulated elastic strain dissipates in the form of branching on specific crystallographic planes, leading to the formation of highly symmetric shapes of the spicules. These findings suggest that spicule morphogenesis is a spontaneous process that is carefully orchestrated by the organism by controlling the conditions in which the filament is self-assembled. This work establishes a novel biomineralization mechanism unique to sponge skeletogenesis.

Keywords: Demosponges - Spicule - Morphogenesis - Self assembly – Branching

T2 O15

Crystallographic Characteristics of Vaterite in Fish Otolith

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Teleost fishes have three types of otoliths called lapillus, sagitta, and asteriscus, which adopt different polymorphs of calcium carbonate (CaCO3); lapillus and sagitta are aragonite and asteriscus is vaterite. The reason why metastable vaterite can be formed and stably exist in asteriscus is not yet understood.

In this research, crystallographic characteristics of vaterite in asteriscus have been investigated to elucidate its formation mechanism. Goldfish (Carassius auratus) was selected as a typical teleost fish species, and inorganically synthesized vaterite was also analyzed for comparison. The crystal structure of vaterite was analyzed as a hexagonal system (P63/mmc, a = 4.13, c = 8.49 Å) of the averaged unit cell proposed by Kamhi (1963).

A goldfish asteriscus is almost disk-shaped. Electron back-scattered diffraction (EBSD) analysis of the cross-sections indicated that the ai axes extend radially from the center. This radial structure is composed of domains of about ~10 □m in width. Transmission electron microscopy (TEM) observation of cross-sectional thin films of the domain prepared by focused ion beam (FIB) revealed that the domain is composed of single crystals of 0.5-3 □m separated by small-angle grain boundaries. These crystallographic features such as elongation along the ai axes and mosaic texture were also found in synthesized vaterite, suggesting that vaterite constituting the asteriscus grew according to the intrinsic crystallographic characters of vaterite.

To investigate the nucleation site of the vaterite crystal, asteriscus (~20 □m in size) in goldfish fry was collected and observed by TEM. The center of the asteriscus contains organic matter and a single crystal of vaterite, implying that this organic matter is relevant to the selection of vaterite.

T2 O16

3D interrelationship between hierarchical canal network and the gradient mineralization of shark tooth osteodentin

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Osteodentin is a dominant mineralized collagenous tissue in the teeth of many fishes, with structural and histological characteristics resembling those of bone. Osteodentin, like bone, comprises osteons as basic structural building blocks, however, it lacks the osteocytes and the lacuno-canalicular network (LCN), which are known to play critical roles in controlling the mineralization of the collagenous matrix in bone. Although numerous vascular canals exist in osteodentin, their role in tooth maturation and the matrix mineralization process remain poorly understood. Here, high resolution micro-computed tomography (micro-CT) and focused ion beam-scanning electron microscopy (FIB-SEM) were used to obtain 3D structural information of osteodentin in shark teeth at multiple scales. We observed a complex 3D network of primary canals with a diameter ranging from ~10 μm to ~120 μm, where the canals are surrounded by osteon-like concentric layers of lamella, with 'interosteonal' tissue intervening between neighboring osteons. In addition, numerous hierarchically branched secondary canals extended out radially from the primary canals into the interosteonal tissue, with a decreasing diameter from ~10 μm to hundreds of nanometers. Interestingly, the mineralization degree increases from the periphery of primary canals to the interosteonal tissue, suggesting that mineralization begins in the interosteonal tissue. Correspondingly, the hardness and elastic modulus of the interosteonal tissue is higher than those of the osteonal tissue. These results demonstrate that the 3D hierarchical canal network is positioned to play a critical role in controlling the gradient mineralization of osteodentin, also providing valuable insight into the formation of mineralized collagenous tissue without osteocytes and LCN.

T2 O17

Novel crystalline phases derived from amorphous calcium hydrogen phosphate

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Calcium (ortho)phosphate (CaP) is an essential mineral component in organisms and extensively employed as a biomedical material. Different CaP phases have distinct physical/chemical properties, biological effects and physiological applications, which encourages us to explore the new CaPs with novel microstructures and properties. However, since the main crystalline CaP phases were discovered before the 19th century and structurally determined in 20th century, the progress in this field finds little progress.

In this study, by controlling the crystallization of amorphous calcium phosphate ($CaHPO_4 \cdot xH_2O$, ACHP) in the water/organic solvents, we discover five crystalline CaPs (denoted as HLCPs, one of them has been published [1]). With chemical formula $CaHPO_4 \cdot X$, where the X represents a molecule of H_2O or organics, the HLCPs have a layered structure, and the X molecules locates between the $CaHPO_4$ layers. Different HLCPs are able to transform into each other under specific conditions. The HLCPs show potential to carry the biomedical molecules in the structure as a drug delivery system. Moreover, the HLCPs display a piezoelectricity behavior, which is rarely seen in other CaP counterparts.

This work not only extends the family of CaP, but also provides new candidates for building functional biomaterials.

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T2 O18

Changes in bone chemistry, mineralogy and structure during heating

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Background

The study of bone changes during heating is highly relevant for forensic and archeological material analyses. However, bone is a complex nanocomposite material composed of an inorganic phase (nanocrystalline carbonated apatite) mineralizing an organic matrix (largely type I collagen fibrils) and water. Moreover, bone composition and structure is highly heterogeneous and varies in different parts of the skeleton depending on bone function.

Methodology

We have studied in detail how bone transform during thermal treatments using different analytical techniques such as thermogravimetry (TGA-DSC), electron microscopy, X-ray diffraction and infrared spectroscopy.

Results and discussion

We show that bone mineral and organic matrix characteristics in specific types of bone tissues strongly influence bone transformation during thermal treatments. For instance, in cortical bone the apatite nanocrystals are integrated within the highly organized collagen fibrils and are coated with phosphorylated proteins (osteopontin). The organic matrix protects the apatite crystals and recrystallization only occurs when organic matter is lost at around 600 C. Also during recrystallization, foreign ions (Mg2+, Na+) are expelled from the apatite lattice to the crystal surface and the degree of preferential orientation of apatite crystals (in cortical bone) increases as larger well oriented apatite crystals grow epitaxially to the expense of smaller randomly oriented crystals. In medullary bone the organic matrix is rich non-collagen proteins and proteoglycans and recrystallization set at much lower temperatures than in cortical bone (around 400 C). Also, the calcination process creates additional microporosity in bone increasing its surface area and reactivity. The information obtained in this study can help to better understand the dynamic of bone transformation during alteration in natural processes (digenesis) and how bone mineral characteristics can be modified for specific applications (bone grafts, implants).

T2 O19

Infrared and Raman spectroscopy for the fingerprinting of microbially-induced calcium carbonate precipitation

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The use of Fourier Transform Infrared (FTIR) and Raman spectroscopy as identification tools for calcium carbonate (CaCO3¬) is extensive, spanning inorganic chemistry to mineralogy and marine zoology. A lesser explored field, is the spectroscopic identification of CaCO3¬ during the self-healing of cracks in construction mortars. Cracks can self-heal over time as CaCO3 precipitates in them. Numerous methods exist to speed this reaction, including the use of microbially-induced carbonate precipitation. Still, it is not possible to readily identify whether the CaCO3 produced is biogenic in origin. Furthermore, the use of FTIR and Raman spectroscopy to fingerprint bacterially precipitated CaCO3 has been limited with little application to explaining its biogenic properties. Nonetheless, in other biomineralization contexts, these spectroscopic techniques are widely used, such as in studies of mollusk shells.

Here we present the potential of FTIR and Raman spectroscopy for differentiating between biotic CaCO3 from two bacteria, Sporosarcina pasteurii and Acinetobacter tjernbergiae, and chemically synthesized CaCO3. Raman spectra identified calcite bands with organic presence due to fluorescence. Furthermore, FTIR spectroscopy shows bacterial CaCO3 as less crystalline and stark interspecies crystal lattice differences. Furthermore, amine and polysaccharide bands were detected between 1700-1550 cm -1 and 1070 cm-1, respectively. Biotic crystals were treated to remove all organic matter to show that the bands are from inter and intra-crystalline occlusions. SEM and TEM was used to identify these occlusions, which to the author's knowledge have not been reported for bacterial carbonates before.

The work applies established analytical solutions in an innovative manner for the fingerprinting of biotic CaCO3 crystals, setting the basis for the use of FTIR and Raman spectroscopy as an identification technique of biotic crystals in mixed environments such as construction mortars.Infrared and Raman spectroscopy for the fingerprinting of microbially-induced calcium carbonate precipitation

Poster presentations

Poster 4

Diagenetic alterations undergone by Rhynchonelliform brachiopod shells throughout fossilization – Multiscale and analytical approach

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The atomic force microscopy (AFM) allowed understanding the hierarchical organization of the layers of extant brachiopod shells (Gaspard & Nouet, 2016). But after death, modifications take place leading to light or heavy alterations in these archives throughout the process of fossilization according to free lying or quick burial.

Our observations were carried on equivalent extant as extinct two or three-layered shells. They are: *Compsothyris racovitzae* from Antarctica (recent) and *Cyclothyris difformis* (Early Cenomanian), Rhynchonellida; *Tichosina sp*. from the Caribbean Sea, *Moutonithyris dutempleana* (d'Orb.) (Early Cenomanian), *Sellithyris sella* (Sow.) (Aptian), *Sellithyris cenomanensis* Gasp. (Mid. Cenomanian), *Phaseolina phaseolina* (Lamk) (Upper Cenomanian), respectively a recent and fossils Terebratulida. In the shell thickness the inorganic material is intimately organized with organic materials allowing consider the shell as a "biomaterial". Several means of observation using the scanning electron microscope (SEM), mapping elements and the (AFM) allow highlight the modifications in these low-Mg calcite shells at different scales. Cross sections in the shells are the supports for these observations.

Early diagenesis can be observed soon after death. It is possible to found fossils with the organic material entirely or partly preserved (*S. sella, S. cenomanensis*). Recrystallizations appear sometimes lightly (*S. sella*, *S. cenomanensis*) or heavily (*C. difformis, M. dutempleana, Ph. phaseolina*) even both in the same shell. When the organic material disappears, a bacterial activity is often present. In case of secondary calcite or silicic alterations, the nanoparticles, well individualized in the elements forming the inner layers (fibrous and prismatic/columnar) in recent shells, tend gradually to be agglomerated in fossils leading finally to nodules. Physical/chemical palaeoenvironmental context act for these alterations that appear partly or in the whole shell.

Poster 5

Mechanical origin of the crossed-foliated ultrastructure in mollusks

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Solving the puzzle of what drives of the morphogenesis of various molluscan shell ultrastructures has been the intrigue of biologists, mineralogists and material scientists alike for decades if not centuries. Whereas, historically, the majority of research was focused on the nacreous and the prismatic assemblies, the most common crossed ultrastructures, such as crossed-lamellar and the crossed-foliated architectures, were mostly overlooked. This is because of the spatial complexity of these assemblies that include multi-level hierarchical organization of the mineral building blocks and an intricate crystallographic relationship between them.

In this work, we describe the formation of the crossed-foliated ultrastructure in the shell of the limpet *Patella vulgata*. Detailed electron microscopy based imaging and diffraction analysis in 3D is used to provide a comprehensive structural and crystallographic information on the formation and spatial organization of the mineral building blocks during the growth of this ultrastructure. These data were then used to develop a model for the hierarchical self-assembly of this ultrastructure driven by mechanical forces generated as the result of a complex interplay between the formation of a single mineral unit and the growth of the shell as a whole. The model is supported by first-principles mechanical simulations.

Poster 6

Random crystal orientations in coral skeleton centers of calcification become more ordered with acidification

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We measured crystallographic c-axis orientations of aragonite crystals in the centers of calcification of *Stylophora pistillata* coral skeletons grown at two different pH values, 7.2 and 8.0[1], using polarization-dependent imaging contrast (PIC) mapping[2] with PhotoEmission Electron Microscopy (PEEM)[3, 4] at the oxygen K-edge[5, 6]. We observed that the crystallites in the centers of calcification of coral skeletons grown at pH 8.0 contained a more even and random distribution of orientations when compared to those grown at pH 7.2. These results are consistent with previously published electron back-scatter diffraction (EBSD) data from the same species of coral[7], but push the envelope further with 60 nm spatial resolution and quantitative analysis of c-axis orientation distributions across 4 samples.

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Poster 7

Ultrastructures and mechanical properties of a flexible plate of calcium carbonate in door snails

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Commonly known as “door snail”, Clausiliidae possess a synapomorphy called a clausilium, a plate attached to the columella by a stalk. Even though clausilium is composed mainly of aragonite-type calcium carbonate, it is characterized by flexibility. However, the microstructures providing the flexible nature have not been clarified. In this study, we examined in detail the microstructure of the shell and the clausilium of *Stereophaedusa japonica* and *Euphaedusa tau* to elucidate their flexible structure.

From detailed observation of the cross-section of the flexible plate and stalk, clausilium is composed of three layers consisting of aragonite blocks with various shapes, such as cylindrical columns shorter than 3 μm, long needles with lengths of several tens of micrometers, and spherulitic grains with diameters of several hundred nanometers. In the outermost layer, the columns are arranged perpendicularly to the plate surface. In the second layer, the long needles that are parallel to the surface are partially stacked like a cross-lamellar structure. The central layer of the plate and stark is composed of randomly arranged spherulitic grains with a relatively large amount of organic matter. The nano-indentation test showed that the hardness and modulus depend on the microstructure of the layers. The hardness of the central layer is lower than that of the other layer. The combination of various layers would provide unique mechanical properties of clausilium.

Poster 8

Biomineralization of avian eggshell through the study of abnormal eggshell formation

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Remarkable progress in biomineralization knowledge has been reached. However, there are many questions still waiting to be addressed. Among other biomineralized systems, the avian eggshell shows distinctive features, i.e. the absence of cells intermixed with the mineralized structure and the rapidity of mineralization. In fact, 5 g of calcium carbonate are deposited in an 18 h period by a precise arrangement of spatio-temporal sequential deposition of macromolecules and inorganic ions. Three main approaches have been utilized to understand the role of eggshell components for controlling inorganic crystal nucleation, growth, structure and texture of the eggshell: (i) mechanical, crystallographic and microstructure characterization, and chemical composition, (ii) characterization of the assembly process during biological development, including oviduct microstructure, gene expression and sequential secretion, (iii) characterization of the *in vitro* effect of isolated or modified eggshell macromolecules on calcium carbonate nucleation and growth and eggshell reconstitution. Several specific proteins and proteoglycans have been isolated, characterized and postulated to be involved in the control of eggshell biomineralization. However, detailed mechanisms of the interaction of organic specific macromolecules and the inorganic moiety is far from being elucidated. Main studies on eggshell formation have been done with normal egg. However, there are special circumstances in which hens lay abnormal egg. Either changes in the organic composition of such eggshell or the experimental induction of eggshell formation by the administration of determined molecules, are scarcely reported. Here we report the reversible effects of specific molecules mimicking eggshell abnormal formation.

