→ Biological Materials
→ Biological and Bio-inspired Materials

BIOMATERIALS

Research in the Department of Biomaterials

Peter Fratzl 13.09.1958
1980: Diploma (Ingénieur Diplômé de l'Ecole Polytechnique, Paris)
1983: PhD, Physics (University of Vienna)
Thesis: Investigation of an Al-Zn-Mg alloy using diffuse neutron scattering
1981-1985: Research Scientist (Austrian Academy of Sciences, Vienna; Laboratoire Leon Brillouin, Saclay, France); Visiting Research Fellow (Hahn Meitner Institute, Berlin; New York University)
1986-1998: Assistant and Associate Professor (Institute for Materials Physics of the University of Vienna, Austria)
1988 and 1989: Visiting Professor (Rutgers University, New Jersey, USA)
1991: Habilitation, Solid State Physics (University of Vienna)
Thesis: Precipitation in alloys – small-angle x-ray scattering and computer simulation
Since 1993: Research Associate (Ludwig Boltzmann Institute of Osteology, Vienna).
1993-1994: Visiting Research Fellow (Heriot-Watt University, Edinburgh)
1997: Visiting Professor, (Physics Department of the University of Munich)
1998-2003: Chair of Metal Physics (University Leoben, Austria)
Director (Erich Schmid Institute for Materials Science of the Austrian Academy of Sciences)
Since 2003: Director, Department of Biomaterials (Max Planck Institute of Colloid and Interfaces, Potsdam-Golm)
Since 2004: Honorary Professor of Physics at Humboldt University Berlin
Since 2009: Honorary Professor (Physics of Biomaterials) at the Potsdam University
Since 2010: Acting Director of the Experimental Department II at the Max Planck Institute for Microstructure Physics, Halle

The Departments focuses on biomaterials research in a somewhat broader sense:

- by using materials science approaches for studying structure-function relationships in biological systems, with potential applications in biology or medicine;
- by studying the "engineering design" which arose during the evolution of natural materials and to extract useful principles for the development of new bio-inspired materials;
- by developing new materials for contact with biological tissues, leading to implantable biomaterials or with applications in tissue engineering.

Together we define this as Biological Materials Science which is inherently multidisciplinary between physics, chemistry and biology. All three areas mentioned above are addressed in the Department with a significantly stronger emphasis on the first two. To tackle such questions, the members of the Department have very diverse scientific backgrounds, including mathematics, physics, chemistry, materials science, physical chemistry, biochemistry, wood science, botany, zoology and molecular biology.

In the course of evolution, load-bearing biological materials have generally not evolved towards perfection and maximum strength, but instead developed high defect tolerance and adaptability **[1]**. Adaption occurs at various levels, see figure 1. While evolution leads to adaptation of entire species, each individual has mechanisms which confer some self-repair properties even at smaller scales to cope with a variety of environmental challenges. Healing and regeneration occur at the level of organs, but many biological materials are damage-tolerant at the supra-molecular level or have (passive) self-repair properties (**see Fig. 1**).

Research Topics

The Department addresses the adaptation of natural materials according to all three levels mentioned in Figure 1, both to advance the understanding of these biological systems, as well as to extract concepts for the development of adaptive, self-healing or multi-functional materials. The adaptation by evolution (cycle (a) in **Fig. 1**) can be studied by comparing the details of material structure (such as teeth, bones, or shells) in closely related species and comparing these to the variability in function due, for example, to differences in habitat. This perspective is taken in particular by *Mason Dean* in the context of fish skeletons and by *Michaela Eder*, studying to plants which, for example, adapt to frequent fires (see their reports).

Bone remodeling is also a process by which damaged tissue is continuously replaced by newly synthesized material and, thus, an interesting case of adaptation (cycle (b) in **Fig. 1**). Bone remodeling generally depends on a dense network of mechanosensitive cells, called osteocytes. This network is such that all mineralized tissue in bone is not further away from the next osteocyte canaliculum than about one micron, making the network an extremely effective transport system **[2]**. While bone continuously repairs damage through remodeling, it needs a more complicated process to heal after a fracture occurred. The healing process is a matter of intensive research in the Department in collaboration with the Charité University Hospital in Berlin and other partners. The questions of healing, remodeling and mechanical adaptation of bone are addressed by the groups of *Wolfgang Wagermaier* and *Richard Weinkamer* (see their reports). In addition, scaffold-supported healing is studied in-vivo **[3]** as well as in-vitro **[4]**, primarily to elucidate the interaction between growing tissue and the geometric constraints from the scaffold material (see report by *John Dunlop*). Other clin-

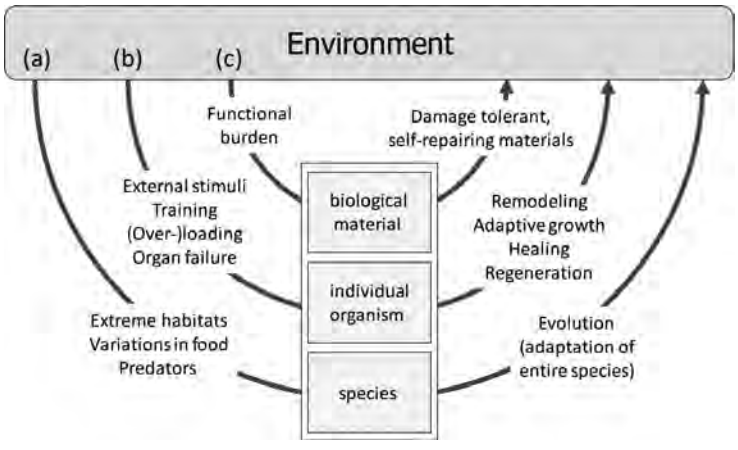

Fig. 1: Three levels of natural adaptation to environmental influences [1]. (a) Darwinian evolution acts on the species level to adapt to long-term challenges, such as habitat, food type or predators. (b) Remodeling, healing or regeneration operate at the organ level within an individual organism. (c) Biological materials, such as bone, extracellular tissue or protein fibers are damage tolerant and often have self-repair mechanisms that operate on the supra-molecular level.

ically oriented research on bone diseases, such as osteoporosis and osteogenesis imperfecta (brittle bone disease) is carried out in close collaboration with the Ludwig Boltzmann Institute of Osteology in Vienna, Austria.

While bone remodeling and healing are processes operating at the organ level (similarly to many kinds of wound healing in animals or plants), there are also intrinsic material properties which provide damage tolerance and self-repair (cycle (c) in **Fig. 1**). Examples are deformable interfaces connecting stiff protein or polysaccharide fibers or mineral platelets and capable of absorbing large deformations in tissues, such as tendons or plant cell walls **[5]**. In some cases, damage is fully recovered over a short or a longer period of time, thus providing some type of self-repair. This is a major topic in the research group of *Matthew J. Harrington* (see his report).

Natural materials are not only based on proteins or cellulose, but in many organisms also on chitin. Arthoropods, such as spiders for example, use their chitin cuticle to house a wide range of sensors and tools which are highly exciting examples of unusual engineering solutions for a variety of technical problems. The group of *Yael Politi* is primarily focusing on this type of research (see her report). The interaction of water with all these biomolecules (proteins and polysaccharides alike) plays an important role for their mechanical behavior, including materials properties, such as stiffness and toughness, but also actuation and the generation of internal stresses. This topic is addressed by *Luca Bertinetti* and partially also in the group of *John Dunlop* (see their reports).

Biomineralization is a further strong topic of the Department. Its director has just been chairing the Gordon Research Conference on Biomineralization in 2012. *Damien Faivre*, who's research group is being essentially supported by an ERC Starting Grant from the European Research Council, works on elucidating how bacteria control the growth of magnetite nanoparticles through the interaction with specialized proteins (see his report). Together with partners at the Weizmann Institute (Prof. Lia Addadi), we were awarded a 5-year grant from the German Science Foundation (within the DIP-Program) to study the origin of the stability of amorphous bio-minerals **[6,7]**. *Wouter Habraken* is strongly active in this project (see his report) and Yael Politi's group is also involved in some of this research. Until spring 2012, Barbara Aichmayer was heading a group concentrating on biominerization of calcium-based minerals. She already left in summer 2012 and no report is included. Some of her publications are, however, mentioned here [6,8,10,11] and in other places of this report (see for example the section by *Admir Masic*).

Methodological Approaches

Generally, the experimental approach is based on multi-method imaging where different probes are used to image the same specimen. This provides information on different features of the materials such as micro-structure, chemical composition, or mechanical properties in a position-resolved manner with micron-range resolution. We are currently developing and using multi-method characterization approaches combining x-ray tomography; scanning electron microscopy and scanning x-ray diffraction to characterize micro- and nanostructure and many levels of structural hierarchy (see report by *Wolfgang Wagermaier*). We have established polarized and confocal Raman imaging to provide information on chemical composition and fiber orientation, which is now being combined in-situ with synchrotron x-ray scattering (see report by *Admir Masic*). We use nano-indentation as well as acoustic microscopy to estimate local mechanical properties. Currently, *Igor Zlotnikov* is establishing modulus mapping which pushes the lateral resolution of mechanical characterization into the nanometer range (see his report). The strength of this multi-method approach is that the different parameters measured on the same specimen can be correlated at the local level with micron (or even smaller)-scale spatial resolution. This facilitates the extraction of structure-property relationships even in extremely heterogeneous materials with hierarchical structure.

In a second type of approach, we study in situ changes in various materials (e.g. due to mechanical stress or to chemical or thermal processing) by time-resolved scattering or spectroscopy during mechanical deformation or thermal or hygroscopic treatment. This gives insight into the molecular and supramolecular mechanisms which are responsible for the noteworthy properties of these materials. In some cases, such measurements can be performed in the laboratory (e.g. with Raman or infrared spectroscopy or in the environmental scanning electron microscope), but in many cases synchrotron radiation is needed (e. g. for x-ray diffraction or small-angle scattering). A dedicated beamline end station for scanning small- and wide-angle scattering and fluorescence spectroscopy is operated at the synchrotron BESSY at the Helmholtz Zentrum Berlin **[8]**.

These efforts are complemented by a significant effort in mathematical modeling, which is always closely tied to the experimental work in the department. Typically, modeling and experimentation go hand in hand with the research projects (see for example the reports by *John W.C. Dunlop* and *Richard Weinkamer*).

References:

[1] Weinkamer, R., Dunlop, J.W.C., Bréchet, Y., Fratzl, P. All but diamonds: Biological materials are not forever, Acta Mater 61, 880 - 889 (2013).

[2] Kerschnitzki, M., Kollmannsberger, P., Burghammer, M., Duda, G.N., Weinkamer, R., Wagermaier, W., Fratzl, P. Architecture of the osteocyte network correlates with bone material quality, Journal of Bone and Mineral Research (2013) [Epub ahead of print] doi: 10.1002/jbmr.1927.

[3] Woodruff, W.A., Lange, C., Chen, F., Fratzl, P. , Hutmacher, D.W., Nano- to macroscale remodeling of functional tissue-engineered bone, Advanced Healthcare Materials 2, 546 - 551 (2013).

[4] Bidan, C.M., Kommareddy, K.P., Rumpler, M., Kollmannsberger, P., Fratzl, P., Dunlop, J.W.C. Geometry as a factor for tissue growth: towards shape optimization of tissue engineering scaffolds, Advanced Healthcare Materials 2, 186 - 194 (2013).

[5] Dunlop, J.W.C., Weinkamer, R., Fratzl, P., Artful interfaces within biological materials, Materials Today 3, 70 – 78 (2011).