Keywords: Biomineralization, chicken, eggshell, misshaped eggs

Poster 9

Cryo-stopped flow methodology for studying pre-nucleation species via solid-state Nuclear Magentic Resonance

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The nucleation and growth of crystalline solids occurring in precipitation events from aqueous solutions is a central focus impacting many scientific areas, from organic to inorganic chemistry or material science.[1] Recently, the classical nucleation and growth theory (CNT) has been challenged by the observation of stable and soluble pre-nucleation species (PNS) prior to the formation of many solid materials, implying that the non-classical crystallization pathway (NCP) might play a role in this process. PNS are (meta)stable and soluble species – highly dynamic nanometric ionic entities in equilibrium with free ions that spontaneously form in solution before the nucleation of the first solid phase. In a biomineralization context, PNS have been described and reported for calcium phosphates,[2] carbonates[3] and oxalates phases.[4] Such species are challenging to characterize due to their soluble nature, nanometric size, dynamic behaviour and short lifetime. Thus, new methods are required to monitor these species' evolution in real-time at nanoscale resolution.

Solid-state Nuclear Magnetic Resonance (NMR) has been used to study the structure and composition of biominerals. However, using NMR to access phases and species out of equilibrium, such as PNS, can be challenging. Thus, in this work, we introduce the cryo-stop flow methodology to follow the evolution of calcium phosphate PNS at different time points to gain more insights into their structure and composition. This cryo-stop flow methodology enables the addition and mixing of independent Ca^{2+} and phosphate solutions at controlled concentrations, volumes and rates. The subsequent freezing at defined time points enables to stop the reaction and analyze transient PNS by low-temperature solid-state NMR. Here, we describe the set-up and experimental conditions optimization to study calcium phosphate PNS at the early stages of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) formation, the main component of bones and teeth.

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Poster 10

Impact of foreign elements on the phase transformation of amorphous calcium carbonate - a bioinspired in vitro study

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Many calcifying organisms use amorphous calcium carbonate (ACC) as a precursor phase which transforms into a stable carbonate phase such as calcite and aragonite [1]. One family of dinoflagellates, the *Thoracosphaeraceae*, forms highly structured calcite shells as part of their life cycle. In these microalgae, intracellular MgCaP-rich bodies take part in the Ca-transport and concentrating mechanism and can be considered a calcite precursor [2]. According to a working hypothesis, these bodies encompass a Mg- and P-containing ACC that transforms into a low-Mg calcite inside the extracellular matrix. So far, the influence of Mg and P on ACC structure and transformation has only been investigated separately [3]. The goal of this project is to investigate the influence of foreign elements on synthetic ACC and its transformation into other polymorphs. Inspired by calcification in dinoflagellates, we synthesised a Mg-P-ACC using different phosphorus sources (e.g., inorganic P_i, polyphosphates) and studied its transformation. This approach will be applied to other elements, such as zinc, iron, and strontium, to gain an understanding of metal incorporation in calcium carbonates. In some organisms, e.g., aragonitic shells of marine bivalves, these serve as a proxy for environmental reconstruction [4]. After optimisation of ACC synthesis, the ACC was transformed into a stable calcium carbonate by heat treatment. Both, the ACC precursor, and the transformed product are comprehensively characterised by vibrational spectroscopy (IR, Raman, and ssNMR), TGA, ICP-OES, and SEM.

Overall, this project aims to constrain the influence of foreign cations on ACC structure and their effect on subsequent phase transformations. These *in vitro* studies provide a synthetic model system that will facilitate the understanding of more complex biological calcification processes.

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Poster 11

Deciphering the role of organic matter in the biomineralization process of marine sponge spicules: a solid-state NMR investigation

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Introduction

Sponges, one of the most primitive animals, are exclusively aquatic sessile organisms. Siliceous sponges are mainly represented by two classes, Demospongiae (Sollas, 1885) and Hexactinellida (Schmidt, 1870). Their internal skeleton is composed of spicules that serve to stiffen the soft body, helping the animal with protection and anchoring. During the biomineralization process, silica plays a dominant role in skeleton formation, but nothing would be possible without organics intercession.

Objectives

In this study, we used solid-state NMR spectroscopy to better understand the role of organics during the biosilicification process. Our goal is to more precisely depict the organo-silica interface to discriminate the different species among the great variety of specimen.

Materials and Methods

Solid-state NMR is one of the most versatile tools of characterization in materials science. However, this technique suffers from low sensitivity and long acquisition times.

Natural specimen of spicules contain a big amount of silicon but a small amount of carbon (*e.g.*, 2-7 wt% C). As no isotopic enrichment is possible and ^{13}C isotope is only 1.1 at% nat. ab., it is difficult to collect exploitable data from carbon.

Results

^{29}Si and ^{13}C NMR spectra were compared to assess the importance of the taxon. Then, two-dimensional (2D) experiments showed heteronuclear correlations that depict the organo-mineral interface but suffer from a poor signal-to-noise ratio.

Conclusion

We now plan to improve the study by using DNP-NMR to enhance the signal and perform a ^{1}H-^{29}Si-^{13}C triple resonance experiment.

Poster 12

Understanding bone diagenesis: Effect of fluorine substitution under hydrothermal controlled conditions

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Bone diagenesis is a complex phenomenon that involves the modification of the physicochemical, histological, and mechanical properties of bone remains in each burial environment. One of the most common diagenetic processes in archaeological and paleontological sites is the incorporation of fluorine in bone mineral which has been widely recorded on many sites [1-5]. However, information is lacking about how bone mineral changes with ion substitution processes during recrystallization in different environmental conditions. In this study, we induce bone fluoridation under controlled hydrothermal conditions. Fragments of an adult pig femur (*Sus scrofa domestica*) were treated with sodium fluoride (NaF) solutions (control-0.0, 0.01, 0.1, and 1 M) and at different temperatures (25, 50, 100, and 200°C) for 24 hours. Changes in bone chemistry and structure were characterized using complementary analytical techniques such as infrared spectroscopy (FTIR), X-ray diffraction (XRD) and electron microscopy. The results showed that fluorine is incorporated into the apatite structure and its substitution increases with temperature and NaF concentration as deduced from the contraction of unit cell size (from 532 to 524 $Å^3$). Hydrothermal conditions also produced an increase in apatite crystallite size with temperature with is greater in fluorine bearing solutions. Other notable changes were the progressive increase of the 575 cm^{-1} band intensity in the $\nu_4(PO_4^{-3})$ region and the inversion of the 605 cm^{-1} band relative to 563 cm^{-1} bands which the incorporation of fluorine in apatite structure. These results are important for understanding the changes occurring in bone during burial and/or diagenesis in stratigraphic sequences of archaeological and paleontological sites.

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Keywords: Bone diagenesis, Apatite, Fluoridation, recrystallization, hydrothermal

Poster 13

Magnetotactic Bacteria: more than nanomagnets

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Magnetotacic bacteria are known to intracellularly biomineralize the iron oxide magnetite and / or the iron sulfide greigite. Alternatively, metal sulfides are a common group of extracellular bacterial biominerals. Only few cases of intracellular biomineralization have been reported in this group, mostly limited to greigite (Fe_3S_4) in magnetotactic bacteria.

Here, we report the intracellular but periplasmic biomineralization of copper sulfide by the magnetotactic bacterium *Desulfamplus magnetovallimortis* (strain BW-1) that is known to mineralize greigite and magnetite (Fe_3O_4) in the cytoplasm. BW-1 produces hundreds of spherical nanoparticles, composed of 1-2 nm substructures of a poorly crystalline hexagonal copper sulfide that remains in a thermodynamically unstable state. Differential proteomics suggests that periplasmic proteins, such as a DegP-like protein and a heavy metal-binding protein, could be involved in this process. The unexpected periplasmic formation of copper sulfide nanoparticles in BW-1 reveals previously unknown possibilities for intracellular biomineralization.

Poster 14

APPLICATION OF CALCIUM PHOSPHATES: IN VIVO TEST IN SHEEP

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Treatments for bone loss are research topics and involve different areas of scientific knowledge, engineering, physics, chemistry, biology and biomedicine. The biomaterials that stand out as replacement in bone structure treatments are hydroxyapatite, β and α calcium phosphate, biphasic hydroxyapatite/calcium phosphate β and α and hydroxyapatite matrix nanocomposite biomaterials. These biomaterials stand out as bone substitutes because they present a crystallography similar to that of human skeleton bone apatite, being bioactive and biocompatible. Nanostructured biphasic bioceramics are researched and show potential to be bone substitutes in surgical repairing procedures and reconstruction of bone tissue. This project was developed based on research of granular biomaterials of HA, β-TCP and biphasic composition HA/β-TCP=20/80%. All biomaterials were characterized by different techniques: scanning electron microscopy, atomic force microscopy, confocal microscopy, optical microscopy, polarized light microscopy. The interest of this research was to evaluate the performance of biomaterials in in vivo tests for the time period of 180 days, in relation to osseointegration and the formation of neoformed bone tissue and determine which biomaterials presented potential as bone replacement for biomedical applications. The study was conducted on sixteen healthy half-breed Texel sheeps with 12 months old. Two 4-mm non-critical defects were produced on the medial tibial diaphyseal of each tibia with the aid of a dental drill. For the adequate implantation of the biomaterials, initially the granulated material was deposited in a sterile vat and hydrated with the animal's own arterial blood. The animals were euthanized 180 days after implantation and implanted bone fragments were collected. The results found are encouraging and demonstrate that the granulated microporous biomaterials of calcium phosphate proves to be ability to repair and bone reconstruction for the test in vivo evaluated, revealing the osseointegration and bone formation. The results obtained from the biopsies containing the biomaterials revealed similar osseointegration behaviours, with excellent biomaterial/bone tissue interface and greater bone formation, presenting smaller amounts of remaining biomaterial. It is due to the contribution of porosity and phase composition parameters to the biodegradation process.

***Keywords**:* β-calcium phosphate, hydroxyapatite*, in vivo* testing, bone neoformation.

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Topic 3 Vertebrate mineralized tissues in health and disease

Keynote lecture

Enzymatic patterning of bone mineralization at the microscale for crossfibrillar mineral tessellation: The Stenciling Principle

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The Stenciling Principle for extracellular matrix mineralization describes an enzyme-substrate, double-negative regulatory process (inhibition of inhibitors) that promotes mineralization in bone and other mineralized tissues, whereas the default condition of inhibition alone prevents mineralization elsewhere in soft connective tissues. It relates to both small-molecule (e.g. pyrophosphate) and protein (e.g. osteopontin) inhibitors of mineralization, and promoters (enzymes, e.g. TNAP, PHEX) that degrade the inhibitors to permit and regulate mineralization. In this process, an organizational microscale motif for bone mineral arises that we call crossfibrillar mineral tessellation where mineral formations – called tesselles – geometrically approximate prolate ellipsoids and traverse multiple collagen fibrils (laterally). Tesselle growth is directed by the structural anisotropy of collagen, being spatially restrained in the shorter transverse tesselle dimensions. Temporo-spatially, the tesselles abut in 3D (close ellipsoid packing) to fill the volume of lamellar bone extracellular matrix. Poorly mineralized interfacial gaps between adjacent tesselles remain discernable even in mature lamellar bone. Tessellation of a same, small basic unit to form larger structural assemblies results in numerous 3D interfaces, allows dissipation of critical stresses, and enables fail-safe cyclic deformations in healthy bone. Incomplete mineral tessellation in osteomalacia may explain why soft osteomalacic bones buckle and deform under loading.

Oral presentations

T3 01

Biological control of enamel mineralization is dependent on phosphorylation of amelogenin.

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Amelogenin (AMELX), the major enamel matrix protein, has a sole post-translational modification – phosphorylation at Ser16. *In vitro* mineralization studies revealed that phosphorylated AMELX stabilizes amorphous calcium phosphate (ACP) and inhibits formation of apatite. To assess the role of this sole phosphorylation in amelogenesis we generated a knock-in (KI) mouse model with a Ser16 to Ala substitution. Using multiple approaches, we have characterized structural, chemical and mechanical properties of forming and mature incisal enamel in KI and WT mice. KI mice exhibit a severe enamel phenotype, manifested by a thin enamel layer; the lack of enamel rods, the hallmark of mammalian enamel; and ectopic calcifications. The *c*-axes of enamel crystals in rod-less inner KI enamel are co-oriented at the Dentin-Enamel Junction (DEJ) and become progressively misaligned toward the surface. The outer KI enamel comprises spherulites, that are structurally consistent with abiotic formation. Our studies of enamel formation also revealed that the rate of transformation of ACP to an apatitic crystal phase is accelerated in KI enamel. Furthermore, we found that mineral density at the onset of enamel formation is twice higher in KI vs WT. These observations are consistent with the results of our *in vitro* mineralization experiments, showing that phosphorylated AMELX controls the rate of mineralization and ACP-apatite transformation. Moreover, while at the onset of enamel secretion ameloblasts look normal, they never develop Tomes' processes, consistent with the absence of enamel rods, and exhibit progressive cell pathology, manifested by loss of polarization and formation of secretory aggregates throughout the ameloblast cell layer. These and other results to be presented demonstrate that the sole phosphorylation of AMELX is crucial for biological control of enamel mineralization, and in maintaining ameloblast phenotype and function. Supported by NIH DE DE029211 (HCM & EB).

Keywords: enamel, amelogenin, phosphorylation, biological control

T3 O2

Low phosphate diet differentially affects bone mineralization in mice deficient in Bone Sialoprotein, Osteopontin or both.

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SIBLING proteins DMP1 & MEPE play a role in phosphate (PO_4) metabolism. We postulated that cognate Bone Sialoprotein (BSP) and Osteopontin (OPN) may also be involved in body PO4 handling. We generated mice deficient ($^{-/-}$) in either BSP[§], OPN or both (DKO)[§ §], and found that $BSP^{-/-}$ mice are hypophosphaturic while the $OPN^{-/-}$ are not. $BSP^{-/-}$ mice display normal PO_4 serum levels, normal/low serum FGF23 together with features of impaired PO_4 gut absorption. Thus, we hypothesized that a low PO_4 diet would induce a more severe mineralization defect in $BSP^{-/-}$ bones.

5 month-old WT, $OPN^{-/-}$, $BSP^{-/-}$ and DKO male mice were fed diets containing either normal (ND, 0.55%) or low (LP, <0.05%) inorganic phosphorus content. At d7 they were put in metabolic cages for 1d for urine/feces collection. After killing at d14, femur and blood were sampled. Bone mass was assessed by µCT and bone remodeling by histomorphometry (HM).

Under LP, we found at d7 a dramatic drop in urinary PO_4 levels compared to ND, with lower values in $BSP^{-/-}$ mice while serum levels remained normal in all groups. Bone mass was 38 and 20% lower than ND in $OPN^{-/-}$ and $BSP^{-/-}$ mice, respectively. HM results [median(IQR)] are presented in the table (*p<0.05 vs respective ND, $p<0.05 vs BSP and OPN under LP).

	WT		$BSP^{-/-}$		$OPN^{-/-}$		DKO	
Diet	ND	LP	ND	LP	ND	LP	ND	LP
OS/BS, %	12.2 (7.8)	34.1 (4.2)*	7.4 (6.6)	30.9 (23.4)*	3.9 (2.5)	22.5 (13.5)*	3.1 (2.4)	30.5 (23.5)*
O.Th, µm	1.1 (1.1)	1.7 (1.0)	2.5 (0.8)	3.8 (2.0)	2.7 (0.7)	6.8 (1.3)*	1.7 (0.7)	8.6 (1.8)*$
MAR, µm/d	0.99 (0.55)	0.47 (0.25)	1.14 (0.21)	0.31 (0.44)*				
sLS/BS, %	5.7 (2.6)	17.4 (13.8)*	6.8 (9.5)	17.8 (89.8)*				
dLS/BS	3.5 (2.2)	6.4 (2.4)	5.1 (4.3)	1.9 (2.4)				
MS/BS, %	7.4 (3.2)	15.5 (9.3)*	9.9 (8.1)	10.1 (6.3)				
MS/OS, %	0.60(0.18)	0.42 (0.29)	0.73(0.53)	0.26 (0.27)*				

Conclusion: LP induces a more severe osteomalacia in mice deficient in BSP, OPN or both compared to WT with the worst phenotype in DKO. Overall, our results indicate that both OPN and BSP are involved in PO_4 metabolism, with no/limited vicariance between the 2 proteins

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T3 O3

Altered bone health in laying hens is associated with FGF23 overexpression in the medullary bone

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For the production of each egg, the hen must allocate about 2 g of Ca for eggshell formation, which originates from its diet and from bone mobilisation. As the hen lays between 300 and 500 eggs during its carrier, the challenge to Ca homeostasis is high, and pathological signs such as osteoporosis, deviations or fractures of the wishbone, or even fractures of long bone, show an increasing prevalence at the end of the production cycle. In their less serious forms, they compromise animal welfare, but they can also lead to culling and losses, or to the early termination of a flock. This sets the need to better understand the physiological mechanisms of calcium homeostasis in hens, particularly those involving bone tissue. At sexual maturity the hen develops a specific type of medullary bone, which is subject to daily cycles of bone remodelling and thus serves as a temporary store of Ca. The team's recent work provides new data on the effectors of calcium transport through the intestinal epithelium, on the mechanisms of bone turnover and their regulation. It is consistent with previous ones in highlighting the central role of vitamin D and its metabolites. Moreover, it also highlights a factor that had not yet been studied in details in the laying hen, Fibroblast Growth Factor-23 (FGF23). It is expressed by the medullary bone when eggshell synthesis is active in the uterus and bone mobilisation is maximal. The expression of its putative receptors (FGFRs) and coreceptor (KL) in the kidney suggest that it could exert its action by promoting the urinary excretion of P resulting from osteolysis. In addition, by inhibiting the synthesis of the active form of vitamin D3 or by activating its degradation, it would exert a negative feedback on Ca and P retention. FGF23 is overexpressed during in the late laying hen compared to the peak laying hen. This opens up the possibility of research into whether FGF23 is the cause or only a marker of deregulation of phosphocalcic metabolism

T3 O4

Multicellular spheroids containing synthetic mineral particles to investigate breast precancer malignancy potential according to the mineral type

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Abstract

Mineral particles that form in soft tissues in association with disease conditions are heterogeneous in their composition and physiochemical properties. Hence, it is challenging to study the effect of mineral type on disease progression in a high-throughput and realistic manner. For example, most early breast precancer lesions, termed ductal carcinoma *in situ* (DCIS), contain microcalcifications (MCs), calcium-containing pathological minerals. The most common type of MCs is calcium phosphate crystals, mainly carbonated apatite; it is associated with either benign or malignant lesions. *In-vitro* studies indicate that the crystal properties of apatite MCs can affect breast cancer progression. A less common type of MCs is calcium oxalate dihydrate (COD), which is almost always found in benign lesions. We developed a 3D tumor model of multicellular spheroids of human precancer cells containing synthetic MC analogs that link the crystal properties of MCs with the progression of breast precancer to invasive cancer. Using this 3D model, we show that apatite crystals induce Her2 overexpression in DCIS cells. This tumor-triggering effect is increased when the carbonate fraction in the MCs decreases. COD crystals, in contrast, decrease Her2 expression in the spheroids, even compared with a control group with no added MC analogs. Furthermore, COD decreases cell proliferation and migration in DCIS monolayers compared to untreated cells and cells incubated with apatite crystals. This finding suggests that COD is not randomly located only in benign lesions—it may actively contribute to suppressing precancer progression in its surroundings. Our model provides an easy-to-manipulate platform to better understand the interactions between mineral particles and their biological microenvironment. A better understanding of the effect of the crystal properties of MCs on precancer progression will potentially provide new directions for better precancer prognosis and treatment.

T3 O5

Ultrastructure of mineral ellipsoids at bone-calcium phosphate interface in bone healing process

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Bone structure at the micro and nanoscale has been revisited in recent years (1-4). An architecture based on sub-micrometer ellipsoid features mineralized within collagen bundles has been proposed as a new hierarchical level of bone (5-6). In this work, we combine synchrotron x-ray (SRuCT) tomography with focused ion beam-scanning electron microscopy (FIB-SEM) and transmission electron microscopy (TEM/HRTEM) to characterize the ellipsoids ultrastructure in the woven bone formed during a healing process in rat tibia defect 21-day implanted with a biodegradable carbonated hydroxyapatite/alginate microspheres. After implantation, microsphere fragmentation improves osteoconduction, and the newly trabecular bone-like network grows, filling the space between the microsphere fragments and attaching to the fragment surface. Mineral tissue is nucleated inside micrometer and sub-micrometer pores. FIB-SEM slice and view tomography showed that the trabeculae volume is filled with osteocyte-lacunae and mineralized ellipsoidal structures oriented in different directions. The presence of these motifs over the entire FIB-SEM series suggests that these structures represent a regular pattern of mineral deposition nucleated in association with collagen bundles. TEM/HRTEM images showed that each ellipsoid comprises a packet of non-ordered mineralized collagen microfibril clusters with a mean length of 1.6 μm and width of 0.7 μm. These structures cover the biomaterial interface, but the ellipsoidal morphology is not identified inside sub-micrometer pores. In this confined condition, the mineralized tissue presents a different ultrastructure than woven trabecular ellipsoids but with a similar electron diffraction pattern. Inside sub-micrometer pores, MT organizes in nearly linear arrangements of mineralized collagen microfibril. The MT nucleation occurs in pores smaller than one micrometer located at more than 5 μm from cells, in a process not apparently controlled directedly by cells.

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T3 O6

The dental and periodontal mineralization defects related to FAM20A gene mutations

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Introduction: FAM20A is a paralog of FAM20C kinase in charge of secreted protein phosphorylation, notably in enamel. FAM20A is a pseudo-kinase which acts indirectly through binding and activating FAM20C. *FAM20A* loss-of-function variants are associated with Enamel Renal Syndrome - ERS (OMIM# 204690) presenting with amelogenesis imperfecta, tooth eruption impairment and gingival fibromatosis.

Our CRMR Center ensures the follow-up of a ERS patient cohort. Our previous studies on teeth and gingiva suggested that an ectopic mineralization process would intervene in ERS pathophysiology. Here, we explored an animal model, the *Fam20a* knock out (KO) mouse. Heads from wild-type and KO mice (3-,5, 7-D PN, 7 week- and 14-month-old) were analyzed (SEM, histology, TEM and amelogenin immunogold labelling). The observed mouse dental phenotype was confronted to our clinical CRMR dataset on ERS patients affected by different *FAM20A* mutations.

KO mouse amelogenesis was biphasic and disordered. The innermost enamel was deposited by ameloblasts, albeit with an abnormal rod-interrod pattern. Starting 5-D postnatally, ameloblasts loose their polarity and intercellular junctions, becoming uncoupled from the forming enamel matrix. They secreted an amelogenin-containing material in ectopic positions : laterally, towards and into the adjoining follicular sac. Ameloblast cell death and embedding in minerals also intervened. The resulting « enamel » agregated electron-dense amelogenin-positive nodules and cell ghosts. Concurrently, a collagen-rich extracellular matrix was deposited, mineralized and fused to « enamel ».

Conclusion: The coronal external layer of ERS teeth would be produced by an ectopic mineralization process involving both the enamel organ and follicular sac. The resulting anchoring of enamel to the periodontium might play a role in tooth eruption arrest, as supported by the characteristic radiological profile of ERS patients with different disease grades.

Keywords: ectopic mineralization, enamel, rare diseases

T3 O7

Effect of MOP consumption on calcium metabolism and gut in ovariectomized rat model

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Osteoporosis is characterized by a bone remodeling imbalance leading to bone mass and mechanical properties loss, increasing fracture risks. Mother of pearl demonstrated protective effect against bone loss when ingested by osteoporosis models as ovariectomized (OVX) mice or rats. This may be related to a direct effect on resorption and bone synthesis as seen on bone cells in vitro. Nevertheless, an indirect effect through the gut-bone axis or calcium metabolism can also be involved. We therefore investigate gut-related effect of MOP consumption in ovariectomized rats. 46 animals were sham operated or OVX at 16-week-old and last ones either fed with normal, 0.25% MOP or calcium (Ca) complemented diet for 3 months. FGF23, PTH, calcitriol, calcium and phosphate serum levels were quantified and didn't show any effect other than ovariectomy, except for serum phosphate increase in MOP group compared to OVX ($p<0,025$). Intestinal samples went through mRNA extraction and QPCR assessment: CaV 1.3 duodenal expression was significantly higher in MOP fed group than in OVX ($p<0,025$), SHAM and Ca groups ($p<0,05$), TRPV6 significantly lower than SHAM and OVX groups ($p<0,05$). Cecal cadherin 17 expression decreased significantly in MOP group compared to Ca and SHAM ($p<0,05$), claudin 12 in Ca ($p<0,05$) and MOP ($p<0,025$) groups compared to OVX and claudin 2 expression increase in Ca ($p<0,025$) and OVX ($p<0,001$) compared to SHAM. Differences between SHAM and OVX groups can be related to estrogen loss while theses between OVX and Ca groups explained by the 0,12% dietary calcium increase. Duodenal changes between MOP and Ca groups could result in MOP-induced higher postprandial calcium absorption and lower reabsorption during fasting while cecal paracellular transport associated proteins expression decrease could diminish epithelial calcium permeability, thus calcium absorption as much as its loss. However, theses expression results should be complete by functional assessments.

Keywords: Calcium absorption; Bone loss; MOP

T3 O8

Lack of Amelogenin Phosphorylation Leads To Acidification Of Secretory Enamel

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Amelogenin (AMELX), the major enamel matrix protein (EMP), has a single phosphorylation on Serine 16. Phosphorylation enhances AMELX's capacity to stabilize amorphous calcium phosphate (ACP) and inhibit apatite crystal formation *in vitro* [1,2]. To study its effects *in vivo* we developed a knock-in (KI) mouse model with a Ser16 to Ala substitution in AMELX, which prevents AMELX phosphorylation. KI mice exhibit a severe phenotype with thin hypoplastic enamel, lack of the decussating rod pattern, and a higher rate of ACP to apatite transformation [3].

Objective: To test the hypothesis that accelerated enamel mineralization in AMELX-Ser16Ala KI mice induces local acidification that affects the EMPs and the forming enamel mineral phase.

Methods: Mandibular incisors from 8-week-old wild-type (WT) and KI mice were isolated and their ameloblast layer was removed. Incisors were freeze-dried and immersed in bromocresol purple (BCP) pH indicator to visualize pH differences in forming enamel of KI and WT. To assess the structural differences in EMPs and enamel mineral phase, we conducted FTIR microspectroscopy in reflectance mode.

Results: BCP staining showed the secretory-stage enamel was more acidic in KI vs. WT. A distinct pattern of alternating low and high pH bands typical of maturation-stage was absent in KI enamel. Consistent with the higher initial mineral density in KI enamel (unpublished), the mineral to protein ratio was greater in KI secretory stage enamel. The ratio of acidic phosphate to phosphate was higher in KI enamel, consistent with mineralization in an acidic environment. Differences in Amide I band were also observed in secretory enamel, implying differences in the EMPs conformation in WT and KI. Together, our observations demonstrate that the lack of AMELX phosphorylation leads to acidification of secretory enamel and affects its mineral phase and EMP conformation. Supported by NIH Grant DE029211 (HCM & EB).

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T3 O9

FT-IR spectroscopic imaging of human aortic valve biomineralization and protein secondary structure combining hard and freeze cutting technique

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Calcified aortic valve disease (CAVD) is the most common valve disease. Its pathology is beside fibrosis characterized by aortic valve (AV) biomineralization. Dystrophic hydroxyapatite intercalation but also neoosteogenesis are research foci. FT-IR (fourier transformed infrared) spectroscopic imaging provides information on mineral composition and secondary protein structure. Thin sectioning of calcified AV tissue w/o prior decalcification for FT-IR spectroscopy is a challenge and offers insights in AV pathogenesis. Aim of the study is the spectroscopic molecular characterization of AV tissue pathomorphology.

After 5 µm sectioning, FT-IR spectra of explanted, formalin fixed and Technovit 9100 embedded AV tissues were acquired in transmission mode (FT-IR-spectrometer, Vertex 70; IR microscope, Hyperion 3000; Bruker-Optik GmbH). Fuzzy k-means cluster and principal component analysis (PCA) are used to evaluate the spectroscopic data (MATLAB, Mathwork. Inc.). Cusps tissue counterparts were fixed, decalcified and cryosectioned for FT-IR spectroscopy in parallel. Movat's Pentachrom and Masson's trichrome stain were used to visualize tissue pathomorphology.

Technovit penetration varies in strongly calcified AV tissues. Hydroxyapatite characteristics are evaluable in areas not affected by the plastic entry. Areas of the thin section not showing noticeable molecular interaction with the embedding medium were selected. Changes of protein secondary structure as well as variations in the spectral regions of phosphate and carbonate bands were analyzed and interrelated. Decalcified, cryosectioned tissue counterparts can further provide chemical information of protein secondary structure in FT-IR spectroscopy.

Molecular characteristics of AV biomineralization and protein secondary structure can be investigated by hard and freeze cutting techniques in a parallel workflow. Nevertheless, the establishment of alternative embedding strategies is envisioned.

T3 O10

Dynamics of extracellular matrix remodeling associated with mineralization during the hypertrophic differentiation of articular chondrocytes

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Abstract

The mineralization of cartilage is a pivotal step in the endochondral ossification process, in which cartilage is replaced by bone. Such a process occurs during the growth of long bones and is reactivated in osteoarthritis. It depends on the hypertrophic differentiation of chondrocytes and is associated with a profound remodeling of the cartilage matrix.

Here, we studied the dynamics of matrix mineralization and modifications during the hypertrophic differentiation of articular chondrocytes thanks to an *in vitro* model of progressive differentiation of immature murine articular chondrocytes (iMACs) into prehypertrophic (Prehyp) and hypertrophic (Hyp) chondrocytes. Kinetics of Prehyp to Hyp differentiation were carried out to follow the mineralization of the matrix and its remodeling by immunofluorescence, biochemical and molecular approaches, as well as by physicochemical approaches, including atomic force microscopy (AFM), scanning electron microscopy associated with energy dispersive X-ray spectroscopy (SEM-EDX), attenuated total reflection infrared (ATR-IR) analyses and X-ray diffraction (XRD).