[6] Gal, A., Hirsch, A., Siegel, S., Li, C., Aichmayer, B., Politi, Y., Fratzl, P., Weiner S., Addadi, L., Plant cystoliths: a complex functional biocomposite of four distinct silica and amorphous calcium carbonate phases. Chem. Eur. J. 18, 10262 - 10270 (2012).

[7] Gur, D., Politi, Y., Sivan, B., Fratzl, P., Weiner, S., Addadi, L., Guanine-based photonic crystals in fish scales form from an amorphous precursor. Angew. Chem. Int. Ed. 52, 388 - 391 (2013).

[8] Paris, O., Aichmayer, B., Al-Sawalmih, A., Li, C., Siegel, S., Fratzl, P. Mapping lattice spacing and composition in biological materials by means of microbeam x-ray diffraction. Advanced Engineering Materials 13, 748 - 792 (2011).

[9] Kolednik, O., Predan, J., Fischer F.D., Fratzl, P., Bioinspired design criteria for damage-resistant materials with periodically varying boundary conditions. Advanced Functional Materials **21**, 3634 - 3641 (2011).
[10] Schenk, A.S., Zlotnikov, I., Pokroy, B., Gierlinger, N., Masic, A., Zaslansky, P., Fitch, A.N., Paris, O., Metzger, T.H., Cölfen, H., Fratzl, P., Aichmayer, B., Hierarchical calcite crystals with occlusions of a simple polyelectrolyte mimic complex biomineral structures. Adv. Funct. Mater. **22**, 4668 - 4676 (2012).
[11] Gilow, C., Zolotoyabko, E., Paris, O., Fratzl, P., Aichmayer, B., Nanostructure of biogenic calcite crystals: a view by small-angle x-ray scaterring. Cryst. Growth Des. **11**, 2054 - 2058 (2011).

Visiting Scholars

Several experienced scientists have been spending significant time in the Department. *Franz Dieter Fischer*, professor of mechanics at the Montanuniversität Leoben (Austria) recipient of the Alexander von Humboldt Award, came for many short visits, which helped advance the mathematical modeling of tissue growth in particular (see report by *J.W.C. Dunlop*) and was involved in theoretical research about the mechanical properties of biological hybrid materials **[9]**. *Hartmut Metzger* arrived in the beginning of 2010 from the European Synchrotron Radiation Facilities (ESRF), where he had been a staff scientist and group head responsible for several beamlines. He brought many years of experience in x-ray diffraction, in particular with grazing incidence and using coherent beams, to our Department. He is by now involved in a number of projects utilizing synchrotron radiation such as the study of biomimetic minerals **[10]** and other topics mentioned in the reports that follow. *Emil Zolotoyabko*, professor of materials science at the Technion (Israel Institute of Technology) spent several months of a sabbatical in the Department on continues to visit on a regular basis. He is also involved in a number of projects on studying biosilica (see report by *Igor Zlotnikov*) as well as other biomineralized tissues **[11]**. *Yves Bréchet*, professor of materials science at the Institut National Polytechnique de Grenoble (INPG) and at the Institut Universitaire de France (IUF) as well as "Haut Commissaire à l'Energie Atomique" received the Gay Lussac-Humboldt Award and is visiting our Department from 2012 onwards. Most recently, *Scott White*, professor at the University of Illinois at Urbana-Champaign received the Humboldt Research Award and is visiting the Department in 2013. His research is focused on developing self-healing and self-remodeling engineering materials. In addition to developing new collaborations, our visiting scholars play an important role in the mentoring of young scientists, and we are most grateful to them for this very important contribution.

The majority of the research in the Department of Biomaterials involves collaborations – within the Department, with other Departments in the Institute and with many outside partners around the world to whom we all extend our sincere gratitude for cultivating and fostering such positive and constructive partnerships.

Peter Fratzl
Director of the Department of Biomaterials

Evolutionary Perspectives on Vertebrate Hard Tissues

The most widely studied hard biomaterials of vertebrates are the bones and teeth of mammals, but these represent just a small proportion of the overall living diversity. Fishes offer a rich research system in providing a huge diversity of skeletal tissues, species (there are more fish than all other vertebrate taxa combined), and ecologies. Also, being comparatively basal ("primitive") lineages of vertebrates, this system allows us to ask wider questions relating to skeletal and dental evolution, both within fishes and vertebrates as a whole. Through collaborations with researchers at the MPI and other institutions, we examine —at multiple scales— the relationships between tissue structure and mechanical performance, allowing derivation of important design principles for biomaterials and manmade composites with structural roles.

How Can Cartilage Perform the Roles of Bone?

I was baffled when I first heard that sharks and rays have skeletons made of cartilage. How could such a material meet similar functional demands to bone, yet without the capacity for remodeling and repair **[1-2]**? In fact, their cartilage is structurally quite unique, comprised of an unmineralized gel like ours but wrapped in a sheath of mineralized tiles (**Fig. 1**) **[2-3]**.

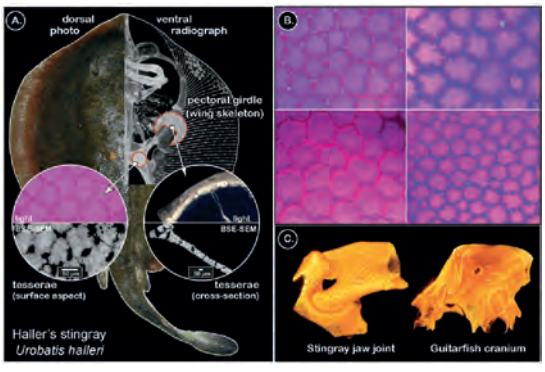

Fig. 1 – Tessellated (tiled) cartilage of sharks and rays.

We are investigating the structure and performance of this tissue composite at a variety of levels: correlating tissue material properties and structure of individual tiles using a combination of scanning acoustic microscopy, x-ray scattering, nanoindentation and backscatter electron imaging (with Dmitri Fix and Wolfgang Wagermeier, MPI); using synchrotron radiation tomography to visualize and quantify ultrastructural growth patterns, which are then used to build physical and theoretical models to test how the geometry of mineralized tiles affects tissue mechanics (with Sébastien Turcaud, MPI; Paul Zaslansky, Charité Hospital; James Weaver, Harvard's Wyss Institute, USA); and applying engineering beam theory to analyze CT scans of whole jaws of sharks with a wide range diets to ask how the mineralized tissue layer is arranged to meet differing functional demands (with John Dunlop, MPI; Laura Habegger, Univ. S. Florida; Dan Huber, Univ. Tampa, USA). By pairing the synthesis of these analyses with studies of organismal performance **[4-5]**, our work will clarify the selective pressures involved in the evolution and maintenance of this ancient skeletal type, providing clues to inform development of low-density, high-stiffness/high-damping engineering composites for human applications.

Is Bone Still "Bone" if it has no Cells?

One of the hallmarks of the bone of mammals is the presence of numerous cells within the tissue (osteocytes), responsible for monitoring bone strains, then orchestrating building and remodelling responses to reduce them. A large proportion of the bones of fish with bony skeletons, however, completely lack these cells and yet these "acellular" skeletons appear to be able to accomplish all the tasks normally attributed to osteocytes in mammals. Through collaboration with Ron Shahar (Hebrew University, Jerusalem), we are working to characterize the material and structural properties of fish bone and its response to load *in vivo*, and to examine these properties within the broader context of vertebrate bone. Our direct tests of various bone types and a metadata analysis of hundreds of literature sources (with John Dunlop, MPI) indicate that compared to other vertebrate bones, both "cellular" and "acellular" fish bone are less mineralized and less stiff, but also can sustain much greater deformations before failing **[6-7]**. Our insights into the structure, physiology and mechanics of fish bone contribute to the discipline of fish skeletal biology, but may answer basic questions of bone biology, in particular relating to the osteocytic function and the regulation of bone deposition and resorption.

The understandings provided by these studies help demarcate the full range of morphologies and functions available to calcium-phosphate based mineralized tissues, allowing us to address much larger questions of how form-function relationships are formed, are constrained and how they evolve.

M.N. Dean and P. Fratzl
mason.dean@mpikg.mpg.de

Mason Dean 17.1.1975
1993-1997: Bachelor of Arts w/ Distinction in Biology (Marine Biology concentration); Duke University (Durham, North Carolina, USA)
1999-2003: Master's of Science in Zoology; University of South Florida (Tampa, Florida, USA)
Thesis: Kinematics and functional morphology of the feeding apparatus of the lesser electric ray, Narcine brasiliensis
2003-2009: Ph.D. in Ecology & Evolutionary Biology; University of California (Irvine, California, USA)
Dissertation: Ontogeny, morphology and mechanics of the tessellated skeleton of cartilaginous fishes
2009-2011: Alexander von Humboldt Fellow / Postdoctoral Scientist, Dept. Biomaterials, Max Planck Institute of Colloid and Interfaces, Potsdam
Since 10/2011: Independent Researcher, Dept. Biomaterials, Max Planck Institute of Colloid & Interfaces, Potsdam

References:

[1] Liu, X, MN Dean, AP Summers & JC Earthman. Mat Sci & Eng C **30**: 1077-1084 (2010).
[2] Dean, MN, LF Hale, CG Mull, SN Gorb & AP Summers. J Anat **215**(3): 227-239 (2009).
[3] Dean, MN, SN Gorb & P Fratzl. Gordon Research Conference – Biomineralization, presented poster (August, 2010).
[4] Habegger, ML, PJ Motta, DR Huber & MN Dean. Zoology **115**(6): 354-364 (2012).
[5] Dean, MN, AP Summers & LA Ferry. J Morphol **273**(5): 461-479 (2012).
[6] Dean, MN & R Shahar. J Appl Ichthyol **28**: 320-329 (2012).
[7] Cohen, L, MN Dean, A Shipov, A Atkins, E Monsonego-Ornan & R Shahar. J Exp Biol **215**:1983-1993 (2012).

Biochemical Strategies in Load-Bearing Natural Materials

Matthew James Harrington
30.08.1980
2002: B.A., Biological Sciences (University of Delaware, USA)
2008: PhD, Marine Science (University of California Santa Barbara, USA)
Thesis: Molecular level structure-property relationships in the byssal threads of marine mussels
2008-2010: Alexander von Humboldt postdoctoral researcher, (MPI of Colloids and Interfaces, Potsdam)
Since 2010: Research Group Leader: Biochemical strategies in load-bearing natural materials

Proteins are the primary building blocks of countless biological materials ranging from spider silk and tendon collagen to hair and cornea. The organization and chemical structure of these building blocks holds important clues to the properties of the materials they compose. Using traditional biochemical and molecular biology techniques combined with those from materials science and chemistry, our group focuses on establishing fundamental relationships between the protein components of natural materials, their hierarchical organization, and the material properties and function. Once these design concepts have been "extracted", they can be applied by polymer scientists to create biomimetic materials with enhanced properties.

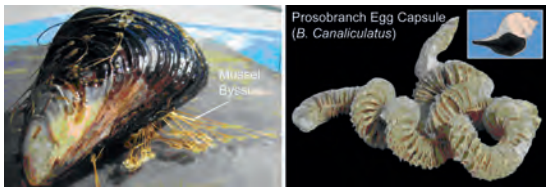

Fig. 1: Marine materials such as the byssus and whelk egg capsule are adapted to be very tough. They are composed almost entirely of protein building blocks, and by understanding the biochemical structure and organization of these proteins, we gain important insights into structure-function relationships that define the materials.

Current research in the group is divided into two primary emphases: 1. Characterization of protein-based biological materials from marine organisms 2. Biochemical investigations of biomolecules with a specific focus on metal-binding proteins. These two foci are separate but complementary aspects of the group, both of which are aimed at understanding the biochemical and structural factors that provide interesting material properties such as underwater adhesion, increased toughness and self-repair.

Characterization of Marine Materials

One prominent aspect of our research is the characterization of structure-function relationships in protein-based materials produced by marine organisms, with a specific focus on those with high toughness or self-repair behaviors. Along these lines, two projects in the group that saw significant advances in the last two years were structural and spectroscopic analyses of mussel byssal threads and whelk egg capsules.

Role of Elastic Framework in Byssus Self-Healing

Mussel byssal threads are protein-based fibers used by mussels to create a strong attachment in wave-swept marine environments. Byssal threads possess notable mechanical properties, including a combination of high stiffness and extensibility that leads to high toughness and the ability to self-heal. *Stefanie Krauß* (former postdoc) has carried out a project to look at *in situ* structural changes in the structural order of the protein building blocks of mussel byssal threads during stretching and subsequent self-healing **[1]**. Our results indicate that the protein making up byssal threads are highly organized axially and laterally into an ordered elastic framework. When stretched, this order is largely lost; however, it recovers elastically almost instantaneously when unloaded. Structural recovery, however, does not lead to mechanical recovery, which requires much longer time scales. The major conclusion was that the structural order facilitates mechanical healing by bringing sacrificially ruptured cross-links back into spatial register so that they can re-form. The results of this study offer potential inspiration for the development of a new generation of self-healing polymers (currently most are isotropic). Current research in the group by *Clemens Schmitt* is focused on spectroscopically characterizing the sacrificial cross-links, which are believed to be coordination bonds between the byssal proteins and metal ions, such as Zn^{2+} and Cu^{2+}.

Marine Egg Capsules: Pseudoelastic Bio-Fibers

Whelks are marine prosobranch gastropods that lay their eggs in protective capsules. The protein-based material that makes up the whelk egg capsule (WEC) has been recently recognized for exhibiting a very remarkable mechanical behavior called pseudoelasticity. This means that when the material is deformed it dissipates large quantities of mechanical energy as hysteresis; however, like an elastic material, it returns instantaneously to its initial length and structure. It is capable of numerous loading cycles without exhibiting fatigue, and in doing so, can dissipate large amounts of mechanical energy from crashing waves or attacking predators.

In this study, performed in collaboration with researchers from the US, UK and Austria, the structural and chemical changes of the component protein building blocks were assessed at various levels of hierarchy using a combination of *in situ* wide-angle and small-angle X-ray scattering and Raman spectroscopy while simultaneously performing mechanical tensile experiments **[2]**. From these experiments, we gained important insights into the molecular level mechanisms of pseudoelasticity in the WEC, including the observa-

tion of a critical phase transition between an ordered -helical protein structure and a disordered protein structure during the yield plateau. Based on these results, we created a simplified mathematical model to describe the equilibrium mechanical behavior of the WEC centered on a molecular phase transition. Further modeling efforts are underway with *Peter Fratzl* and *Dieter Fischer* to help explain the non-equilibrium behavior including strain-rate dependence and hysteresis. Additionally, we are collaborating with *Ali Miserez* (NTSU) in a comparative approach examining the structure and mechanical behaviors of WEC from different species.

Characterization of Biological Building Blocks

The other main focus in our group is the characterization of proteins that compose biological materials in order to develop a more biochemical understanding of how protein sequence, conformation and cross-linking affect material properties, such as underwater adhesion and self-repair. Along these lines, a major focus is the use of protein-metal coordination cross-links by organisms to tune mechanical properties.

Mussel Adhesive Proteins

The adhesive prowess of the mussel byssus under conditions where man-made adhesive simply fail is well known in the literature; however, surprisingly, there is only a cursory understanding of the mechanisms of adhesion at the molecular level. In collaboration with *Dong Soo Hwang* (UCSB), we combined mechanical measurements of adhesion by mussel proteins using a surface force apparatus (SFA) with spectroscopic characterization of the interaction at the adhesive interface using confocal Raman spectroscopy. It was demonstrated that adhesion on TiO_2 surfaces by mussel foot protein-1 (MFP-1) depends largely on the bidentate coordination of the Ti ion by the oxygen atoms on the DOPA catechol ring (**Fig. 2**). TiO_2 is a well known alloy used in biomedical applications and this strong attachment occurred in the presence of a salty buffered solution, demonstrating the potential of mussel inspired chemistry for biomedical applications, such as dental adhesives and coatings for biomedical implants.

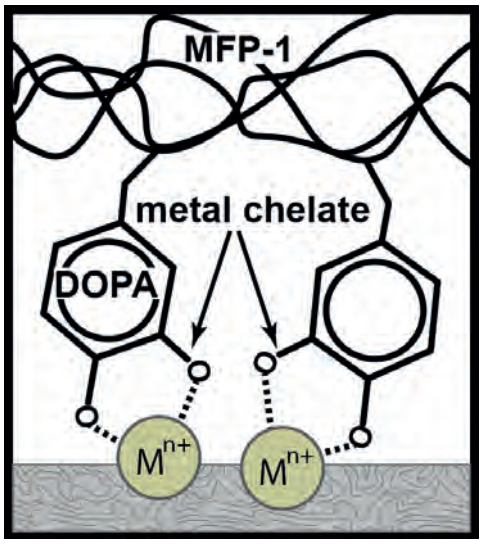

Fig. 2: Marine mussels make prodigious use of DOPA in roles such as adhesion, hardening, and self-repair. Experiments on DOPA containing proteins and polymers indicate that much of this behavior arises from the ability of DOPA to form stable coordination bonds with metal ions such as Fe, V, and Ti.

Mussel Inspired Biomimetic Polymers

In collaboration with *Niels Holten-Andersen* (MIT) continued efforts to create polymers that utilize the DOPA-metal coordination cross-link chemistry of the mussel byssus are underway. Initial efforts produced a PEG-DOPA based hydrogel that demonstrated tough and self-healing behaviors dependent on metal cross-links **[4]**. Currently, we are exploring the effect of metal ion and pH-dependence on the degree of cross-linking and mechanical performance. Apparently, these factors provide a convenient method for mechanical tunability of hydrogels.

M. Harrington, E. Degtyar, G. Milliron, A. Reinecke, S. Schmidt, C. Schmitt and J. Steffen
matt.harrington@mpikg.mpg.de

References:

[1] Krauss, S., Metzger, H., Fratzl, P., and Harrington, M.J. Self-repair of a biological fiber guided by an ordered elastic framework. Biomacromolecules, Accepted (2013).

[2] Harrington, M. J., Wasko, S.S., Masic, A., Gupta, H.S., Fischer, F.D., and Fratzl, P. Pseudoelastic behavior of a natural elastomeric proteinaceous material is achieved via reversible changes in backbone conformation. Journal of the Royal Society Interface **9**, 2911-2922 (2012) .

[3] Hwang, D.S., Harrington, M.J., Lu, Q., Masic, A., Zeng, H. and Waite, J.H. Mussel foot protein-1 (mcfp-1) interaction with titania surfaces. Journal of Materials Chemistry **22**, 15530-15533 (2012).

[4] Holten-Andersen, N., Harrington, M.J., Birkedal, H., Lee, B.P., Messersmith, P.B., Lee, K.Y.C. and Waite, J.H. Mussel-inspired metal-ligand cross-links yield polymer gels with near covalent elastic moduli and self-healing properties. Proceedings of the National Academy of Science, U.S.A. **108**, 2651-2655 (2011).

Biological Chitin-Based Tools and Sensors

Yael Politi 10.9.1976
1999-2001: Bachelor of Science majoring in Biology (with Honours) (Tel Aviv University, Tel Aviv, Israel)
2002-2004: MSc. Thesis: Transient amorphous calcium carbonate in sea urchin skeleton.(Weizmann Institute of Science, Rehovot, Israel)
2005-2009: Doctoral Thesis: The formation of transient amorphous calcium carbonate in biomineralization and its transformation into calcite. (Weizmann Institute of Science, Rehovot, Israel)
2009-2012: Postdoctoral Scientist Department of Biomaterials, Max Planck Institute of Colloids and Interfaces.
2009: Alexander von Humboldt Fellow
Since 07/2012: Research Group Leader, Department of Biomaterials, Max Planck Institute of Colloids and Interfaces

After cellulose chitin is the second most abundant natural bio-macromolecule. For example, it forms the cell walls of fungi, plays major roles in the mollusc skeletal and mouth parts, and is the main building block of all arthropod cuticles. It is therefore possible to find biological chitin based materials with extremely wide range of physical, and in particular, mechanical properties. Due to its widespread abundance and biocompatibility chitin is also extensively used in diverse industrial processes and has found various technological and medical applications **[1]**. The study of chitin and chitin based materials therefore holds a promise for clever bio-inspired materials design.

The cuticle of arthropods is an example for such a family of materials. The large diversity seen in the arthropod phylum is also reflected in an ample diversity of cuticular materials with different physical properties that serve many different biological functions forming the external skeleton, skin, sense organs and more. The cuticle can be described as a fiber reinforced composite material, where -chitin crystallites tightly coated by a protein shell form the fibrous phase and the matrix is composed of a wide range of proteins **[1]**.

The main goals of our newly formed group is to obtain basic understanding of the cuticular material on the one hand and to gain insight into the structure-function relations in specific functional organs such as cuticular tools (e.g. fangs, claws) and mechanosensors, on the other hand; We work in close collaboration with *Prof. Friedrich Barth*, from the University of Vienna (Vienna, Austria) *Prof. Vladimir Tsukruk* from Georgia Institute of Technology (Atlanta, USA) and *Prof. Leeor Kronik* from the Weizmann Institute of Science (Rehovot, Israel).

The current members of the group are *Dr. Clara Valverde Serrano*, *Dr. Maxim Erko*, *Dr. Osnat Younes-Metzler* and *Ms. Birgit Schonert*. In addition *Ms. Ana Licuco* and *Dr. Benny Bar-On* are expected to join the group during the coming semester.

Basic Research: Understanding of the Cuticular Material at the Molecular Level

We study the chitin-protein interaction, the cuticle interaction with water and the properties of the matrix in terms of composition, for example metal ions and halogen incorporation or mineralization (in crustaceans) and their effect on cuticle properties, and the chemical interaction between different cuticular components.

That water sorption has a strong effect on the cuticle is well documented. Maturation processes of the cuticle i.e. sclerotization involve drastic changes in cuticle hydration state, especially in the exocuticle. Cuticle dehydration often results in significant increase in the cuticle stiffness and brittleness. Nevertheless the exact manner in which water is adsorbed in the different cuticle layers (i.e. exo- meso- and endo-cuticle) is still unknown. Water sorption is studied by X-ray scattering, thermo-gravimetric analysis and differential scanning calorimetry and other techniques. Together with Dr. Luca Bertinetti we use a method based on Infrared Lock-In Thermography to spatially resolve and image water sorption in the main cuticular layers (**Fig. 1**).