Results show the formation of a mineral phase 7 days after the induction of hypertrophy, which spreads within the matrices to form poorly crystalline carbonated hydroxyapatite. Hyp differentiation also induces a matrix turnover characterized by a rapid drop in type II collagen and aggrecan and the concomitant appearance of type X collagen. This is accompanied by an increase in the concentration and the enzymatic activity of MMP-13, the main collagenase of cartilage. Chemical mapping also shows that phosphorus is concentrated in cellular debris, which may originate partly from apoptosis. These findings provide new insights into the dynamics of matrix remodeling resulting from Hyp differentiation. They also provide relevant guidelines for the understanding of remodeling events that may occur at the pathological osteochondral junction (e.g. during osteoarthritis).

Keywords: chondrocyte, hypertrophic differentiation, mineralization, cartilage, collagen, aggrecan

Poster presentations

Poster 15

Chicken eggshell quality in the late phase of the laying cycle

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The laying hen uses large amounts of calcium each day to form the eggshell which is composed of 95% of calcium carbonate on calcitic forms and 3.5% organic matter that directly influence the biomineralization process. Shell mineralisation occurs at night when the animal has no access to feed. The calcium is then taken from the bone which is regenerated during the day. For reasons of sustainability, commercial laying hens are being reared later and later, but as they become older, calcium metabolism deteriorates, leading to shell strength defects and spontaneous bone fractures.

In this work, we investigated the effect of such extension of the laying period on the eggshell quality. Commercial laying hens were reared in cage-free system to 32-34 (peak of lay), 71-73 (current laying cycle) and 94-99 (extended laying cycle) weeks of age. Eggshell quality parameters (breaking strength, dry weight, thickness and toughness) were determined on eggs collected at each period of age. As expected, eggshell breaking strength and thickness significantly decreased during the three tested periods of the laying cycle. More surprisingly, eggshell weight is significantly lower at the oldest age (94-99 weeks). Eggshell toughness is decreased in both 71-73 and 94-99-week-old hens, when compared to 32-34-week-old hens.

Eggshell mechanical properties are due to the mass of materials and to the fabric of the shell. The latter comprises the morphology, size and orientation of crystals that define the shell texture and ultrastructure. As the hen ages, shell mass remains constant, while egg weight increases, resulting in a reduction in shell thickness. The decrease of eggshell mechanical quality parameters observed between 32-34 and 71-73 weeks is likely due to this decrease of shell thickness combined with modifications of the shell fabric and eggshell texture as observed by the decrease in shell toughness. In older hens (94-99 weeks of age), shell mass is reduced. This effect on shell mass was not reported earlier likely due to the absence of measurements at this advanced age. This decrease in mass, combined with changes in the ultrastructure of the calcite crystals making up the shell, leads to a further reduction in the shell biomechanical properties.

Structural and molecular studies must be performed to further understand the mechanisms leading to eggshell degradation at 94-99 weeks and to identify potential levers to lower negative impacts on eggshell and bone quality in extended laying periods.

Poster 16

In vitro simulation of the oral consumption of MOP powder and screening of the mineralization capacities of digestion products

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Mother-of-pearl (MOP), the internal layer of mollusks shell, was firstly investigated as a solid and biocompatible bone graft material that led to discover its effect on bone cells. It finally shown in vitro effects on both sides of bone remodeling by repressing bone resorption and stimulating bone formation. That led scientists to investigate it in bone disease characterized by unbalanced remodeling with increased resorption and decreased bone synthesis such as osteoporosis. Indeed, ingested MOP powder or extracts in osteoporotic mice, rat or postmenopausal women showed a limitation of bone loss. However, action mechanism after oral consumption remains unknown. Here, we investigate whether the bone effects of orally ingested MOP could be attributed to previously demonstrated in vitro abilities. MOP dissolution in gastric condition was performed and released organic compounds were extracted and digested according to INFOGEST 2.0's Nature Protocol standardized static digestion protocol. This protocol involves three steps designed to mimic human natural digestion: an oral phase, a gastric phase (pH 3, 37°C, 2h) and an intestinal phase (pH 7, 37°C, 2h). After MOP digestion, products were submitted to absorption assessment on 21 days old intestinal epithelium made of differentiated Caco-2 cells. Undigested, digested and absorbed MOP fractions were then assessed for their pro-mineralization abilities on MC3T3 and primary murine osteoblast. In conclusion some MOP compounds released during gastric dissolution showed a positive effect on mineralization in vitro both before and after their digestion. Absorption assessment didn't allow us to conclude that orally ingested MOP effect on bone loss could be related to a direct effect on bones cells. A fraction of MOP remains undigestible and could be used as a substrate that may influence intestinal microbiota.

Keywords: MOP; digestion; bioactive compounds; bone loss

Poster 17

A novel nacreous bioinspired peptide stimulates endochondral differentiation

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Nacre, also known as mother of pearl, is produced by some molluscs as an inner shell layer. Although nacre is known for its ability to stimulate endochondral differentiation and bone formation, its whole osteogenic composition is still mysterious. Several nacreous proteins have been identified and proven to have an osteogenic power. To our knowledge, there have been no studies exploring the effect of nacre peptides on endochondral differentiation. In this study we aimed to identify nacre peptides that are able to stimulate this biological process. The organic matrix of *Pinctada margaritifera* shell powder has been purified by the cationic approach that we developed previously, and its composition has been analysed. Using tandem mass spectrometry (LC-MS/MS), we found an interesting peptide that has been further produced and several analogs synthesized. The amino acids that may play a role in endochondral differentiation were then highlighted by a new screening tool of a stable cell line expressing an osteogenic reporter gene (ATDC5 pMetLuc2 ColX), that we created. The peptides were tested on a micromass model of ATDC5 cells to investigate their capacity to stimulate endochondral differentiation and mineralization of the extracellular matrix. In conclusion, the present study led to a discovery of a new nacre and synthetic peptides active in endochondral differentiation. Such a discovery can be potentially useful in developing drugs from nacre to improve bone repair.

Keywords: nacre, bone, screening, endochondral differentiation

Topic 4 Imaging methods for biomineralization researches

Keynote lecture

Invited Review: A darker side to X-ray brightness - assessing calcium mediated structural degradation during bone studies

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X-rays are widely used for imaging of bones and other biomineralized tissues. Due to their high penetration power, they traverse rather thick structures yielding important diffraction or tomographic data. The high density of calcium in mineralized collagen and other biogenic matrices almost begs analysis by X-ray radiation methods, where higher resolution and structural sensitivity call for ever increasing high-power and high-energy radiation sources such as synchrotrons. Yet when mineralized samples are exposed, a significant amount of the incoming flux is absorbed by the mineral. Though essential for scattering and image formation, absorption leads to energy deposition in the material.

Absorbed photons induce photon-electron excitations. Synchrotron-based X-ray diffraction and micro-computed tomography methods thus lead to in situ degradation of the collagen fibrils due to primary radiation damage. Following just 40 s of moderate exposure, we observe protein disintegration through dimming of second harmonic generated emission from within the collagen fibers. Diffraction measurements with increasing exposures reveal residual strain relaxation in dry, pre-stressed, apatite nanocrystals, due to expanding c-lattice parameters as collagen is disrupted. We find that ionization of calcium and phosphorous within the nanocrystals yields fluorescence and high-energy scatter electrons that emerge from the mineral to spread multiple micrometers. Ionization then causes structural damage in the organic fibers. Scattered electrons spread beyond regions directly within the field of view of the incident radiation beam. Our observations indicate that damage to bone collagen increases rapidly from the onset of irradiation, suggesting that there is no minimal 'safe' dose that bone can sustain. The talk will present our current understanding of the impact of X-ray methods on the generation and spread of primary radiation damage in bony materials.

Reference:

Sauer, K., Zizak, I., Forien, JB. *et al.* Primary radiation damage in bone evolves via collagen destruction by photoelectrons and secondary emission self-absorption. *Nat Commun* **13**, 7829 (2022). https://doi.org/10.1038/s41467-022-34247-z

Keywords: X-ray, Radiation damage, Collagen backbone destruction

Oral presentations

T4 O1

Dynamic interpretation of the bone mineralization process in the chick embryo based on Cryo FIB-SEM images.

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Cryo focused ion beam with scanning electron microscopy (cryo FIB-SEM) is a powerful tool to image volumes of hydrated frozen specimens at high resolution. Of particular interest, this method enables the visualization of mineral precursors, allowing to shed insights into the bone mineralization process. Here, we focus on the three-dimensional visualization and quantification of the mineral precursors in the femur of the fast-growing chick embryo. Based on volume imaged at different hierarchical with cryo FIB-SEM covering the smallest length scale, we also aimed at providing an interpretation of the mineralization logistics in a dynamic manner, where the speed of transport of the mineral precursors to the site of mineralization is estimated.

In this approach, we used micro-computed tomography (micro-CT) to calculate the osteocytic lacunar density at embryonic day 13 and thus, estimate the volume of bone to be mineralized per cell in one day. 3D volumes were acquired using cryo FIB-SEM to observe the mineral precursors, the cells as well as the mineralized matrix in their close to native states. The results show that numerous vesicles containing mineral precursors are present intracellularly, both in osteocytes and in osteoblasts/pre-osteocytes. The average volume of a vesicle was 0.65 µm3 with a volume of mineral precursor per vesicle of approximately 0.058 µm3. From the micro-CT data, we calculated that a cell is responsible for the mineralization of a volume of about 5100 µm3. Based on the quantification of these 3D snapshots, we estimated that a cell needs to release one vesicle every second to mineralize the required bone volume in one day. Consequently, these vesicles travel at a mean velocity of 0.27 µm/s within the cell.

This dynamic interpretation of the logistics of bone mineralization demonstrates that mineral precursors have to be actively transported within cells before there are shed into the extracellular space, where they move passively via diffusion.

Keywords: cryoFIB-SEM, bone, mineral precursors, vesicles

T4 O2

Towards *in vivo* imaging of the physico-chemistry processes involved in the biomineralization of mollusc shells

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Calcium carbonate biomineralization is widespread across the animal phyla, in particular in the marine environment. It integrates complex physical and chemical processes bio-controlled by the living organisms through ion regulation and organic molecule production. Hard tissues, produced during favourable-condition crystallisation, present a variety of structural, optical and mechanical properties, making the understanding of the exact biomineralization pathways highly desirable. For many species, it has been shown, mostly from post-mortem analysis and biomimetic models, that biomineralization starts with forming an amorphous precursor, which subsequently transforms into a crystal [1]. However, the nature of the transient physico-chemical states and the dynamics of the transformation are still discussed. Direct evidence could be brought by *in vivo* approaches, under the assumption that spatial, temporal and quantitative information is accessible.

Here we present recent developments towards *in vivo* study of calcareous biomineralization:

- To monitor the different chemical states (e.g., amorphous or crystallized carbonates), a highly sensitive coherent Raman microscopy approach is developed. It allows mapping molecular bond concentrations with spatial resolution in the order of 300 nm and demonstrates suitability for the *in vivo* imaging of a shell growing edge (*Pinctada margaritifera*) [2].

- To address the crystallization process, we make use of a novel 4th generation synchrotron source, which allows nanoscale spatially resolved and crystalline-sensitive study of a biomineralizing animal (*Crassostrea gigas* shell). Our results point towards an evolution of the freshly produced crystallites, including growth, coalescence and strain release. Remarkably, the crystallisation occurs without the presence of the mantle, showing that the elements needed for the production of calcite biomineral are already in place in the amorphous precursor [3]. It again highlights the relevance of biomineralization for the design of bio-inspired materials.

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[2] H. Dicko *et al.*, Journal of Structural Biology **214** (2022) 107909.
[3] T. Grünewald *et al.*, *in preparation*.

Keywords: in vivo, microscopy, physico-chemistry, biomineralization, x-rays, Raman, mollusk

T4 O3

Unique features of Caudofoveata (Mollusca) spicules revealed by combined light and electron microscopy techniques

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The Caudofoveata is a class of marine worm-like mollusks that live, in their great majority, embedded in the muddy bottom of marine environments. The Caudofoveata tegmentum is composed of a chitinous cuticle [1] and calcareous micrometer sized spicules [2] which are formed extracellularly within a deep invagination of a single cell, where calcium carbonate is secreted on a cup-like organic template. Morphology and thickness of the Caudofoveata spicules have been considered species-specific [3].

In this work, we investigated the calcium carbonate spicules of Caudofoveata (Mollusca), by several methods of microcopy in different length scales, in a correlative way. By analyzing Electron Diffraction patterns of lamellae obtained by Focused Ion Beam (FIB) from spicules, we concluded that the whole structure is composed of a single crystal of aragonite. However, when analyzing SEM images of NaOH or acid treated spicules, it becomes clear that the bulk of these biominerals is formed by a set of alternating plate-like components, with different textures, most probably crystalline, oriented in parallel. When imaging the dorsal surface of spicules by Atomic Force Microscopy (AFM), it was observed a wavy appearance, composed of myriads of tiny, pointed crystallites oriented along the longer axis of the spicules which coincides with the *c*-axis of the aragonite. Besides, AFM showed that the crystallites presented in some areas a twinned appearance. The correlation among the substructures observed after etching (revealed by SEM), the apparent organization of the tiny crystallites as twinned ones (revealed by AFM), and the single crystalline appearance of the whole spicule (revealed by electron diffraction of FIB prepared lamellae), is still an open question and gives strength for further analyses using Caudofoveata spicules as a model for biomineralization studies, including a molluscan phylogeny in the light of still unexplored characters and their organization patterns.

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[3] Scheltema, A.H., Schander, C., 2000. *Bioll. Bull. Woods Hole* 198, 121-151.

Keywords: Aragonite spicules; Nanostructure; Caudofoveata; Biomineralization; FIB-SEM

T4 O4

Uncovering the complex world of elastic deformations in biological ceramics

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Biological ceramics are complex materials. Understanding their mechanical behavior requires methods allowing quantitative imaging of deformations under contact loads - in operando - rather than providing a snapshot of induced damages. Here, we introduce an imaging method by which mechanical deformations under contact loads can be imaged in 3D and with sub-micron resolution. By taking examples of biological ceramics and their geological counterparts, we then unravel how the crystallographic organization of grains and crystals and organic inclusions influence the formation and evolution of elastic-inelastic strains. Our technique can pave an important step toward a quantitative understanding of the formation and development of elastic deformations and their interplay with the anisotropy and heterogeneity of materials.

Keywords: Micromechanics, Elastic strain, Calcitic shells

T4 O5

Study of the role of trace elements on kidney stones pathogenesis with multi-scale and multimodal scanning X-ray tomography

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Urolithiasis is a product of a pathological biomineralization process affecting ~10% of the population. A significant proportion of these stones develops on Randall's plaque (RP), which is a mineral deposit at the tip of renal papillae. However little is known about the processes involved in RP development and especially about those which trigger or influence early-stage pathological calcification. Some results published in the literature have revealed that certain trace metals, e.g., zinc, are significantly enriched in the carbapatite of RP[1]. However, up to our knowledge, the role of these trace metals in the early stage of biomineralization of Randall Plaques has not been addressed yet, partially because this necessitates non-destructive, high analytical sensitivity studies at nanometer resolution.