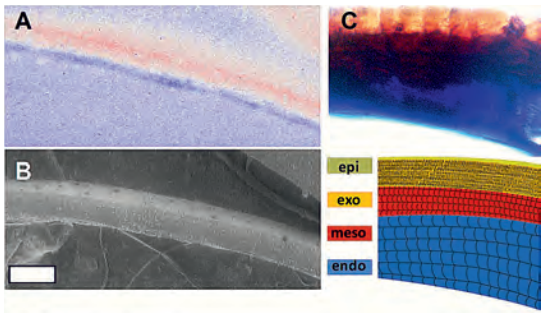

Fig. 1: water sorption in different cuticular layers. (A) Infrared lock-in thermography mapping of a cuticle section from the tibia (leg) of the spider Cupiennius salei. The colours in the image relate to the temperature of the sample resulting from the variable amount of water sorption at the sample surface and its energetics. Highest water sorption occurs at the endo-cuticle, however with low temperature increase (blue), whereas the exo-cuticle adsorbs less water, but with higher increase in temperature and is therefore seen blue. The meso-cuticle is white indicating intermediate level of water sorption/temperature change. (B) SEM image of the sample, scale bar = 1µm. (C) Light microscope image of a tibia section stained with "Mallory stain" which is used to identify the cuticular layers. The epicuticle is un-stained, exo-cuticle is stained as amber, meso-cuticle: red and endo cuticle: blue. (D) a schematic representation of the cuticlar layers in the tibia. Light green: epicuticle, Yellow: exo-cuticle, Red: meso-cuticle and blue: endocuticle.

Incorporation of Metals and halogens incorporation in cuticlar tools is widely used by many arthropods to enhance the cuticle mechanical properties. We have studied[1] this phenomenon in the spider's cheliceral fangs that are used to inject venom into prey. The fangs are rich in Zn, Ca and Cl with specific spatial distribution. Interestingly, the spiders' claws contain high levels of Mn ions. The manner in which these ions are incorporated is however still unclear. It is also unknown, what is the adaptive advantage of using a specific metal ion relative to another in the various tools. Amongst the various approaches we employ in this study, we take use of element-specific spectroscopy and microscopy techniques such as Zn, Ca and Mn K-edge XAS, and N K-edge EELS (in collaboration with *Dr. Eckhard Pippel*, MPI of Microstructure Physics, Halle) (**Fig. 2**) that allowed us to identify the Zn complexation by His residues in the fang matrix.

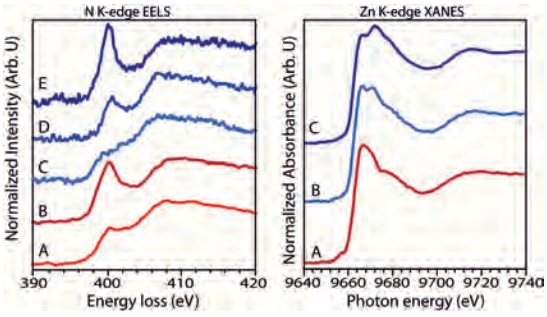

Fig. 2: Element-specific spectroscopy at the N and Zn K-edges showing the histidine-Zn complexation from the point of view of both the metal ion (Zn) and the amino acid (N in the imidazole ring). Left panel: Nitrogen K-edge Energy loss spectra of (A) the protein matrix in the spider fang where no Zn ions are detected. (B) The protein matrix in the spider fang in a Zn-rich region, the first peak, assigned to 1s->π transition is enhanced by the interaction with Zn ions. (C) Spectrum of the protein insulin where most of the nitrogen atoms reside in the peptide bonds. (D) poly-histidine peptide, the two nitrogen atoms present in the imidazole ring, show increased 1s->π*signal, this interaction is increased with Zn complexation, as seend in (E) Poly-histidine peptide complex with Zn ions. Right panel: metal coordination from the Zn point of view by x-ray absorption spectroscopy: Zn K-edge spectrum of the (A) spider fang (B) insulin and (C) polyhistidine+Zn. The spectra series suggests that in addition to hisitidine, other molecules, e.g. water, may be involved in Zn coordination.*

Structure Function Relations in the Cuticle

In fiber-reinforced material such as the arthropod cuticle, fiber orientation is a primary factor determining the anisotropy of the mechanical properties. In addition, lamella thickness and other structural motifs have large effect on the materials response to mechanical load. We aim at establishing direct correlation between organ morphology and chitin fiber arrangement, in terms of microstructure, fiber alignment and orientation and the spatial arrangement of different microstructural motifs within a functional organ/tool. For example, in the spider fang we have characterized various structural motifs and established gradient in mechanical properties that results from changes in degree of fiber alignment, in addition to the influence of metal ions [ref]. We use a similar approach to study the structure-function relation in the study of the spiders mechano-sensors (see below).

Mechano-Sensing in Spiders

The spider cuticle is covered by numerous cuticular-sensors that react with remarkable sensitivity and specificity to a wide range of mechanical stimuli (medium flow, substrate vibration and cuticle strain) **[2]**. Filtering of back-ground noise from relevant information occurs at the material/organ level which makes these structures appealing as models for the bio-inspired design of mechanoresponsive and adaptive nanostructured materials.

In order to exploit fundamental principles found in natural mechanoreceptors for bio-inspired materials, we focus on understanding the mechanism of mechanical signal detection, transmission and filtration for the spider slit biosensory system at the material level. We investigate the direct spatial correlation among cuticle morphology, hierarchical structural organization and micromechanical properties in spider slit-sensilla as well as hair like sensors (**Fig. 3**). We explore the time-dependent micromechanical properties of biological strain receptors embedded in the spider exoskeleton with high spatial resolution (down to a few nanometers) and relate the findings to the function of these organs as sensitive vibrational filters and efficient transmitters of external mechanical stimuli.

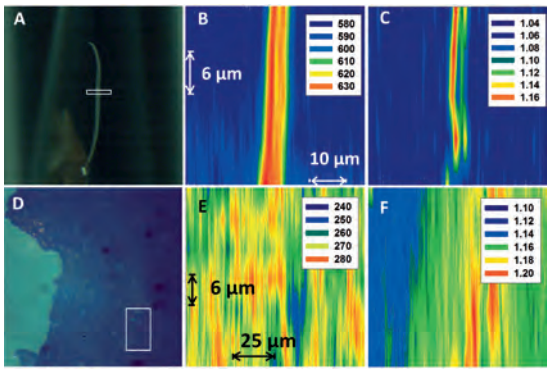

Fig. 3: XRD of two kinds of mechanosensors from the spider leg. Optical microscopy images from (A) a tactile hair and (F) the region around slit-sensilla organ. The white squares represent studied areas. (B, E) chitin scattering intensity of the corresponding regions (arb. Units). (C) and (H) show the degree of fiber orientation within a single hair and in the slit-sensilla region, respectively.

Y. Politi, C. Valverde Serrano, M. Erko, O. Younes-Metzler, B. Schonert.
politi@mpikg.mpg.de

References:

[1] Y. Politi, M. Priewasser, E. Pippel, P. Zaslansky, J. Hartmann, S. Siegel, C. H. Li, F. G. Barth, and P. Fratzl, Advanced Functional Materials **22** (12), 2519 (2012).
[2] Peter Fratzl and Friedrich G. Barth, Nature **462** (7272), 442 (2009).

Advanced Raman Spectroscopic Imaging of Biological Tissues

Admir Masic 16.06.1977
2001: M. Sc. Degree, Chemistry (University of Torino, Italy)
Thesis title: Molecular motions of organometallic compounds included in cyclodextrins studied by means of solid state NMR
2005: PhD, Chemistry (University of Torino, Italy)
Thesis title: Application of innovative techniques for the study of deterioration pathways within objects of cultural and artistic interest
2007: Postdoctoral scientist (University of Torino, Italy).
Since 2008: Researcher (Max Planck Institute of Colloids and Interfaces, Potsdam)

Complex biological materials, such as bone, silk or wood, often exhibit outstanding mechanical properties, a feature that can be directly related to their functional adaptations and interactions at multiple hierarchical length scales. Raman spectroscopic imaging, a non-invasive and label-free approach to obtain both chemical (molecular interactions), and structural (orientation) information with sub-micrometer precision, is a powerful tool for the molecular level characterization of such materials.

The primary focus of our research is the *in situ* study of biological and biomimetic materials at various levels of hierarchy (from the molecular up to the macroscopic scale) taking advantage of advanced spectroscopic imaging techniques.**[1-5]** One of our research goals, for example, is to map collagen fibril orientation in a wide range of different tissue types by evaluating its molecular response to a polarized laser source (**Fig. 1**).**[6]**

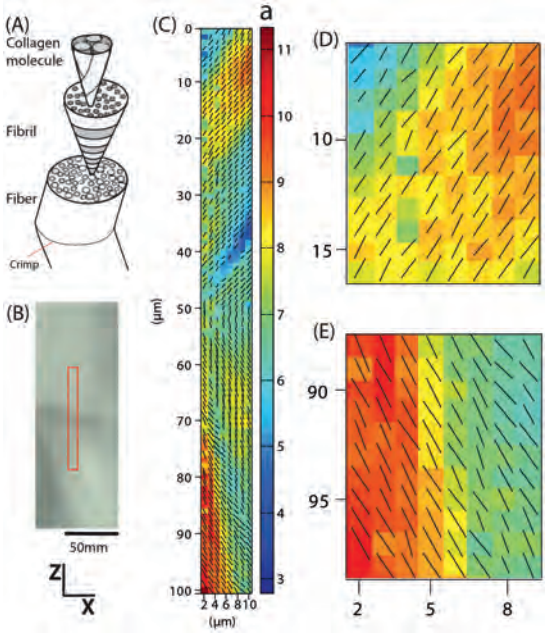

Fig. 1: Polarized Raman mapping of collagen fibril orientation in the crimp region of an un-stretched, fully hydrated rat tail tendon. The hierarchical structure of collagen (A), an optical microscopy image of the crimp region (B) and its corresponding collagen orientation map (C) with magnified regions of interest (D and E). For further details see ref. [6].

We are currently applying this methodology to map both the three-dimensional orientation of collagen in biological materials and the evolution of collagen organization in hard and soft tissues formed in the fracture gap (callus) during the process of bone healing in rats (with *J. Dunlop*, Biomaterials, and *G. Duda*, Charité Hospital Berlin).

The ultimate aim of our work is to link the structural organization and chemical composition to the physical properties of biological material.**[3, 6-9]** One such example is a collaboration with *B. Aichmayer* (Biomaterials) and *A. Berman* (Ben-Gurion University, Israel), where we used Raman spectroscopic imaging to study the chemical composition and microstructure of the ultra-tough and damage tolerant teeth from the freshwater crayfish, *Cherax quadricarinatus* (**Fig. 2**).

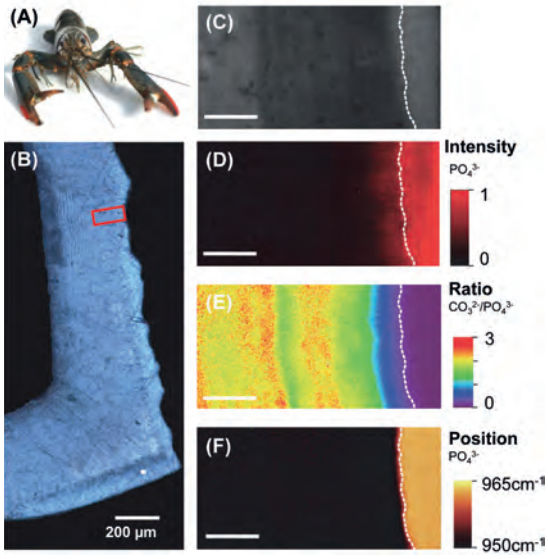

Fig. 2: Raman imaging of the crayfish (A) anterior molar. Light micrographs (B and C) of the analyzed area which covers the transition zone between the apatite and the amorphous mineral phase (indicated by the red rectangle in (B)). Raman imaging of the phosphate distribution (D), carbonate to phosphate intensity ratio (E), and the phosphate peak position (F). For details see ref. [9].

Our results reveal that the crayfish molar is a highly complex, periodically renewable organ, in which a unique architecture of amorphous and crystalline calcium carbonate and phosphate minerals constitutes a tool with mechanical properties comparable to those exhibited by mammalian teeth.

In addition to our work with high performance biological materials, and in collaboration with Federal Institute for Materials Research and Testing (BAM, *I. Rabin*), Helmholtz-Zentrum Berlin (HZB, *U. Schade*), and the University of Torino (*R. Gobetto*), we have also applied these techniques to the investigation of ancient historical manuscripts. For example, by combining polarized Raman, far infrared, and nuclear magnetic resonance spectroscopy techniques we have been able to directly investigate, in unprecedented detail, the changes in collagen structure during the deterioration of the Dead Sea Scrolls.**[11]**

A. Masic, M. Fechler, L. Galvis, B.E. Pinchasik, R. Schütz.
admir.masic@mpikg.mpg.de

References:
[1] Yashchenok, A., et al., Small **9** (3), 351 (2013).
[2] Lee, K., et al., Nature Communications **3**, 725 (2012).
[3] Harrington, M. J., et al., Journal of the Royal Society Interface **9** (76), 2911 (2012).
[4] Bratashov, D. N., et al., Journal of Raman Spectroscopy **42** (10), 1901 (2011).
[5] Schenk, A. S., et al., Adv. Funct. Mater. **22** (22), 4668 (2012).
[6] Masic, A., et al., Biomacromolecules **12** (11), 3989 (2011).
[7] Seto, J., et al., PLoS ONE **7** (10) (2012).
[8] Ermeydan, M. A., et al., Acs Applied Materials & Interfaces **4** (11), 5782 (2012).
[9] Bentov, S., et al., Nature Communications **3**, 839 (2012).
[10] Bauer, F., et al., Biomacromolecules (2012).
[11] Masic, A., et al., Analytical and Bioanalytical Chemistry **402** (4), 1551 (2012).

In-Situ Mechanical Characterization of Internal Interfaces in Biomaterials

Nature is successful in forming complex hierarchical composites with properties far superior to the properties of each constituent. The building blocks at all hierarchical levels are usually joined together by a gluing material to obtain a functional structure. Although internal interfaces between the building blocks comprise only a small volume fraction of the entire structure, mechanical properties of biomaterials are governed by their properties. In most cases, the building blocks are glued together by an organic softer phase. This interface can exhibit interpenetration of the two compounds, more than one order of magnitude change in elastic modulus, roughness, viscoelastic behavior and more. Thus, the main focus in this work is measuring the mechanical and compositional gradation across the interface between a single building block and the surrounding gluing medium, which is important for understanding the overall behaviour of the entire structure. This eventually, will have a significant impact on bio-inspired multi-scale composite material synthesis.

In order to measure gradual change of mechanical properties across an interface, we adapted a recently developed nanoscale modulus mapping technique and combined it with reverse finite element analysis **[1]**. The basis of the modulus mapping technique is the well-established nanoindentation instrumentation employing a Berkovich diamond tip. Thus, when measuring inside nanometric inclusions, the obtained modulus is strongly affected by the modulus of the matrix. Therefore, a detailed simulation by finite element approach is required to extrapolate the real value of the elastic moduli.

This methodology was first used to map the elastic modulus across a 35 nm thick organic layer within biosilica in a giant anchor spicule of the glass sponge *Monorhaphis chuni* **[2]**. *M. chuni*, is a deep sea glass sponge that belongs to the class of Hexactinellida and is among the earliest multicellular animals found as fossils. The most fascinating feature of the sponge is the giant basal spicule around which the animal is assembled. This spicule is used for anchoring the animal to the ocean's bottom and can reach up to 3 m in length and 8 mm in diameter. An organic filament, nearly 2 µm in diameter, provides the central vertical axis of the spicule with biosilica cylinders arranged in nearly concentric layers (2-10 µm wide) around it (**Fig. 1a**), separated by tiny organic layers (**Fig. 1b**).

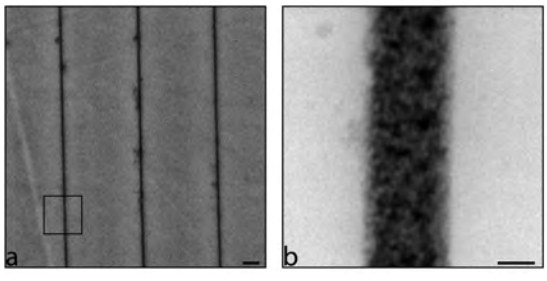

Fig. 1: (a) – SEM micrograph of the spicule cross-section (plane view) showing alternating biosilica-organic layers (scale bar is 1 µm); (b) – HAADF-STEM image of an individual organic layer (plane-view projection, scale bar is 20 nm) taken from the area indicated by a square box in (a).

After iterative simulations of the mapping procedure across the organic layer (**Fig. 2a, b**) we find the best fit to experimental results with modulus of 0.7 GPa in the organic layer as compared to 37 GPa in the bioglass. This indicates an impressive performance of the animal and a drastic increase of its fracture stress **[3]**. Furthermore, a modulus gradient extends 50 nm into the glass layer, probably due to spatial distribution of small organic inclusions (**Fig. 2c**).

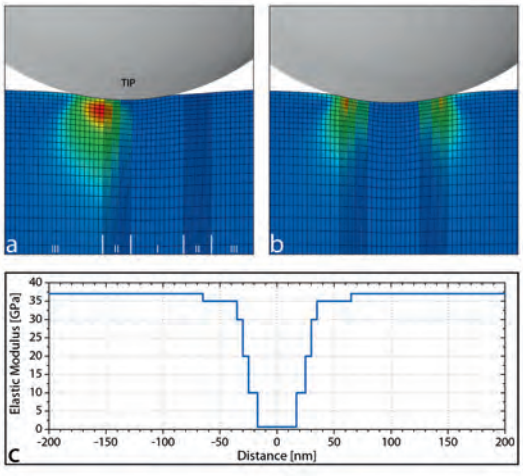

Fig. 2: (a) – a simulated Von Misses stress distribution map when the tip touches the left edge of the organic layer (I – organic layer, II – steep modulus gradient, III – biosilica); (b) – a simulated Von Misses stress distribution map when the tip touches both edges of the organic layer; (c) – resulted elastic modulus distribution across the organic layer.

With this new methodology it becomes possible to determine elastic moduli of nanometric inclusions even when embedded in a 50 times stiffer matrix. Currently, this technique is applied to investigate interface properties in other biostructures such as the calcite/organic interface in the prismatic layer of the giant shell *Pinna nobilis* and to resolve the different ultrathin layers in the cell wall of the spruce tree *Picea abies*.

I. Zlotnikov, B. Bayerlein
Igor.Zlotnikov@mpikg.mpg.de

Igor Zlotnikov 20.07.1980
1998-2003: B.Sc. in Materials Engineering, B.A. in Physics (Technion – Israel Institute of Technology, Haifa, Israel)
2003-2005: M.Sc. in Materials Engineering (Technion – Israel Institute of Technology, Haifa, Israel)
2005-2009: Ph.D. in Materials Engineering (Technion – Israel Institute of Technology, Haifa, Israel)
Since 08/2009: Postdoctoral Scientist, (Department of Biomaterials, Max Planck Institute of Colloids and Interfaces)
Since 7/2012: Independent Researcher (Department of Biomaterials, Max Planck Institute of Colloids and Interfaces, Golm, Germany)

References:

[1] Zlotnikov I., Drezner H., Shilo D., Aichmayer B., Dauphin Y., Zolotoyabko E. and Fratzl P.: Mapping nanomechanical properties near internal interfaces in biological materials. MRS Symp. Proceedings **1345** (2011).
[2] Woesz A., Weaver J. C., Kazanci M., Dauphin Y., Aizenberg J., Morse D. E. and Fratzl P.: Micromechanical properties of biological silica in skeletons of deep-sea sponges. J. Mater. Res. **21**, 2068-2078 (2006).
[3] Fratzl P., Gupta H. S., Fischer F. D. and Kolednik O.: Hindered Crack Propagation in Materials with Periodically Varying Young's Modulus Lessons from Biological Materials. Adv. Mater. **19**, 2657-2661 (2007).

Mechanobiology

Richard Weinkamer 14.08.1967
1995: Diploma, Mathematics (University of Vienna)
Thesis: The modular group: an investigation with methods of combinatorial group theory
1998: Research Stay (Rutgers University, New Jersey)
2000: PhD, Physics (University of Vienna)
Thesis: Diffusion and diffusional phase transformations in binary alloys: Monte Carlo simulations of lattice models
2000-2003: Postdoc, (Erich Schmid Institute of Materials Science, Leoben)
Since 2003: Group Leader (Max Planck Institute of Colloids and Interfaces, Potsdam)
2012: Habilitation in Theoretical Physics (Humboldt University, Berlin) Thesis: Processes in living bone and the resulting structural changes – computational studies

Bone health is intimately linked to the processes of bone mineralization, remodeling and healing. The control of these processes occurs at the level of the cells, not only via biochemical signaling, but also via physical, in particular mechanical stimuli. Nowadays animal experiments cannot directly address cellular regulation, but are limited to the structural changes on the tissue level. Computer experiments can help bridge the gap between the cellular and the tissue level. In the computer model hypotheses about cellular regulation are implemented and the consequences for the tissue are calculated **[1]**. When modeling different bone processes, two aspects are important: (i) structural changes occur at very different length scales, from conformational changes of the collagen molecule to the bridging of a macroscopic bone fracture; (ii) the importance of mechanics demands not only an accurate description of the external loading, but also a characterization of the local mechanical properties of the tissues. Scanning acoustic microscopy is a promising technique to measure functional properties of biological materials in native wet conditions in a non-destructive way.

Collagen Structure, Mineralization and Remodeling

The initial stage of the mineralization process in bone is influenced by the molecular structure of collagen. This structure in turn depends on the presence of water and ions in its close environment. Together with the Theory Department we used Molecular Dynamics (MD) simulations to investigate how various collagen-like peptides change their structure, in particular their helicity, depending on ion environments containing Ca^{2+} or Na^{+}. The simulations showed that the helicity changes with the ion concentration in regions, where the repetitive sequence of amino acids is not retained (**Fig. 1**).

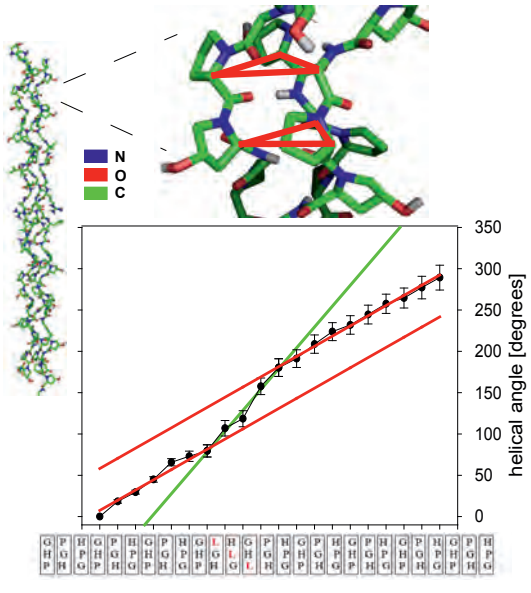

Fig. 1: Molecular model of a collagen-like peptide with 30 amino acids in an ionic environment containing Ca^{2+}. The helicity is calculated based on the triangles formed by the Cα atoms on each chain of the triple helix (top, right). Comparing the amino acid sequence (bottom) and the helicity of the molecule, the latter is increased when leucin (L) interrupts the repetitive sequence of glycine (G), proline (P) and hydroxyproline (H).