In this work, thanks to the new developments in multi-length scale and multimodal scanning imaging and tomography at the Nanoscopium beamline (SOLEIL Synchrotron) [2], we studied the complex 3D elemental, crystalline, and morphological structure of Randal papilla and that of micron-sized calcification spheres in RP, an example for incipient calcification. The obtained results will bring nephrologists a step further to understand and prevent pathological calcification processes

Keywords: Kidney stones, Randall's plaque, Trace elements, X-ray imaging, Pathological calcifications

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T4 O6

Quantitative evaluation of enamel prism arrangement based on image processing technique

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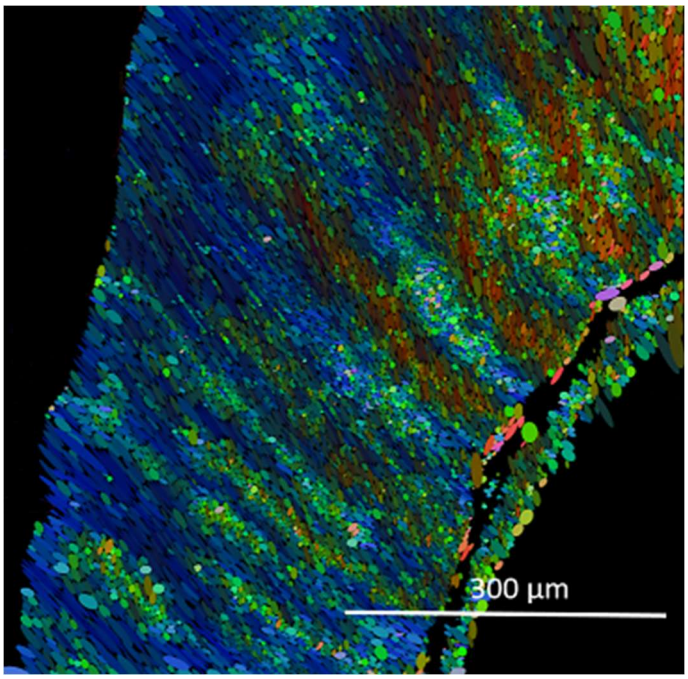
Tooth enamel is the hardest and most mineralized tissue of vertebrates. The complex microsctructure of enamel contains needle-shaped hydroxyapatite (HAP) nanocrystals arranged on several structural level to form the most characteristic unit of mammalian dental enamel, the elongated enamel prism. Although, the c-axis of HAP correlates with the prism direction, prism orientation can not be assigned to any specific crystal orientation This makes quantitative analysis of the prism structure difficult based on diffraction or polarisation based methods.

In present work, we introduce an image processing method based on the analysis of scanning electron microscope (SEM) images of different primary dental enamel sections. The method separates prisms using the systematically changing electron contrast at the prisms' surfaces and approximates different prism sections with elipses. Output of the method is a set of quantitative parameter, which is able to describe and visualize the local prism orientation for various prism arrangements.



Specific prism orientation maps obtained on distal-mesial cross-sections show the size and growth pathways of enamel producing ameloblast. Additionaly, prism orientation in Hunter-Schreger bands, which are mostly identified as periodic dark and light bands under light microscope, was analysed quantitatively and was proved to exhibit nearly identical prism orientations by the prisms arranged into groups. Based on the prism orientation map we determined the orientation dependency of mechanical properties of human dental enamel.

Hegedűs, Máté; Kis, Viktoria; Szabó, Ábel; Kovács, Ivett; Rózsa, Noémi; Kovács, Zsolt, Gradient Structural Anisotropy of Dental Enamel is Optimized for Enhanced Mechanical Behaviour (submitted)

Hegedűs, Máté; Kis, Viktoria; Kovács, Zsolt, Quantitative evaluation of enamel prism arrangement based on image processing technique (in prep.)

Poster presentations

Poster18

Structural analysis of sub- and supragingival mineralized oral biofilms using high-resolution imaging techniques

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Biofilms are living materials that appear when bacteria synthesize and assemble extracellular matrix components after colonizing a surface. They constitute a key feature of bacteria survival in biologically, chemically and/or physically challenging environments. In the mouth, the oral microbial community builds biofilms called dental plaque. Oral biofilms are associated with some of the most common dental diseases, such as caries and periodontitis [1]. Dental plaque mineralizes and forms dental calculus, which is generally classified into two categories according to the location. Supragingival calculus is located above the gingival margin. On the other hand, subgingival calculus is located below the gingival margin in the gingival sulcus or periodontal pocket [2].

In this work, we aim to understand the differences in microstructure, mineralization and composition of the two types of dental calculus. We characterized native human teeth ethically harvested from consenting donors. We used X-ray microtomography to investigate the overall morphology of the calculus at the mesoscale. To further characterize the mineralized biofilm, we used Focused Ion Beam-Scanning Electron Microscopy (FIB-SEM). Eventually, using small- and wide-angle X-ray scattering (SAXS and WAXS), we compared the crystalline composition of the two types of calculus. Results obtained by segmenting the microtomography of entire teeth, showed that supragingival calculus is generally porous, whereas subgingival calculus is tightly packed and presents a layered structure. Analysis of the crystalline composition in the different calculi, revealed a variety of different mineral phases present in the two different calculi. The present results may help to better understand the formation of dental calculus and to design new preventive strategies for oral diseases.

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Keywords: mineralized oral biofilm; Subgingival calculus; Supragingival calculus

Poster 19

Functional membrane interactions regulate silica formation in diatoms

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The silica cell wall formation in diatoms, a widespread group of unicellular microalgae, is a spectacular example of biological control over mineral formation. Diatom silicification occurs intracellularly in a membrane-bound organelle responsible for silica precipitation and morphogenesis. Despite many years of research, the inorganic-organic interactions that drive silica formation inside this organelle are unclear. This is due mainly to the limitations of traditional TEM techniques in elucidating the structural motifs of this organelle. Here we collected cryo-electron tomography datasets of cryo-FIB milled lamellae from diatom cells at various stages during cell wall formation. We visualize the mineral formation process in-situ with nanometer-scale resolution and reconstruct a timeline of mineral formation inside the cell. Our observations show that the silicification occurs in a highly confined lumen, bordered by the organelle membranes, which are in very close proximity to the plasma membrane. The plasma and organelle membranes interact via membrane contact sites, possibly facilitating continuous transport of lipids from the plasma membrane to the growing organelle, and building blocks for mineralization. These membrane-membrane interactions are manifested in the curvature of the distal organelle membrane, which molds the silica. Our findings reveal a new mechanism that regulates silica growth and shaping through membrane crosstalk.

Keywords: Diatom, Silica, cryoEM, Tomography

Poster 20

Use of fluorescent marker of mineralization dynamics : Application to lactating mice.

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Osteoblasts are responsible for bone formation by producing the collagen matrix which gradually mineralizes. In this process, some osteoblasts become enclosed in the matrix and become osteocytes, with their lacunae interconnected by dendrites, forming an important lacuno-canalicular network (LCN).

It has been shown in different models such as PTH injection or lactation (Bonewald et al., 2017) that osteocytes are able to modify the bone matrix in their vicinity through peri-lacunar/canalicular remodeling (PLR). The process has been documented in breastfeeding rodents (Bonewald, 2012, 2020) using bone markers previously validated to label the mineralization fronts (calcein, tetracycline and alizarin) which hardly diffuse throughout the osteocyte network.

Peri-lacunar fixation of these markers, proof of osteocyte remodeling is limited to the first line of young osteocytes and is not able to diffuse in depth to older osteocytes. This method cannot show the existence of this process throughout the osteocyte network at distance from the mineralization front. It would thus be useful to have a label integrated into the young bone matrix.

Our objective was to evaluate the existence of PLR in the LNC of lactating mice, thanks to the use of a temporal multi-labeling with alizarin and a fluorescent molecule circulating throughout the osteocyte network without specific binding to calcium or minerals and spreading into the osteoid tissue and the bone matrix all the more easily as it is little mineralized. During clearance by the kidneys and the digestive tract, only the young bone matrix where mineralization is active remains stained.

During the 10 days following 20 days of breastfeeding, we documented a resumption of bone formation marked by the fluorochrome, at the periosteum and in the entire endosteal surface of the femur cortex, showing a modification of the dynamics of the bone drift compared to non-lactating control mice.

However, we did not observe active mineralization around the lacunae of osteocytes formed before lactation suggesting that lactation/weaning is not associated with in depth PLR.

In conclusion, the fluorochrome used could be useful to study the dynamics of matrix mineralization because classical calcein/alizarin molecules are not able to diffuse through the LCN and the matrix of mineralizing bone tissue.

Keywords: Breastfeeding, osteocyte network, dynamic mineralization

Poster 21

Light Channeling and Damage Localization in *Pinna nobilis* Calcitic Prismatic Layer

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Pinna nobilis is a marine bivalve mollusk and its shell is composed of outer single-crystal-like calcitic prisms and inner aragonitic nacre. The latter is known for its contribution to the outstanding mechanical property of the shell; hence, its absence at the upper part of the shell, composed solely from the prismatic layer, raises the question of whether the upper part of the shell can serve any functional purposes while sacrificing its mechanical performance. Here, we reveal that the prismatic layer of *Pinna nobilis* exhibits remarkable optical properties, wherein each prism acts as an individual optical fiber that guides light to the inner shell cavity through total internal reflection. This "fiber optic" of prisms enhance spatial resolution and contrast, allowing more precise tracking of moving objects in the surrounding of the shell. We then use our devised "in operando 3D strain mapping" method and show how the architectural organization of the prisms can promote damage localization. Our discovery provides insight into the evolutionary perspective of light sensing in biological materials and offers a conceptual framework for the development of bio-inspired multifunctional ceramics and architecture light-tracking materials.

Keywords: *Pinna nobilis*, light tracking, damage localization, in operando 3D strain mapping

Topic 5 Immunity in biomineralized barriers

Keynote lecture

Invited review: Dynamics of the chicken chorioallantoic membrane and the eggshell during chicken embryonic development: a fine regulation between eggshell decalcification and maintenance of egg defences

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The avian eggshell is a highly ordered mineralized structure that isolates and protects the embryo from environmental fluctuations during its development. This calcitic eggshell, which also contains 3% protein including antimicrobial proteins and peptides, constitutes the first level of egg defence, acting as a physical and molecular barrier against environmental changes, pathogenic microorganisms or dehydration. However, during the second half of the chicken development, the avian embryo uses the minerals of the eggshell, mainly calcium, for the mineralization of its skeleton. The thinning and weakening of the eggshell resulting from the demineralization of its inner surface facilitates chick emergence at the end of incubation but is likely to increase the susceptibility of the embryo to microbial contaminations. The hypothesis is that the antimicrobial proteins occluded in the mineral phase of the eggshell may be released together with the calcium during eggshell solubilisation and may form a local protective proteinaceous film onto the inner surface of the eggshell. Indeed, some authors have pointed out the potential dual role of certain eggshell matrix proteins in both mineralization (during eggshell formation in the hen uterus) and antimicrobial protection (during embryo development).

Decalcification of the eggshell is mediated by the chorioallantoic membrane (CAM), a highly vascularized extra-embryonic membrane that develops on the inner surface of the eggshell from the fifth day of incubation, onwards. It covers the entire inner surface of the eggshell by the eleventh day of incubation. Besides its major role in calcium metabolism and mineral transport, the CAM is likely to play a major role in innate immunity. Indeed, its strategic position (in contact with the inner surface of the eggshell) and its well-developed vascularization allow the local recruitment of immune cells in case of bacterial penetration through a defective eggshell. The CAM also expresses antimicrobial molecules as well as other components of innate immunity (cytokines).

Using the chicken embryo as a model of mineralized structure, this review will shed light on the mechanisms that allowed calcitic eggs to adapt to terrestrial environments, and the fine regulation between the mineralized structures of the egg and the extra-embryonic cellular structures to maintain protection of the embryo throughout incubation.

Oral Presentations

T5 O1

Shedding light on the nature of sea urchin pigment-bearing vesicles

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Red-spherule cells (RSCs) take part into the innate immune system of sea urchins likely through the release of poly-hydroxylated naphthoquinones (PHNQs) [1], initially enclosed in the cytoplasmic vesicles as dense red granules. RSCs can be very abundant in the body fluid of larvae and adult sea urchins during infection as well as in the soft tissues of regenerated spines after fracture [2]. Known for their demonstrated antimicrobial activity, PHNQs are also responsible for the multiple colors observed in sea urchin biominerals [3, 4]. However, the mechanism by which PHNQs are eventually released from the RSCs and incorporated into the biomineral structure during its growth is not known. Using complementary microscopic studies, we here investigate the biomineral pigmentation pathways in *Paracentrotus lividus* sea urchins. From FIB-SEM data on regenerated spines, we propose that pigment vesicles are released by RSCs at the vicinities of growing micro-spines during fracture healing. To go further on the structure and composition of the RSC and their pigment vesicles, we then performed TEM and histological observations of both pluteus larvae and adult coelomic fluid as well as regenerated spines. TEM observations reveal that RSCs' pigment vesicles have different PHNQs granule densities and are internally coated by a polysaccharide layer possibly acting as a cellular protective barrier against acidic pH [5]. Finally, histological staining with pH-sensitive dyes reveals a lower pH for RSCs in regenerating spines as compared to the extracellular matrix. Staining of the urchin larvae confirms the presence of acidic areas (pH ~ 6.0) where RSCs are abundant. These findings indicate that pH could play a major role upon releasing and incorporation of PHNQ molecules into the growing mineral, suggesting an intricate association between pigmentation and biomineral formation.

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Keywords: red spherule cells; poly-hydroxylated naphthoquinones; pigmentation

T5 O2

Induced mineralization in *E. coli* biofilms: the role of extracellular matrix

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Biofilms are living microbial tissues emerging that occur as a survival strategy in challenging environments, after bacteria colonize a surface and synthesize extracellular matrix. In addition to the organic matrix, some biofilms precipitate mineral particles such as calcium phosphates and calcium carbonates. Previously, we cultivated *E. coli* K-12 W3110 on an agar medium containing calcium ions and organic phosphates. This *E.coli* strain produces an extracellular matrix mainly made of amyloid curli fibers and we reported the precipitation of calcium phosphates in the form of hydroxyapatite in the [1]. Focused ion beam with scanning electron microscopy performed in cryogenic conditions (cryo-FIB-SEM) enabled us to identify both mineralized bacteria and mineralized portions of the extracellular matrix. This can indicate an interplay between different biomineralization processes related to microbial activities. With few exceptions, microbial mineralization usually results from adventitious precipitation of inorganic compounds led by their interactions with different metabolic processes [2] and with the macromolecules present in their surroundings [3]. In this context, we investigated the influence of different macromolecules in the biofilm on the formation of calcium phosphate crystals. On nutritive agar substrates inducing mineralization, we cultivated diverse *E. coli* strains, which would produce as extracellular matrix only amyloid curli fibers, only phosphoethanolamine cellulose fibers, both of them or none of them [4]. We estimated crystal dimensions using wide-angle x-ray scattering enabling us to assess crystal strains at the nanoscale. Cryo-FIB-SEM was exploited to study crystal aggregation at a larger scale. Uncovering the influence of the biomolecules present in the extracellular matrix is an important piece of knowledge to engineer living composites.