The processes of bone mineralization and remodeling result in a patchwork structure of bone on the length scale of roughly 50 µm, which can be imagined in the electron microscope using the backscattered mode (qBEI). In our mathematical description of this material heterogeneity, we corrected for the finite acquisition time during the qBEI-measurement **[2]**. The model can then predict the evolution of this heterogeneity in scenarios of bone diseases and medical treatment. For diagnostic purposes the discrimination between scenarios of a changed rate of bone remodeling and a disordered mineralization process is of particular importance. The spatial heterogeneity of the mineral content in bone can also be used to test current theories about the control of bone remodeling **[3]**.

In vivo micro-computed tomography (micro-CT) opens a new possibility to monitor structural changes in the bone of living small animals. In collaboration with the ETH Zürich, we developed an evaluation method of micro-CT images to quantify the (de)mineralization kinetics in mice after deposition and before resorption of bone, respectively (**Fig. 2**). Measurements on mice, where the investigated vertebra was mechanically loaded, compared to the unloaded control group, indicate that loading accelerates the incorporation of mineral into the bone (**Fig. 2**) **[4]**.

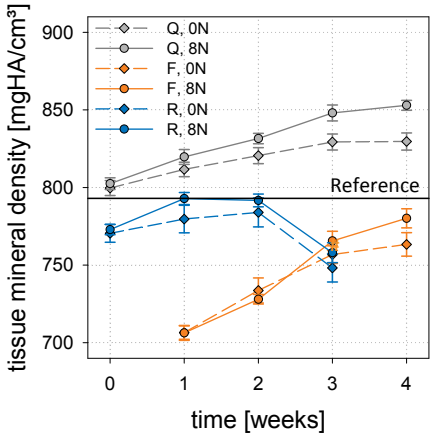

Fig. 2: Time evolution of the mean mineral content of formed (F), resorbed (R) and quiescent (Q) bone for trabecular bone loaded with 8N and unloaded control (0N). Bone was formed within the first week (therefore no data point at week 0) and resorbed within the last week (therefore no data point at week 4).

Micro-CT images of human trabecular bone of different age can also be used to learn about the control of trabecular bone remodeling. In the model the changes in the thickness of trabeculae during remodeling are described by a Markov chain. The calculated probabilities for bone deposition or resorption as a function of the thickness of the trabeculae show that the mechanical regulation of remodeling can be well described by a threshold above which bone deposition sets in **[5]**.

In cortical bone, remodeling leads to the formation of cylindrical structures called osteons, which house a blood vessel in its central osteonal canal for nutrition supply. We quantified the order in the arrangement of osteons in the cor-

tices of horses and dogs, finding variations in the order not only between different bones of one animal, but also for different anatomical locations within the same bone. Model calculations showed that the measured order could be well understood under the assumption that osteonal canals are surrounded by an "exclusion zone", which inhibits the formation of other canals within this zone **[6]**, ensuring an efficient supply with nutrients.

Mechanical Heterogeneity of Bone

The heterogeneity of the mineral content as described in the last section together with the anisotropic structure of the material, results in a mechanical heterogeneity of bone. Scanning acoustic microscopy (SAM) allows measuring this heterogeneity with a lateral resolution of roughly 1 µm. The measured acoustic reflectivity from the bone surface depends on two local characteristics of the sample, the effective stiffness and the mass density. Via combination of an electron backscattered image (qBEI) containing the information about the local density and of two SAM-measurements with acoustic lenses of different resolution the effective stiffness of compact bone in a human femur was calculated. In the evaluation we separated the younger bone of an osteon formed by remodeling process from the surrounding older so-called interstitial bone (**Fig. 3**). The average value for the effective stiffness of the interstitial bone is more than 25% larger in the osteon, which can be largely explained by its higher mineral content. For both, osteons and interstitial bone, SAM maps show oscillations in the effective stiffness with a wavelength of approximately 5 µm, which is the typical thickness of a bone lamella. This mechanical heterogeneity can be understood based on the anisotropic arrangement of the mineralized tissue. An evident clinical application of SAM is to complement structural images of bone biopsies with functional images of the mechanical properties to assess more directly bone quality.

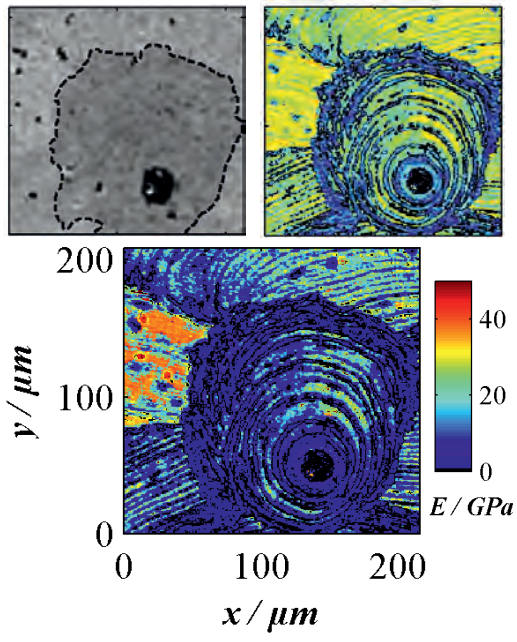

Fig. 3: Top, left: quantitative backscattered electron image (qBEI) of human cortical bone (measured by Paul Roschger, Ludwig Boltzmann Institute of Osteology, Vienna). The dashed line separates the osteon from the interstitial bone; top, right: scanning acoustic microscope (SAM) image obtained with an 820 MHz lens of the same sample region. Bottom: effective stiffness of osteons and interstitial bone as the combined result of qBEI and SAM-measurements.

Bone Regeneration and Healing

The regenerative property of bone allows healing of macroscopic defects as occurring, for example, after bone fracture. Via the transient presence of additional tissue called the callus, successful healing leads to a return to the pre-fractured state. One peculiarity of the process is that not only new bone is formed within the callus, but transiently also soft tissue like fibrous tissue and cartilage. Another peculiarity is that the reconnection of the broken bone ends does not occur "directly" via a bridging of the fracture by new bone formation. Bone healing rather occurs "indirectly" with the broken ends first reconnect outside of the fracture gap.

To address the above mentioned peculiarities of bone healing, we developed two complementary models. With the first we want to explain the spatio-temporal patterns of different tissues as observed experimentally using simple mechanobiological rules. The essence of these rules is a threshold of the mechanical stimulus, below which either cartilage or bone is formed, or bone resorption starts. The model considers the strong mechanical heterogeneity of the newly formed bone **[7]**. The simulated tissue patterns are compared with a succession of six images obtained from histological sections of a sheep experiment performed at the Julius Wolff Institute, Charité. Best agreement with the experiments is obtained when the volumetric strain is assumed as mechanical stimulus **[8]**. Intermediate stages of the healing process are strongly influenced by the stochastic influences on the control. In a separate study, the same mechanobiological rules could explain the asymmetric development of the bony callus on the inner (medial) and outer (lateral) side **[9]**.

With the second more generic model we ask the question which factors in the local control determine, whether healing occurs directly or indirectly. The mechanical stimulus is assumed to be a combination of the local mechanical strain and the local stiffness of the material. Healing occurs when the stimulus is within a predefined window. For the case that the size of the window is strongly restricted, the simulations show that indirect healing is preferred.

R. Weinkamer, D. Fix, P. Kollmannsberger, C. Lukas, S. Pabisch, C. Pilz, F. Repp, N. Timofeeva, A. Vetter
richard.weinkamer@mpikg.mpg.de.

References:

[1] R. Weinkamer, Habilitation Thesis, Humboldt University, Berlin (2011).
[2] C. Lukas, P. Kollmannsberger, D. Ruffoni, P. Roschger, P. Fratzl, R. Weinkamer, The heterogeneous mineral content of bone — using stochastic arguments and simulations to overcome experimental limitations, J. Stat. Phys. **144**, 316-331 (2011).
[3] M.A. Hartmann, J.W.C. Dunlop, Y.J.M. Bréchet, P. Fratzl, P. and R. Weinkamer, Trabecular bone remodeling simulated by a stochastic exchange of discrete bone packets from the surface, J. Mech. Behav. Biomed. **4**, 879-887 (2011).
[4] C. Lukas; D. Ruffoni, F.L. Lambers, F.A. Schulte, G. Kuhn, P. Kollmannsberger, R. Weinkamer; R. Müller, Mineralization kinetics in murine trabecular bone quantified by time-lapsed in vivo micro-computed tomography, submitted.
[5] M. Rusconi, A. Valleriani, J.W.C. Dunlop, J. Kurths, R. Weinkamer, Quantitative approach to the stochastics of bone remodeling, EPL **97**, 28009 (2012).
[6] R. Shahar, C. Lukas, S. Papo, J.W.C. Dunlop and R. Weinkamer, Characterization of the spatial arrangement of secondary osteons in the diaphysis of equine and canine long bones, Anat. Rec. **294**, 1093-1102 (2011).
[7] A. Vetter, Y. Liu, F. Witt, I. Manjubala, O. Sander, D.R. Epari, P. Fratzl, G.N. Duda, R. Weinkamer, The mechanical heterogeneity of the hard callus influences local tissue strains during bone healing: a Finite Element study based on sheep experiments, J. Biomech. **44**, 517-523 (2011).
[8] A. Vetter, F. Witt, O. Sander, G.N. Duda and R. Weinkamer, The spatio-temporal arrangement of different tissues during bone healing as a result of simple mechanobiological rules, Biomech. Model. Mechan. **11**, 147-160 (2012).
[9] F. Witt, A. Petersen, R. Seidel, A. Vetter, R. Weinkamer, G.N. Duda, Combined in vivo / in silico study of mechanobiological mechanisms during endochondral ossification in bone, Ann. Biomed. Eng. **39**, 2531-2541 (2011).

Plant Material Adaptation

Michaela Eder 1972
2003: Diploma in Wood Science and Technology (BOKU – University of Natural Resources and Applied Life Sciences, Vienna, Austria)
2007: Doctoral Thesis: Structure, properties and function of single wood fibres of Norway Spruce (Picea abies [L] Karst.) (BOKU, Vienna, Austria)
2007-2011: Postdoctoral Scientist Department of Biomaterials, Max Planck Institute of Colloids and Interfaces.
Since 09/2011: Research Group Leader, Department of Biomaterials, Max Planck Institute of Colloids and Interfaces

Plants are sessile. This means they are bound to a certain location in a given environment. To be successful under these circumstances, plants have developed sophisticated strategies which are typically reflected in the material forming the plant body. A plant is composed of different tissues which themselves consist of cells, each of them encased by a more or less rigid polymeric cell wall (**Fig. 1**).

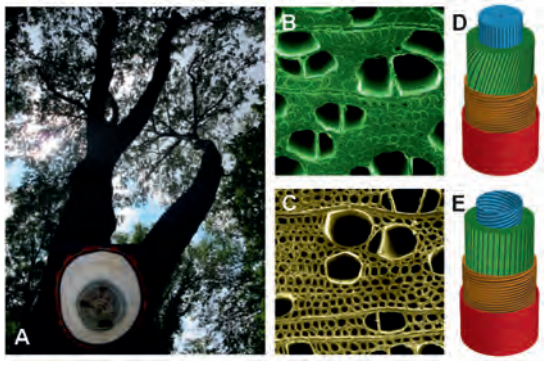

Fig. 1: Wood, an example for a plant forming material. The inserted cross section (A) shows alternating layers of earlywood and latewood (tissue) and excentric growth due to reaction wood formation. SEM images show reaction wood, here tension wood (B) and normal wood (C), cell wall cartoons depict proposed cell wall structure of (D) tension wood and (E) normal wood (black lines indicate the orientation of cellulose fibrils in the different cell wall layers.

Our research interests are plant material structure, (mechanical) properties, the function for the plant and how and/or whether the environment is reflected in the material. Selected plant systems are/will be studied in detail. In terms of applications, revealed material optimization strategies for certain functions could be used for the development of new materials. Furthermore a deeper understanding of plant based material is essential for sustainable and targeted use of the abundant resource plant material.

Plant Cell Wall Properties

Knowledge about cell wall structure is essential to understand plant material. A growing cell is surrounded by a primary cell wall which is both flexible enough to allow cell expansion and mechanically stable to resist internal and external forces. After cessation of growth, many cells form additional layers, the mechanically robust secondary cell walls. Cell walls in general can be seen as fibre-reinforced structures: stiff and strong cellulose fibrils are embedded in a more pliant hemicellulose-pectin matrix (primary cell walls) or in a hemicellulose-lignin matrix (secondary cell wall). The arrangement of the stiff cellulose fibrils determines cell wall mechanics and anisotropy to a large extent. Still, both the processes of cellulose synthesis and the arrangement of cellulose fibrils in growing cells is not fully understood yet. One outcome of the research activities on the model plant system Arabidopsis thaliana is the availability of numerous cell wall mutants. Structural and mechanical investigations of their dark grown hypocotyls are a promising approach to a deeper understanding of primary cell wall formation, structure and finally cell growth. However, detailed knowledge on the hypocotyl properties of wildtype plants, especially on how they change with hypocotyl growth (age) is essential.

We made structural and mechanical investigations on 4,5,6 and 7 day old dark grown hypocotyls (**Fig. 2**). The cellulose orientation in different regions along the hypocotyl was studied by a newly developed synchrotron X-ray method, the mechanical properties in the lower region of hypocotyls was determined by microtensile tests **[1]**. In the future these methods will be applied to study hypocotyls with targeted modification (in collaboration with *Staffan Persson*, MPIMP, Potsdam).

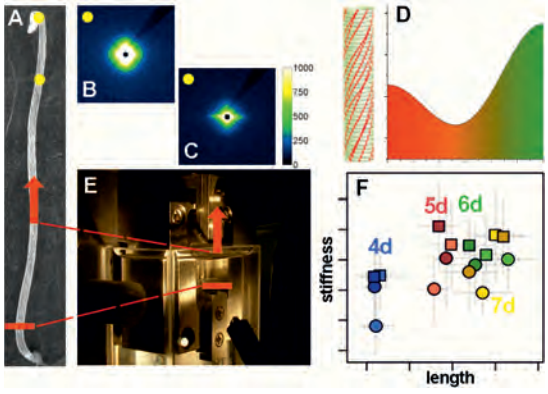

Fig. 2: Structural and mechanical studies on Arabidopsis hypocotyls (A), x-ray diffractograms of the apical hook (B) and ~ 5 mm below (C), diagram showing microfibril angle distribution (D). (E) lower part of a hypocotyl in the microtensile tester in liquid medium, (F) stiffness values of 4, 5, 6 and 7 day old hypocotyls, determined in the lower part of the hypocotyls.

To study the mechanically more robust secondary cell walls we apply a multitude of different methods, eg. **[2,3]**. An example for an interesting secondary cell wall system which we started to investigate in more detail is given below:

Plants and Fire - Storage and Protection of Banksia Seeds in Follicles

So-called serotinous plants are seen in some fire-prone environments. The term "serotiny" describes the trait to retain mature seeds on the plant instead of releasing them. The plant benefits from increased competitiveness after fire: a massive seed stock is released at once into the post-fire nutrient-rich soil. Prominent examples for this plant trait are species of the ancient Australian genus Banksia: seeds can be stored for more than 15 years in woody follicles (**Fig. 3**) on shrub- or tree-like plants with species-dependent triggers for opening, ranging from very high temperatures, to a combination of heat plus cyclic wetting and drying. To be a beneficial (adaptive) functional trait these follicles must meet at least two requirements (i) seed protection for long periods demanding (structural) stability against weathering, microorganisms and animals and (ii) the ability to open rapidly upon the appropriate environmental trigger or disturbance, most commonly fire.

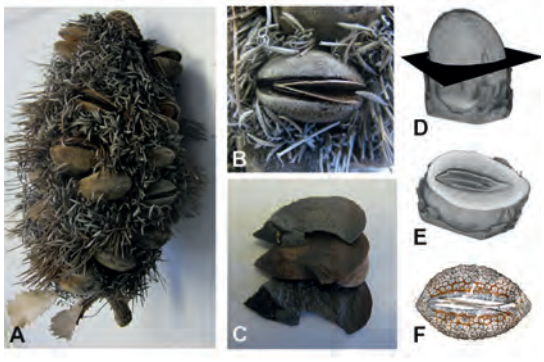

*Fig. 3: Banksia serrata: (A) infructescence with closed and open follicles surrounded by remnant flowers; (B) open follicle with protruding separator; (C) two winged seeds and separator lying between; (D) CT scan of a closed follicle (E) cross section of CT scan showing internal follicle structure (F) unstained light micrograph of a cross section **[4]***

We expect sophisticated material properties of the follicles including dimensional and mechanical stability, durability and flame retardant properties. Banksia follicles of selected species will be studied in detail at different length scales (in collaboration with *David Merritt*, BGPA, Perth, Australia and *Christoph Neinhuis*, TU Dresden).

M. Eder, G. Bauer, M. Ermeydan, N. Horbelt, J. Huss, F. Saxe, V. Schöppler, S. Weichold, C. Weisskopf, G. Wienskol
Michaela.Eder@mpikg.mpg.de

References:

[1] Saxe, F., Significance of cell wall ultrastructure for growth and mechanical properties of Arabidopsis thaliana, Doctoral thesis, University of Potsdam, 2012.
[2] Eder, M., Arnould, O., Dunlop, J.W.C., Hornatowska, J., Salmén, L., Experimental mechanical characterization of wood cell walls, Wood Sci Technol, **47**, 163-182, (2013).
[3] Gierlinger, N., Luss, S., König, C., Konnerth, J., Eder, M., Fratzl, P., Cellulose microfibril orientation of Picea abies and ist variability on the micron-level determined by Raman imaging, J Exp Bot, **61**, 587-595 (2010).
[4] Huss, J., Mikro- und ultrastrukturelle Charakterisierung von Banksia serrate Follikeln, Bachelorthesis, University of Freiburg, 2012.

Hierarchical Structure of Biological and Biomimetic Materials

Wolfgang Wagermaier 19.06.1974
2001: Diploma, Material Science (Montan-University, Leoben)
2003-2006: PhD, Material Science (MPI of Colloids and Interfaces, Potsdam and Montan-University, Leoben) Thesis: Synchrotron X-ray diffraction studies of nanoscale bone structure and deformation mechanisms
2007-2009: Postdoc, (GKSS Research Center, Center for Biomaterial Development, Teltow)
Since 2009: Group Leader (Max Planck Institute of Colloids and Interfaces, Potsdam)

Many biological materials have excellent mechanical properties and they often show unique capabilities such as the ability to regenerate. Thereby the material is adapted to environmental conditions at all hierarchical levels by the activity of cells. In our group, we use specific combinations of materials science methods to answer biologically driven questions. We characterize biological materials at various levels, from the nano- to the centimeter range. In our research, bone serves as prototypical system for a hierarchically structured biological material. It can be considered as a composite material, consisting of collagen I molecules and mineral particles at the nanometer scale **[1]**. The research on bone is performed in cooperation with partners from the Julius Wolff Institute at the Charité in Berlin as well as the Ludwig Boltzmann Institute of Osteology in Vienna, Austria.

Furthermore, we investigate specific properties and basic formation mechanisms of synthetically produced complex materials and compare them with those of biological materials.

Our central experimental methods are X-ray scattering (SAXS, WAXS), X-ray fluorescence (XRF), polarized light microscopy (PLM), confocal laser scanning microscopy (CLSM), electron microscopy, micro-computed tomography (µCT) and nanoindentation. For X-ray scattering experiments we use our lab sources as well as synchrotrons, in particular the MPI µspot beamline at BESSY II (Helmholtz-Zentrum Berlin für Materialien und Energie, Berlin Adlershof).

Bone Formation and Healing

Bone formation takes usually place in two stages. First, a rather unorganized bone tissue (woven bone) is generated by bone-forming osteoblasts. Second, lamellar bone grows on top of the woven bone and partially replaces it. Hence, intramembranous bone formation requires an intermediate step in which bone with a lower degree of orientation serves as a substrate for osteoblasts **[2]**. This is followed by a cooperative action of osteoblasts resulting in the deposition of lamellar tissue. During bone formation some of the osteoblasts get embedded within the collagen matrix and differentiate thereby into osteocytes. They are then located in cavities called lacunae, and form cell processes within small tubes (canaliculi). These structures form a dense network through the entire bone matrix representing a fingerprint of bone formation, as the position of osteoblasts during the embedding can be deduced. In **Fig. 1a**, the osteocyte network of plexiform sheep bone shows a layer with a relatively small amount of woven bone surrounded by layers of lamellar bone. These structures can be very well visualized with CLSM **[2]**, which enables the interpretation of the bone forming process.

A similar two-step process like in bone formation was also found during bone healing in a sheep callus **[3]**. To explore if this process during bone healing can be generalized we currently investigate also the material structure in small animal (rat and mouse) osteotomy models **[4]**. In a study on ovine bone with a critical size defect filled with a porous scaffold, we find that the scaffold architecture guides new tissue formation **[5]**. At first, the scaffold supports the formation of a structured fibrous tissue across the defect. This fibrous network guides the mineralization process and consequently enables bone ingrowth into a critical-sized defect.

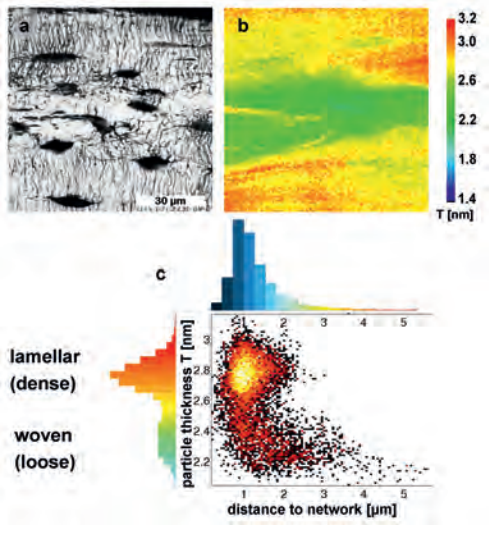

Fig. 1: Structure and properties of plexiform ovine bone. (a) CLSM image showing compact bone. Areas with a lower degree of orientation in the middle are surrounded by lamellar layers, represented by an oriented arrangement of osteocyte lacunae and canaliculi. (b) Mineral particle thickness (T-parameter in nm) from the same bone area as shown in (a); every pixel represents one measurement with small angle x-ray scattering with 1 µm resolution. (c) Correlation of mineral particle thickness (T-parameter) with the distance of these mineral particles to the cell network. The T-parameters shows a bimodal distribution in relation to the different bone areas.