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T5 O3

Morphology and size of mineral particles may be affected by bacteriophages

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Until now, the properties of mineral particles have been thought to be influenced by bacteria, fungi and photosynthetic microorganisms. The latter have been recognised as one of the main factors that can influence biogenic carbonate formation in microbial mats. However, their role was limited to photic and oxic environments. On the other hand, sulphate-reducing bacteria, as well as methanogenic archaea involved in the anoxic oxidation of methane can play a role in carbonate formation in anoxic environments.

However, the abundance of these microorganisms is relatively low compared to viruses, which are an order of magnitude higher. Their role in the formation of various minerals has not been widely recognised. It is still not known how viruses affect the process of mineral formation.

In our experiments [1]-[3], we demonstrated that (i) viruses may induce framboid-like mineral formation; (ii) the electrokinetic potential of minerals can be altered during mineral precipitation in the presence of bacteriophages; (iii) viruses can alter the size of mineral particles precipitated in the presence of bacteriophages; (iv) viruses can bind to the surface of various minerals, altering their properties.

Bacteriophages are thought to affect mineral precipitation in a variety of environments. Their influence on the agglomeration or aggregation and physicochemical properties of mineral particles appears to be of particular interest. We conclude that the interaction between bacteriophages and mineral particles may play an important, but underestimated role in mineral formation in sedimentation environments.

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Poster Presentations

Poster 22

What is the role of the shell organic matrix in molluscan defense against microorganisms ?

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Mollusks are soft bodied animals that have developed a peculiar set of graded defenses against chemo-physical and biological threats: among them, the shell constitutes the first shield, and the mucus layer, an additional cleaning system, while hemocytes are involved in repair mechanisms and immunity at cellular level. There are however persistent clues that the shell is not solely a physical barrier. To this end, we investigate here the role of the calcifying organic matrix, a cocktail of macromolecules that regulate the deposition of the crystalline units and remain occluded in the shell. Until recently, they were thought to regulate only the mineral deposition process. High-throughput proteomics techniques backed by transcriptomes show the complexity of this calcifying matrix and the abundance of basic peptides, suggesting potential antimicrobial properties. This research seeks to demonstrate *in vitro* the bactericidal capacity of calcifying matrices extracted from mollusk shells of economic interest. We intend to valorize - through sophisticated applications - an abundant co-product of the sea which is only recycled with low added value. For this purpose, we selected different bacterial strains: generalist bacteria (*E. coli, P. aeruginosa, B. subtilis spp*.) and common marine pathogens (*Aliivibrio salmonicida*, *Vibrio tapetis, V. mytili, V. harveyi, V. aestuarianus*). For each mollusk species, the effects of different acetic acid-soluble shell fractions on bacterial growth were measured via disk diffusion and metabolism (phenol red) assays; the insoluble fractions were also tested via the first method. In parallel, all fractions (soluble and insoluble) were analyzed by proteomics (LC-MS-MS). Through potential applications at the interface of aquaculture, conservation and health, this project aims to establish a virtuous circular economy and to better understand the complex role of the mollusk shell as a protective barrier.

Keywords: mollusk, shell, biomineralization

Topic 6 Biomineralization and biomimetics

Keynote lecture

Biological Adaptations and Blueprints for Extreme Environments

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Organisms have derived specific sets of traits in response to common selection pressures that serve as guideposts for optimal biological designs. A prime example is the evolution of toughened structures in disparate lineages within plants, invertebrates, and vertebrates[1]. Extremely tough structures can function much like armor, battering rams, or reinforcements that enhance the ability of organisms to win competitions, find mates, acquire food, escape predation, and withstand high winds or turbulent flow.

Some of these natural systems have developed well-orchestrated strategies, exemplified in the biological tissues of numerous animal and plant species, to synthesize and construct materials from a limited selection of available starting materials. The resulting structures display multiscale architectures with incredible fidelity and often exhibit properties that are similar, and frequently superior to, mechanical properties exhibited by many engineering materials[1,2]. In specific instances, comparative analyses of multiscale structures have pinpointed which design principles have arisen convergently; when more than one evolutionary path arrives at the same solution, we have a good indication that it is the best solution. This is required for survival under extreme conditions. We describe a few of these systems that show convergent design and describe how controlled syntheses and hierarchical assembly using organic scaffolds lead to these integrated macroscale structures[3-8]. We describe their function and translation to biomimetic and bioinspired materials used for engineering applications[9-11].

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Oral Presentations

T6 O1

Structure-function studies of intrinsically disordered proteins-iron oxide interaction

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Biomineralization is a complex process that involves specialized proteins to mediate and control mineral sedimentation. These biomineralization proteins often have intrinsically disordered regions that are critical for their function. To gain insights into this process, we investigated the Mms6 protein, an intrinsically disordered protein from magnetotactic bacteria that are involved in magnetite nucleation, using various techniques such as NMR, X-ray, and cryo-EM.

In our study, we utilized the cavity of ferritin as a nanoreactor to mimic the magnetosome lumen, where Mms6 binds to iron and participates in the nucleation event. Using these approaches, we observed the interaction between M6A, an active intrinsically disordered iron-binding domain peptide derived from Mms6, and an iron oxide particle, at high resolution. Our findings suggest that the folding of M6A correlates with detecting mineral particles in its vicinity. We also found that M6A interacts with the iron oxide particles through its C-terminal side, stabilizing a helix at its N-terminal side.

Importantly, our results demonstrate the ability of intrinsically disordered proteins to respond to signals from their surroundings by undergoing conformational changes. These findings shed light on the mechanism underlying the control of biomineralization protein over mineral microstructure, where the unstructured regions of these proteins become more ordered upon interacting with the nascent mineral particles. Our study provides crucial insights into the function of intrinsically disordered proteins and their role in biomineralization processes.

T6 O2

The calcium phosphate-based biomineral clusters for rapid remineralization of tooth enamel

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Using biomineral-based materials to grow hydroxyapatite (HAP) crystals on tooth enamel surfaces is a common strategy for repairing early demineralization. However, the effectiveness of these materials is usually reduced by the dynamic oral environment, which includes continuous saliva flow and friction from cheek muscles. To address this, a rapid remineralization method is needed to avoid or diminish these influences. The challenge has been finding stabilizers that do not resist transformation during remineralization. We have discovered a stable species of calcium phosphate biomineral with ultra-small size (1-2 nm) that can rapidly repair enamel via water-triggered transformation in both static and dynamic environments. The calcium phosphate biomineral can easily penetrate nano/micro-sized enamel defect sites and transform into HAP nanorods immediately, going through an intermediate phase of an amorphous calcium phosphate nanowire. The resulting nanorods stand vertically to the enamel surface and form a compact HAP repair layer within 30 minutes, which is much faster than conventional materials that take hours or days. In vitro and in vivo studies have shown that the recovers mechanical properties of samples are similar to those of sound enamel. The calcium phosphate biomineral with ultra-small size has the potential to be a widely used strategy for dental remineralization due to its rapid repair capacity, simple preparation process, low cost, and remarkable biocompatibility.

T6 O3

Structural and mechanical anisotropy in crustacean claws mineralized with amorphous calcium phosphate

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Crustacean exoskeletons are not only fascinating from the point of view of functional morphology; their versatility, representing adaptations to a variety of functions, may inspire biomimetic materials with outstanding performance. The crustacean exoskeleton consists of the cuticle, which is secreted by epidermal cells and can be viewed as a composite material consisting of chitin fibres and a mineralized matrix. We studied the claws on the walking legs of isopods, which are minute and thin structures, yet are capable of supporting the entire animal during walking and climbing.

In the terrestrial isopod *Porcellio scaber*, the claws are only a few tens of microns in length. We analysed the architecture of the organic matrix and mineral components in the claw at the nanoscale with the use of electron microscopy. Our results showed that the isopod's claws are mineralized exclusively with amorphous calcium phosphate. Furthermore, the claw skeleton is highly structurally anisotropic, with chitin fibres aligned in a single direction, matching the principal direction of stress. The claw possesses a mineralized core enveloped by a thick layer of non-mineralized cuticle, an organization that likely confers greater fracture resistance. To understand the mechanical consequences of these features, we studied the sea slater *Ligia pallasii*. Its larger size allowed us to perform additional imaging using micro-computed X-ray tomography and the determination of the cuticle mechanical properties using nanoindentation. The organization of the matrix and the chemical nature of mineral components in the claw are similar as in *P. scaber*. Micro-CT demonstrated that the leg cuticle includes non-calcified regions that may act as shock absorbers. Nanoindentation allowed us to confirm that the claw is highly mechanically anisotropic and strengthened in the direction of predominant loading, reflecting its structural anisotropy.

T6 O4

Bio-Inspired Fluorescent Consolidants for the Conservation of Gypsum Plasterwork

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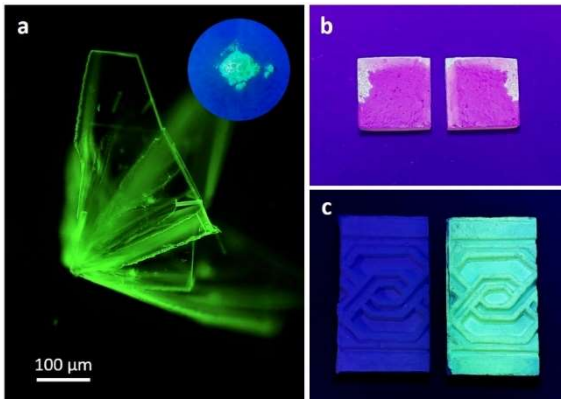
In Nature, living organisms show an unmatched ability to originate sophisticated inorganic/organic hybrid materials and composites with a wide variety of physicochemical properties, such as enhanced mechanical strength or optical functionalities. In particular, fluorescence occurs in some species increasing their visibility, which is a powerful tool we can use in our favor for multiple purposes (*e.g.*, drug delivery control).

Although it is still a challenge, biomimetic and/or bio-inspired strategies have been developed in order to recreate biomaterials at the synthetic scale using organic polymers that imitate the role of proteins and/or polysaccharides during biomineralization processes.

In this work we investigated the effect of calcein (*i.e.*, an organic Ca^{2+} specific fluorescent chelating agent) on the crystallization of $CaSO_4 \cdot nH_2O$ phases, and we elaborated a solvothermal procedure for the synthesis of calcein-loaded bassanite ($CaSO_4 \cdot 0.5H_2O$) nanoparticles with luminescent properties for their use as consolidants for the conservation of decayed gypsum plasterwork. Fluorescence allowed to distinguish the newly deposited nanoparticles from the original plaster, a problem that is extremely difficult to address using other imaging techniques, as both consolidant and substrate share the same composition.



Fluorescence photomicrographs (a) demonstrating that $CaSO_4$ crystallizes in the presence of calcein acquiring fluorescent properties. Treated plaster specimens irradiated with UV light (b and c) showing that the synthesized luminescent bassanite nanoparticles penetrate inside the substrate and can be easily detected

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T6 O5

Iron-based biominerals of marine sponge origin as inspiration for biomimetics

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Research into iron-based biominerals derived from skeletal spongin of marine bath sponges fits perfectly both into the fields of biomineralogy and biomimetics[1]. Crystalline mineral phases of lepidocrocite (γ- FeOOH) origin on proteinaceous spongin microfibres have been already reported in 1968[2]. Is it possible to use spongin as renewable source of unique 3D scaffolds for it functionalization using iron ions for large scale application?

For this purpose, a new bioinspired by biocorrosion of iron biomimetic method has been developed to form lepidocrocite on a spongin scaffold *in vitro* for the first time. The present study investigates the interaction of iron ions with a spongin scaffold in an artificial seawater as corrosive environment. Consequently, the novel Iron-Spongin composite was developed (Fig. 1A). Alternatively, the extreme biomimetics approach was used to form goethite on a spongin scaffold. This experiment involved mixing crystalline iodine and powdered iron with spongin to create a new FeI-Spongin biocomposite at room temperature (Fig. 1B). Obtained composites remain stable after sonication during 5 h. Furthermore, mechanisms for the formation of both iron oxides on spongin fibres were suggested. Both composites were characterised using instrumental techniques including microscopic imaging (optical, SEM/EDX, HRTEM), spectroscopic analysis (FTIR, Raman), X-Ray diffraction and thermogravimetry. For the first time we successfully used these unique 3D composites as sensors for dopamine detection. The next step will include the first attempt in the history of materials science to use the obtained iron-containing spongin composites to obtain a higher-order metallic phase by melting them at 1450°C.

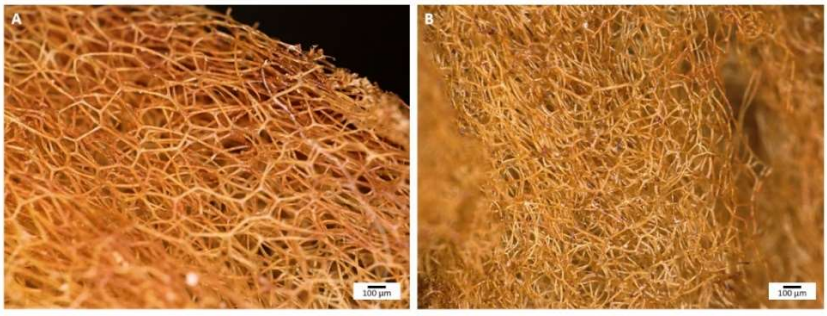

Figure 1. Images taken with a digital microscope at 100 µm magnification. (**a**) Iron-Spongin; (**b**) FeISpongin.

Acknowledgments

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T6 O6

Controlled crystallization of guanine nanocrystals

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Guanine is one of the most widespread organic crystals existing in organisms, which has superior optical properties and exhibits structural colors. The excellent optical properties of biogenic guanine crystals contribute to the exquisite control on the polymorph, morphology, size and highly-ordered hierarchical microstructure assemblies of guanine crystals and cytoplasm. Controlled crystallization of guanine crystals with particular polymorphs, morphologies, exposing crystal faces was investigated in both water and organic solvents in the presence of additives. Controlled crystallization of guanine monohydrate, anhydrous guanine α and β form in pure phase were realized for the first time under mild conditions in aqueous solution without the presence of additives by changing the guanine concentrations and pH values. Amorphous guanine was synthesized by a one-step synthesis process in large scale, which transformed to anhydrous guanine β form in pure phase. Characterizations using FTIR and ss-NMR showed that anhydrous guanine β and amorphous guanine had similar short-range order structure, which might be the reason for the controlled transformation from amorphous phase to thermodynamically meta-stable anhydrous guanine β form. The controllable synthesis of twinned crystalline guanine microplatelets and dye-doped guanine microplatelets were also was realized for the first time in the presence of polymer additives.

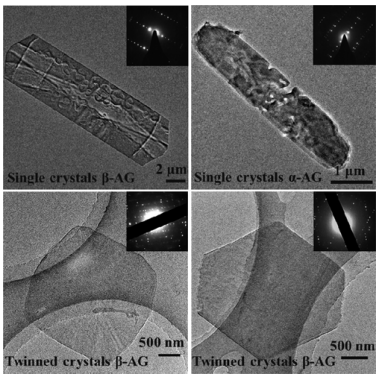

Fig. 1 Controlled synthesis of single crystalline and twinned crystalline guanine nanoplatelets.

Keywords: guanine; optical materials; biomimetic crystallization; functional biomimetics; nanocrystals

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T6 O7

Role of Polyamines in the condensation of silicic acid for efficient silicification and its study with pH

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Living organisms form mineral salts in highly-controlled biological processes. Biomineralization in marine organisms is a fascinating example of adapting various minerals to accommodate the essential functions of cells. Diatoms can condense soluble silicic acid into an insoluble silica barrier around their cell wall.[1] The silicification occurs within an acidic compartment called silica deposition vesicle (SDV), enriched with macromolecules like polyamines or lysine and arginine-rich.[2] Although these molecules effectively promote synthetic silicification, bio-silicification results in a more efficient process yielding 90% silica content. The inspired bio-silicification processes show a direction for silicification and studying the silicification in the diatoms. Since a recent study showed a role for phase separation in bioinspired silicification, in our current work, we look at liquid-liquid phase separation of ionic polymers and silicic acid, as a model system for efficient silicification.[3] We are currently finding organic amines' role in silicification processes with various factors such as concentration, pH, and ionic strength. We use commercially available cationic polyallylamine (PAH), and study how neutral or basic pH conditions might produce more silica precipitation than acidic conditions. We use dynamic light scattering (DLS) to check the concentration-dependent interactions between PAH and silicic acid in various solutions. Silica precipitation was quantified in each set of experiments to analyze total silica content by FTIR, TGA and SEM microscopy. The result of this study reveals the chemistry behind the initiations of silica condensation in the presence of cationic polymers.

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Poster Presentations

Poster 23

Novel Mms7-mediated biomimetic magnetite nanoparticles

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Magnetic nanoparticles (MNP) are magnetite crystals whose large surface-to-volume ratio, ability to be coupled with molecules and response to magnetic fields make them great supports to carry and concentrate a wide variety of compounds. However, commercial MNP present drawbacks that need to be improved, such as the need of post-production coating that provides functional groups to immobilize molecules, or their poor magnetic properties. These problems have been solved by the development of biomimetic magnetic nanoparticles (BMNP), which are produced by mimicking the biomineralization process carried out by magnetotactic bacteria to synthesize their magnetosomes. This biomineralization is mediated by magnetosome-associated proteins (MAPs) that bind iron and control crystal growth.[1] The addition of MAPs to chemical precipitation experiments *in vitro* enhances de nucleation and growth of magnetite crystals, providing BMNP with improved magnetic and surface properties.[2] MamC from *Magnetococcus marinus* MC-1 has been successfully used in the synthesis of BMNP, but it is necessary to elucidate the effect of other MAPs. In this work, Mms7 (*M. marinus* MC-1) was purified and used to synthesize BMNP. Transmission electron microscopy images show that the size of Mms7-mediated BMNP depends on the concentration of Mms7. X-ray diffraction and Fourier-transform infrared spectroscopy indicate that these magnetite nanoparticles contain Mms7, although it is unknown whether it is attached or embedded. The isoelectric point of Mms7-mediated BMNP is ~4.7, and they are less aggregated than MamC-mediated BMNP at pH values in the range 3-10. As occurs with MamC-mediated BMNP or inorganic MNP, Mms7-mediated BMNP can increase the temperature following upon laser exposure in the NIR or exposure to an alternating magnetic field. However, their response to external magnetic fields is less efficient since they present more organic matter (Mms7-mediated BMNP ~13%, MamC-mediated BMNP ~7%, MNP ~5%).

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Poster 24

Keeping cartilage covered: Quantifying growth rules in stingray tessellated cartilage

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Sharks and rays have cartilaginous skeletons covered by a continuous layer of abutting mineralized tiles (tesserae). Since these skeletons never stop growing, the presence of tesserae creates a challenging growth constraint: how can a continuous tiled covering be dynamically maintained while the volume of the underlying cartilage increases? In principle, maintaining an enlarging tiled surface could be supported either by an increase in the number of tesserae or by their growth, or by both. However, a constant mineral apposition rate at the surface of tesserae does not necessarily result in a continuous growth of the tessellation and may even lead to geometric incompatibilities. To understand how an organism has solved this conundrum, we examined the development of a stingray skeletal element (hyomandibula) in microCT datasets, which allow the first illustration of geometrical changes occurring at the level of an entire tesserae 'population', to quantify structural aspects of tesserae over 100% animal size increase. As animals age, the hyomandibula grows roughly isometrically and, although new tesserae are added, most skeletal growth is accomplished by proportional growth of individual tesserae. Tesserae are typically brick-like in cross-section (3-4x wider than they are thick), but several skeletal regions exhibited distinct trends: multilayered, thin tesserae at the cranial articulation; irregular, patchy tesserae where muscles attach; and columnar tesserae forming stout ridges. Across ontogeny, tesserae ranged from 4- to 8-sided shapes, but were predominantly hexagonal (i.e. with 6 neighbours), especially in flat regions (zero mean curvature), whereas either individual very large or multiple small tesserae were employed to tile curved surfaces. These results provide quantitative insights into how nature can craft complex shapes from tiled architectures and into the dynamic interplays governing growth in tessellated cartilage, where topological requirements (e.g. filling of gaps generated by growth) are balanced by both geometrical and mechanical constraints (e.g. neighbouring tesserae, muscular forces).

Topic 7 Biomineralization in Aquaculture

Keynote lecture

Invited Review: Contribution of Biomineralization Research to the Advancement of Pearl Aquaculture

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In this presentation, the relationships between pearl quality and the function of a pearl sac will be discussed, emphasizing the importance of physiological and cell biological research on pearl sac epithelial cells. 1. Quality of a pearl is determined by a pearl sac: A pearl is formed in a follicle called a pearl sac derived from the outer epithelial cells of the mantle. Changes in the pearl sac function recorded in the crystal layers cause variations in pearl quality. The initial formation of a prismatic calcite layer on the nucleus affects the size and cloudiness of a pearl. The shape of aragonite tablets in the nacre affects the color and luster of a pearl. An abnormal pearl sac formation process leads to a deformed pearl. 2. Control of the function of a pearl sac: When tissue debris is included in a pearl sac, organic substances from pearl sac epithelial cells are produced. After this step, the pearl sac cells shift their function to aragonite or calcite formation. To realize the changes in the function of a pearl sac, clarification of the molecules controlling the differentiation of pearl sac epithelial cells is essential. In the great pond snail, Lymnaea stagnalis, insulin-related neuropeptides control body and shell growth. Orthologous peptides in bivalves are candidates for the endocrine hormone controlling the growth of a pearl. 3. In vitro culture of pearl sac epithelial cells: Cell culture of the mantle outer epithelial cells has been tried, and transient cell proliferation is observable. But it is difficult to maintain the growth and normal functions of the cells mainly because information on the suitable culture media and substrates is insufficient. 4. Biomineralization research and pearl aquaculture: Physiological and cell biological studies on pearl sac cells will contribute to the advancement of pearl culture technologies. How do changes in environmental factors and nutritional conditions affect pearl formation? Novel research is anticipated.

Oral Presentations

T7 O1

Estradiol and bisphenol A effects on early skeletogenesis in the European sea bass *D. labrax*

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First identified as sex hormones, oestrogens have been shown to play a key role in gonadal development, but also in skeletal development and homeostasis. In mammals, cytosolic estrogen receptors are expressed in osteoblasts, osteoclasts, osteocytes as well as in chondrocytes and chondroblasts, regulating their activity to build and maintain cartilage and bone. To extend our knowledge of estrogen signaling in skeletal development beyond mammals, we used the European sea bass Dicentrarchus labrax. Because natural and synthetic estrogens are pollutants in aquatic ecosystems, they can have important consequences for estrogen-sensitive functions, such as skeletal development, when waterborne exposure occurs during early life stages when blood estrogen concentrations are low. To better understand how (xeno)oestrogens can affect the skeleton, sea bass larvae aged 6-23 days post-hatch were experimentally exposed to two concentrations of a natural estrogen (E2), and a xenoestrogen, the bisphenol A (BPA). The levels of mineralisation of the cranium, vertebrae and fins were investigated by Alizarin Red staining. In addition, the RNA expression levels of several genes playing a key role in skeletogenesis and estrogen signaling pathways were quantified. Our results showed that E2 and BPA have contradictory effects on gene expression and mineralisation phenotype. We also show that their effects depend on the time of exposure, either before or after the initiation of bone mineralisation.

Keywords: estrogens, skeleton, mineralization, European sea bass

T7 O2

An alternative method for extracting the organic matrix from the skeleton of the red coral Corallium rubrum

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As in other animals, skeleton formation in corals is a biologically controlled process in which the organic matrix (OM) corresponds to the organic part of the biomineral produced and secreted by the animal. An essential step in the biochemical characterization of the OM is to isolate it, even though it is enclosed in the biomineral. Its extraction therefore requires several tedious steps usually including (cryo)grinding, demineralization, as well as multiple bleaching and rinsing steps, followed by concentration; a methodology that often results in moderate reproducibility.

Here we present an alternative and simple OM extraction method for the red coral Corallium rubrum that requires only a few equipment and steps. The entire calcium carbonate skeleton is directly demineralized to produce a gelatinous material called a ghost, which corresponds to the structured OM. This water-stable ghost is then rinsed and "melted" at 80 °C. To validate our approach, we compared the OM of C. rubrum produced by the standard method and our alternative method: The comparative analyses by electrophoretic migration, Western blot and FTIR spectroscopy show that the "alternative OM" is equal or even better in quality than the "standard OM".

Our more direct and faster method is certainly suitable for the red coral, but probably also for other OM-rich biominerals of other organisms.

T7 O3

Biogenic organic crystals are solid solutions: HPLC-Quantification of purine-bases

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Guanine is an ubiquitous molecule in nature, illustrating its essential roles by e.g., conserving genetic information in the form of DNA or RNA, acting as a high-capacity and rapid-turnover metabolite, or as a signaling molecule. Furthermore, there are numerous examples where the high refractive index of anhydrous guanine-crystals is employed to serve a light-manipulative or dyeing purpose [1].

Jantschke et al. was the first to examine guanine deposits in the dinoflagellate *Calciodinellum operosum aff.* using Raman imaging and 2D/3D-cryo-(FIB)-SEM [2]. Later, Mojzeš et al. identified crystalline guanine in phylogenetically distinct groups of microalgae [3] that sometimes contained a significant amount of other purines [4]. Powder-XRD and solid-state NMR analyses revealed that biogenic guanine crystals are rather solid solutions that bear the flexibility to incorporate up to 20 % of other purines, especially hypoxanthine, without altering the crystal structure significantly [5]. Yet, a method for the precise quantification of the purine composition of those crystals remained to be found.

Herein, we present an HPLC method for the determination of various purine concentrations within cells, including uric acid, xanthine, hypoxanthine, and guanine, allowing the precise quantification of all analytes in the µM-range. Samples were prepared by acidic hydrolysis using concentrated perchloric acid. The method was optimized regarding column material, temperature, flow rate, and mobile phase. Here we found that employing an Agilent Zorbax SB-Aq column with a linear gradient using ammonium formate buffer (10 mM, pH=5) and methanol, at 30 °C, yielded clearly separated analyte peaks.

This method underlines its elegance by opening an easy and universal pathway to analyze the purine-composition of various samples and understand the purine variability in biogenic guanine crystals.

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Keywords: Microalgae, HPLC, Purines, Solid Solutions

T7 O4

Carbonic anhydrase activity identified in the powdered nacreous layer of Pinctada fucata.

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The Japanese pearl oyster, Pinctada fucata, is used for pearl aquaculture in Japan. The shells of P. fucata, composed of CaCO3 and several organic molecules, have the outside prismatic layer and the inside nacreous layer. The organic molecules containing chitin and proteins play significant roles in shell mineralization. Nacrein is the most abundant acid-soluble protein in the shell of P. fucata. Nacrein has a carbonic anhydrase (CA) domain that catalyzes the reversible conversion of CO2 to HCO3-. We discovered that the nacre powder (NP) directly obtained from crushed P. fucata shells displayed CA activity, with a Km of 3.44 mM and kcat/Km of 920 M-1 s-1. The addition of the NP in solutions increased the rate of CO2 uptake. The NP was also thermally stable at temperatures over 70°C and reusable after ten cycles of catalysis. The fluorescence detection study revealed that nacrein was exposed on the surface of the NP, indicating that nacrein localized on the NP's surface and contributed to the NP's CA activity. We conducted further examination of CA activity in other industrial waste shells. The powdered prismatic layer of P. fucata and the shell of Mizuhopecten yessoensis exhibited CA activity, whereas the powdered shell of Crassostrea gigas did not. CA has gained attention as a potential material for CO2 sequestration to combat global warming. As the shell of mollusks is a waste from the aquaculture, the shell powder can be produced inexpensively. Therefore, we propose usage of the shell powder as an effective, thermally stable, reusable, and low-cost biocatalyst for CO2 sequestration.

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Keywords: carbonic anhydrase, nacre powder, CO2 sequestration

Poster Presentations

Poster 25

The very first days of cultured pearls from French Polynesia

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The quality of natural and cultured pearls depends on the size, shape and properties of the surface (colour, lustre) of the nacreous layer. In 1893, a method allowing for production of cultured pearls was elaborated in Japan: the grafting method which involves two pearl oysters of the same species. One is used to produce the grafts, pieces of living tissues cut from the nacre producing area of the mantle. Then, each graft is deposited onto a spherical body (nucleus) in another animal. After wrapping of the nucleus by the expanding graft, the mineralization restarts. As the graft was producing nacre before being cut, it is expected that the nucleus will be covered by nacre, allowing for production of the much prized round shaped pearls.

Nevertheless, structural irregularity and heterogeneous mineralogy of pearls have long been noticed: non-nacreous material such as “aragonitic” prisms and calcite are common. Such units are not known in the shell of Pinctada. In situ characterization methods at a micro- and nanoscale on Tahitian pearls produced by P. margaritifera allow for a direct examination of samples grown during the very earliest days of the mineralization process of pearls. Correlative data on mineralogy, crystallinity, structure and elemental composition are obtained.

First day deposits show a great variability of the inner structures and composition. There are no two identical samples. Moreover, the arrangement is irregular within a single pearl, and some structures (aragonitic pseudo-prisms) do not exist in the shell. Thus, the assumption that the cultured pearl is a nucleus covered by a thin organic layer, a thin prismatic layer and a thick nacreous layer similar to what is known in the shell of the oyster is, in most cases, erroneous.

Keywords: nascent pearl, structure, composition, in situ analyses

Poster 26

In vivo injection method to study control of calcification in Octocorals.

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Corals have evolved with the ability to build biominerals structures resulting in the formation of sclerites and/or axial skeletons made of calcium carbonate and organic molecules. The term 'coral' has been extensively used to describe tropical corals, or 'reef-building corals' defined in the class Hexacorallia, yet another class diverged 550-650 million years ago in the pre-Cambrian resulting in the Octocorallia class (soft corals, sea pens and sea fans). Our study focusses on corals from the Octocorallia class, excellent comparative models. Despite information at the cellular and physiological level, knowledge of the mechanisms controlling coral calcification remain elusive due to the lack of appropriate techniques.

Long term exposures to exogeneous molecules are very challenging in aquaria facilities because of the seawater renewal, costs of compounds, their quantity, solubility, and diffusion in seawater. Here, we provide a viable alternative method through *in-vivo* injection of substances of interest dissolved in a liquid vehicle which solidifies upon injection. Our experimental study evaluates slow-release and localised injection as a novel method for delivering an internal emulsion with molecules of interest. Local diffusion of the injected products in the organism was followed using visual tracers. Specifically, we followed two classes of fluorescent markers, one of which investigated the internalisation into cells, while the others were used as an application to monitor the calcification process.

This methodology was optimised on *Sarcophyton sp*. (order Malacalcyonacea) for validation of the injection procedures and characterise the diffusion of the markers, while proof of the transferability of the methodology was performed on the precious coral *Corallium rubrum* (order Scleralcyonacea).

Keywords: *Corallium Rubrum*, *Sarcophyton*, injection of exogeneous molecules

Topic 8 Biomineralization and environmental changes

Keynote lecture

Invited Review : Impacts of climate change on mollusc biomineralisation pathways

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Marine molluscs are vulnerable to the impacts of ocean acidification driven by oceanic CO2 absorption, and coastal acidification driven by land run off. These drivers of environmental change have deleterious effects on shell growth. Ocean acidification and global warming, limit the ability of molluscs to produce their calcium carbonate protective shells. The mechanisms of coastal and ocean acidification are very different, however similarities occur including increases in dissolved inorganic carbon and reduced availability of carbonate. Limited carbonate under acidification has been shown to inhibit shell growth and the material properties of shells, suggesting an alteration in carbon sequestration. Molluscs control their shell growth through biomineralization, but the mechanisms behind biomineralization and their response to acidification are relatively unknown. It is unclear how much carbon is taken into the shell from the environment compared to metabolic sourcing. Shell production is energetically costly to molluscs and metabolic processes and energetic partitioning may affect their ability to perform biomineralization under environmental change. The mechanisms of shell growth through carbon uptake have been examined under both experimental and naturally occurring coastal acidification. Carbon isotopes (δ13C) have been used to examine the change in biomineralization pathway in molluscs under environmental change. The carbon uptake by δ13C tracing and deposition into mantle tissue and shell layers has been examined in several different species of mollusc with varying calcium carbonate polymorphs. These biomineralization pathways are compared in Magallana gigas, Mytilus sp.. and Saccostrea glomerata grown under acidification and warming to assess the vulnerability of molluscs to environmental change. Are molluscan biomineralization pathways under environmental change species-specific and what can we learn from experimental versus coastal acidification?

Keywords: Molluscs Biomineralization ocean acidification warming

Oral Presentations

T8 O1

Secondary aragonite recrystallization during otoliths diagenesis: natural and experimental evidence

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Biogeochemical signatures recorded in calcareous biominerals are used as environmental and physiological proxies. Reliability of these signatures can be compromised during the fossilization, due to diagenetic processes that affect especially aragonite biominerals, which are typically transformed into the more stable calcite during burial. Thus, aragonite is rarely preserved in fossils, and its exceptional occurrence is considered as evidence of pristine preservation. In this study, we show that fossil otoliths from several Cretaceous and Cenozoic deposits from Poland, despite of aragonite preservation, show clear evidence of some crystallographic and structural modifications such as: coarsening of aragonite crystals, generation of porosity, and lattice parameters modifications. This suggests that, although the mineralogy of fossils remains the same, the aragonite is altered. To test this hypothesis, we performed diagenetic experiments in aragonite otoliths (saggitas) of Micromesistius poutassou, in simulated burial water for 7 and 14 days (100°C and 175°C). We compared pristine and altered otolith structures, mineralogy, and biopolymer content. During the experiment, no substantial organics loss occurs, but biopolymers become more complex. Alteration of organics is suggested to slow down the aragonite transformation kinetics, making otoliths more resistant to hydrothermal alteration (containing < 2.5 wt.% calcite after 14 days at 175°C). However, secondary aragonite forms as parches via a slow dissolution - precipitation reaction facilitated by fluid migration toward the interior. The secondary aragonite keeps the original crystallographic orientation, but undergoes changes in lattice parameters, organics content, solubility, and porosity. These results suggest that aragonite "pristine" preservation of fossils requires careful reconsideration.

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T8 O2

Primary coral polyps responses to decreasing seawater pH: observations from cell to the complete organism

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In light of increasing stress and mass mortality of reef-building corals due to climate change, the resilience of some marine ecosystems relies on successful coral larva recruitment. Knowledge of the acclimatory and/or adaptive potential as a response to environmental challenges such as ocean acidification (OA) in the earliest life stages is limited. In this study, we report on *Stylophora pistillata* larvae and primary polyps cultured under acidic pH conditions. We investigated the response of the endosymbiotic algae and the coral host across biological levels, from cellular to organismal. By combining transcriptomic analysis with physiological and morphological measurements, we identified that while the number of survival and settlement of coral larvae were reduced under OA conditions, the surviving recruits adjusted well to the challenging conditions. Coupling synchrotron X-ray μCT with artificial intelligence, we found that OA has a morphologically-altering effect on coral skeletal features. Although skeleton growth is reduced, coral recruits possess acclimatory mechanisms allowing them to survive under OA conditions. These include the transition to a less-mineralized/increased-tissue phenotype exhibiting greater incorporation of organic matrix proteins within the skeletal fibers. Moreover, we found that the algal photosynthetic activity is stimulated under OA, as well as the transfer of photosynthates to the coral host, potentially sustaining the host energetic expenses.

Keywords: Coral, Ocean acidification, X-ray μCT

T8 O3

Investigating the impact of ocean acidification on $CaCO_3$ crystal growth rates in the reef coral *Stylophora pistillata*

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Keywords : extracellular calcifying medium, coral biomineralization, growing edge, light enhanced calcification, fluorescent markers

The aragonite skeleton of scleractinian corals forms in the extracellular calcifying medium (ECM) which is separated from seawater by the calicoblastic epithelium and the overlying tissue layers. The calicoblastic cells exert biological control on the composition of the ECM via regulation of transcellular and paracellular ion transport. Despite biological control of the ECM and its physical separation from the surrounding seawater, coral calcification is sensitive to environmental changes, such as ocean acidification. The mechanisms explaining the environmental sensitivity of coral calcification are poorly understood. Our research addresses this knowledge gap by investigating the links between cellular physiology and calcification of corals in vivo. To achieve this, we focus our investigations on the 'growing edge' of Stylophora pistillata, an area of the coral colony where the calicoblastic cells and the ECM can be analysed directly, and lateral growth can be observed through the formation of isolated CaCO3 crystals at the edge of the skeleton. Our previous analysis of the growing edge has provided in vivo insight into how ocean acidification disrupts pH regulation of the ECM and calicoblastic cells, and causes changes in paracellular exchange between seawater and the ECM. To understand how these physiological changes may affect calcification, we are currently characterizing the formation of the isolated crystals at the growing edge under different conditions of experimental ocean acidification. Our analysis involves using confocal microscopy and fluorescent marker dyes that allow us to quantity the growth rates of a range of crystal morphologies and sizes. Because coral calcification is sensitive to light exposure, via a poorly understood process termed 'light enhanced calcification', we are investigating coral crystal growth rate at the growing edge in both light and darkness in conditions of acidification.

T8 O4

Organization and magnetic properties of magnetic ectosymbiotic bacteria optimize a collective magnetotaxis behavior in a microbial holobiont

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Magnetotactic bacteria (MTB) are a diverse type of motile, single-cell prokaryote well known for their capacity to biomineralize chains or clusters of magnetosome organelles that contain magnetic nanocrystals of magnetite or greigite [1]. They are ubiquitously found in micro-/anaerobic conditions and can easily locate such habitats thanks to the collective magnetic moment of their magnetosomes, which is referred to as magnetotaxis. Though they have been known to create multicellular consortiums [2], it was only recently discovered that non-motile MTB were symbionts of a flagellated microeukaryotic host, together known as a holobiont [3]. This magnetotactic holobiont (MHB) performs a magnetic field-assisted motility guided by a chemaerotaxis system similar to individual free-living MTB. This complex behavior raises many questions regarding how magnetic properties of symbionts determine holobiont magnetism and motility. This work employed a suite of light-, electron- and X-ray-based microscopy techniques (including X-ray magnetic circular dichroism (XMCD)) to reveal that symbionts optimize the motility, the ultrastructure, and the magnetic properties of MHBs from the microscale to the nanoscale [4]. The surface organization of magnetic symbionts is explicitly presented with electron and X-ray microscopy, depicting bacterial membrane structures that ensure longitudinal alignment of cells. Magnetic dipole and nanocrystalline orientations of magnetosomes were shown to be consistently oriented in the longitudinal direction, maximizing the magnetic moment of each symbiont. The magnetic moment transferred to the host cell is in excess (100-1000X stronger than free-living MTB), well above the threshold for the host cell to gain a magnetotactic advantage. With an excessive magnetic moment given to the host cell, the benefit provided by magnetosome biomineralization beyond magnetotaxis can be questioned.

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Keywords: Magnetosome, magnetite, X-ray microscopy, magnetotaxis

T8 O5

Cellular pH regulatory systems underlying calcification in the sea urchin larva

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To generate their skeletons, sea urchin larvae like many other marine calcifyers acquire carbonate ions that largely derive from the hydration of metabolic CO_2. In this process protons are liberated that need to be removed from the calcification front to promote calcification and to defend cellular acid-base balance. Thus, biological calcification and pH regulation are intrinsically linked processes that remain relatively unexplored.

Using a wide range of techniques ranging from life cell imaging and intracellular pH recordings over protein-biochemical methods to molecular technologies our work identified a set of PMC specific ion transporters and channels that mediate a carbon concentration mechanism (CCM) and removal of protons liberated by the calcification process. During skeleton regeneration pH_i and intracellular HCO_3^- levels are increased accompanied by a change in proton transport pathways. During active mineralization, resting PMCs are recruited into the calcifying state seen in their physiology and increased expression of of key acid-base transporters including HCO_3^--transporters (Sp-Slc4a10), Carbonic anhydrases (Cara7) and the proton channel Otop2l.

Using the sea urchin larva as a model organism we are able to resolve fundamental mechanisms of acid-base transport in calcifying systems. Energy efficient mechanisms of intracellular pH regulation to generate $CaCO_3$ from a metabolic waste product – carbon dioxide- demonstrates the elegance of nature in utilizing resources in the most sustainable manner. In this way, insights into the cellular calcification mechanisms may help to develop novel approaches for biology-inspired technologies to sequestrate carbon in times of rapid climate change.

Keywords: Cell physiology, membrane transport, pH regulation, carbon concentration, environmental change

Poster Presentation

Poster 27

Bone as a bioadsorbent for bacteria removal

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Contamination of domestic water by potentially dangerous bacteria and other microbes is a common problem, which may cause gastrointestinal and short-term diseases. The development of procedures that use environmentally friendly materials (preferably residues) with high bacterial retention capabilities is an interesting approach. In this context, chicken bone is a residue from the food industry, mainly composed of an inorganic phase (nanocrystalline carbonated apatite) mineralizing an organic matrix (mainly type I collagen). Thermal treatments can change the characteristics of the mineral and the organic matrix, resulting in a material with increased microporosity and enhanced surface reactivity. Thus, thermally treated bone can be used as an efficient adsorbent for different compounds and/or microorganisms. With increasing temperature bone properties change due to dehydratation, loss of organic components and recrystallization of the mineral. To obtain deproteinate bone mineral, bones are treated at 600ºC, since combustion of most organic components occurs up to 600ºC. This calcination process eliminates all pathogens and creates additional microporosity and a large surface area. Our results show that calcined bones placed in a bacterial culture, have a higher adsorption capacity of microorganisms than untreated bone. Also, we have studied the bacterial adsorption ability of different types of bone (cortical and medullary), subjected to different calcination temperatures (400ºC, 600ºC and 800ºC) that differ in their porosity and reactivity, as a proof of concept of green and sustainable technology involving bone waste recycling for bacterial adsorption from aqueous samples.

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Keywords: Chicken bone, thermal treatments, microporosity, bacterial adsorption

Topic 9 Evolution of molecular tool kits

Keynote lecture

Invited review : Few thoughts on the evolution of calcifying matrix proteins in metazoans

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In metazoans, the deposition of calcium carbonate biominerals is regulated by an organic matrix, secreted at the interface between the mineralizing cells and the forming calcified tissue (skeleton, or non-skeletal tissue). This matrix – or a part of it - remains occluded in the calcified tissue once formed.

Recent high-throughput screening techniques (i.e., combination of transcriptomics and proteomics) have revealed that this matrix is a complex system composed of dozens to hundreds of proteins that belong to several families of elusive molecular functions. Similarly to proteins of the extracellular matrix (ECM) of vertebrates, many of these proteins are modular, i.e., their primary structure is organized in a succession of different functional domains. While some domains are identified by sequence similarity (like enzymatic domains), the function of others is unknown, or 'guessed' but not experimentally tested. Of particular importance are Low Complexity Domains (LCDs) that can account for a large proportion of all domains in a matrix, but also domains putatively involved in protective functions sensu lato (like immune or protease inhibitor functions). Transmembrane domains and domains of cytoplasmic / nuclear origin are also of notice. Today, the matrix is considered as a molecular toolbox for calcifying, with a high evolutionary plasticity. Through few examples, this talk will sketch how functional domains were recruited for calcification, and how some calcifying matrix proteins are lineage-specific while others are ancient and may have been recruited multiple times. This talk will also address the question of the emergence of complex and stable-through-times forms under the control of 'molecular tools' (proteins) that evolve fast and, consequently, are unstable-through-time. It will also reemphasize the central role of the calcifying cells/tissues and the likely dialog between these living entities and the growing mineral.

Keywords: **Proteomics - Metazoan - Skeleton - Evolution - Matrix**

T9 O1

The vertebrate skeleton through the eye of a cartilaginous fish: Evolutionary perspectives raised by the study of the small spotted catshark Scyliorhinus canicular

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Bone together with other mineralized tissues evolved early in the vertebrate lineage (more than 415 Myr ago), even before the evolution of jaws. Endochondral bone though has evolved as a general rule in the bony fish lineage only, to which most model organisms for developmental biology belong (tetrapods or teleost fishes). In the cartilaginous fish lineage however, bone is considered secondarily lost despite conspicuous mineralisation of the skeleton in the form of tessellated cartilage. Recently, an integrative set of data has informed the developmental dynamics, the cellular characteristics and the genetic actors of skeletal mineralisation in sharks and rays, in particular through the study of a non-model organism, the small spotted catshark *Scyliorhinus canicula*. These data have highlighted several aspects of cartilaginous fish-specific evolutionary events, including gene duplication, diverged developmental processes and probably cell type identity. Despite prolonged independent evolution in the bony fish and cartilaginous fish lineages, the comparative analysis of these data also helps identify an ancestral genetic toolkit, still to be discussed regarding other mineralised tissues in extant jawed vertebrates.

Keywords: EvoDevo; vertebrate skeleton; shark; genetics