The Role of Osteocytes in Bone

In bone, the physical properties of the extracellular matrix are closely correlated with cell functions. Osteocytes are known to orchestrate bone remodeling, but their precise role during mineral homeostasis and its potential impact on the quality of the bone material is not yet fully understood. To understand the interaction of the extracellular matrix with osteocytes we examined the network organization with respect to the properties of the surrounding material **[6]**. The osteocyte network was visualized by CLSM and characterized by topologically quantifying the distance of the bone matrix from the cell network (lacunae and canaliculi). By means of synchrotron SAXS with a 1 µm beam (ID13, ESRF, Grenoble, France) we determined the size and arrangement of mineral particles in the same bone sections. **Fig. 1b** shows the size (T-parameter in nm) of mineral particles in relation to the geometry of the osteocyte network. An important finding in this study was that these properties depend on the distance to the cell network (**Fig. 1c**). The most surprising insight was that the majority of the mineral particles reside within less than one micrometer from the nearest cell network channel. By this combination of research methods it could be shown that the osteocytes have potential access to a vast reservoir of minerals in the bone and therefore might contribute to the mineral homeostasis **[6]**.

References:
[1] Wagermaier, W. and Fratzl, P.: Collagen. In: Matyjaszewski, K. and Möller, M. (eds.), Polymer Science: A Comprehensive Reference, Vol **9**, pp. 35–55, (2012).
[2] Kerschnitzki, M., Wagermaier, W., Roschger, P., Seto, J., Shahar, R., Duda, G.N., Mundlos, S., Fratzl, P.: The organization of the osteocyte network mirrors the extracellular matrix orientation in bone. J. Struct. Biol., **173**, (2), 303-311, (2011).
[3] Kerschnitzki, M., Wagermaier, W., Liu, Y., Roschger, P., Duda, G.N., Fratzl, P.: Poorly Ordered Bone as an Endogenous Scaffold for the Deposition of Highly Oriented Lamellar Tissue in Rapidly Growing Ovine Bone. Cells Tissues Organs, **194**, (2-4), 119-123, (2011).
[4] Seidt, B.: Investigating material structure and mineralization processes during bone healing in Muridae osteotomy-models by X-ray scattering and electron microscopy. Diploma thesis, University of Kassel, (2012).
[5] Cipitria, A., Lange, C., Schell, H., Wagermaier, W., Reichert, J. C., Hutmacher, D. W., Fratzl, P., Duda, G. N., Porous scaffold architecture guides tissue formation. Journal of Bone and Mineral Research, **27**, (6), 1275-1288, (2012).

Mineralization in Healthy and Diseased Bone

New insights into the mineralization of bone could be achieved by applying a unique combination of quantitative X-ray scattering and fluorescence methods to fetal and postnatal mouse bone **[7]**. Our results revealed strong differences in size and orientation of the mineral particles between fetal and postnatal bone, with bulkier, randomly oriented particles at the fetal stage, and highly aligned, much longer particles after birth. **Fig. 2** shows the amount of hydroxyapatite (HA-002-peak area) in fetal and postnatal samples measured by WAXS as a function of the calcium content determined by XRF. The correlation between HA and calcium is not linear and a linear regression of the fetal data (dashed line) reveals a calcium offset. This leads to the interesting observation that the tissue at all stages of development contains more calcium than is present in hydroxyapatite.

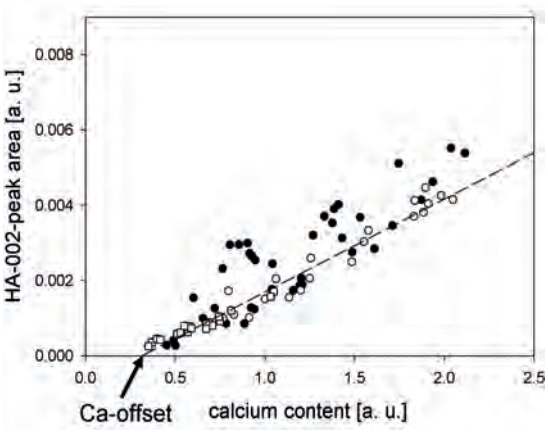

Fig. 2: Comparison of the WAXS (amount of hydroxyapatite, HA-002-peak area) and XRF (calcium content) data for two representative fetal samples, F16.5 (open squares, open circles) and one representative postnatal sample, P1 (closed circles). The plot shows the amount of hydroxyapatite (HA-002-peak area) as a function of the calcium content.

Currently we are investigating medullary bone which serves as model system for rapid bone turnover rates as it is a calcium source for daily egg shell formation in hens **[8]**. One of the main discoveries there is that there are three different bone types. Additionally to the two known bone types (cortical and medullary bone) a third type (termed 'nebular bone') has been discovered, which may represent an intermediate phase during mineralization. Understanding the structure of medullary bone at different points in time during egg shell formation might be a key to gain further insights into mineralization mechanisms in bone.

Osteogenesis Imperfecta (OI) is a genetic mutation resulting directly in a disturbed collagen formation and indirectly in a disordered bone with increased bone fragility, low bone mass, impaired bone material properties and unusually high bone matrix mineralization. In human bone of children, we compared the mineral crystal size in OI with a control group and found that the increase in mineral density in OI is not due to an increase in particle size, but due to an increase in the number of particles **[9]**.

Microlens Arrays with Uniform Size and Focal Length

Biomineralized tissues, such as sea shells and bones, grow in a genetically programmed way to obtain specific compositions and shapes, which define their unique functionalities. The growth of biominerals usually takes place in aqueous media at ambient conditions. While such natural systems and processes are usually very complex, tailor-made model systems can be used to explore basic processes. We developed a simple synthesis of unique micro-optical devices: microlens arrays **[10]**.

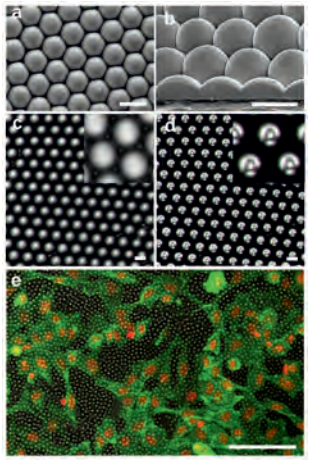

Fig. 3: Morphology and optical properties of the microlens arrays. (a) SEM image of the homogeneous and well-ordered $CaCO_3$ microlens array from top view. (b) SEM image of cross-sectioned microlens array from tilted view. The microlens array was etched by focused ion beam milling. (c, d) Optical microscope images of $CaCO_3$ microlens array and inversely projected 'A' array. The magnified images are shown in the insets. (e) Overview of NIH3T3 fibroblast cell growth on the microlens array. The actin filaments within the cells are stained in green and the nuclei in red with fluorescent dyes (overlay of fluorescent and phase contrast images). Scale bars: a-d: 5µm, e: 20µm

To produce these optically functional $CaCO_3$ structures, we used saturated calcium solution and CO_2 in air for the mineral precipitation. The formation process is regulated by an organic surfactant whose amphiphilic molecules play a crucial role at the early stage of self-assembly. Within one to two hours micrometer-sized $CaCO_3$ structures with hemispherical shape and uniform size are formed as a thin film on the surface of the solution (**Fig. 3a and b**). By means of light microscopy multiple images of a micron-sized 'A' could be projected through the array of microlenses, proofing that the hemispherical $CaCO_3$ structures work as micron-sized convex lenses (**Fig. 3c and d**). In this project the biocompatibility of the $CaCO_3$ microlens arrays was demonstrated by seeding fibroblasts on the array (**Fig. 3e**). The study was performed at the Max Planck Institute of Colloids and Interfaces in Potsdam and is a joined work with KAIST in South Korea.

W. Wagermaier, G. Benecke, R. Hoerth, M. Kerschnitzki, C. Lange, K. Lee, C. Li, B. Seidt, I. Schmidt, S. Siegel, T. Zander and I. Zenke,
wolfgang.wagermaier@mpikg.mpg.de.

[6] Kerschnitzki, M., Kollmannsberger, P., Duda, G.N., Weinkamer, R., Wagermaier, W., Fratzl, P.: Architecture of Osteocyte Communication Channels Correlates with Bone Material Quality. Journal of Bone and Mineral Research, accepted 2013, **DOI: 10.1002**/ jbmr. 1927.
[7] Lange, C., Li, C., Manjubala, I., Wagermaier, W., Kuhnisch, J., Kolanczyk, M., Mundlos, S., Knaus, P., Fratzl, P., Fetal and postnatal mouse bone tissue contains more calcium than is present in hydroxyapatite. Journal of Structural Biology, **176**, (2), 159-167, (2011).
[8] Zander, T.: X-ray scattering and electron microscopy studies of mineralization processes in medullary bone. Master thesis, TU Berlin, (2012).
[9] Schmidt, I.: Charakterisierung der Mineralpartikelgrößen und Mineralpartikelorientierung in Knochen von Patienten mit Osteogenesis imperfecta Typ I mittels Röntgenstreumethoden. Diploma thesis, TU Berlin, (2012).
[10] Lee, K., Wagermaier, W., Masic, A., Kommareddy, K. P., Bennet, M., Manjubala, I., Lee, S. W., Park, S. B., Colfen, H., Fratzl, P.: Self-assembly of amorphous calcium carbonate microlens arrays. Nature Communications, 3, Art.Nr. **725**, (2012).

Synthesis and Thermodynamic Stability of Amorphous Minerals

Wouter Habraken 04.12.1979

1998-2003: Master of Science at the Department of Chemical Engineering and Chemistry at the Eindhoven University of Technology (Eindhoven, The Netherlands).

2003-2008: Doctoral Thesis: "Development of Biodegrable Calcium Phosphate Cement for Bone Tissue Engineering" at the Radboud University Nijmegen Medical Center (Nijmegen, The Netherlands) Supervised by John .A. Jansen in cooperation with Antonios G. Mikos (Rice University, USA).

2008-2010: Postdoctoral Scientist at the Laboratory of Materials and Interface Chemistry and Soft Matter Cryo-TEM Research Unit (Nico A.J.M. Sommerdijk, Eindhoven University of Technology, Eindhoven, The Netherlands)

2010-2012: Postdoctoral Scientist at the Bio-Inspired Hybrid Materials and Synchrotron Research group (Barbara Aichmayer, Department of Biomaterials, Max Planck Institute of Colloids and Interfaces, Golm, Germany)

Since 7/2012: Independent Researcher (Department of Biomaterials, Max Planck Institute of Colloids and Interfaces, Golm, Germany)

References:

[1] Dmitrovic, V.; Habraken, G.J.; Hendrix, M.M.; Habraken, W.J.; Heise, A.; de With, G.; Sommerdijk, N.A. Random Poly(Amino Acid)s Synthesized by Ring Opening Polymerization as Additives in the Biomimetic Mineralization of $CaCO_3$. Polymers **4** 1195-1210 (2012).

[2] Habraken, W.J.E.M.; Tao, J.; Brylka, L.J.; Friedrich, H.; Bertinetti, L.; Schenk, A.S.; Verch, A.; Dmitrovic, V.; Bomans, P.H.H.; Frederik, P.M.; Laven, J.; Schoot, P. v. d.; Aichmayer, B.; de With, G.; DeYoreo, J.J.; Sommerdijk, N.A.J.M. Ion association complexes unite classical and non-classical theories for the biomimetic nucleation of calcium phosphate, Nature Comm., accepted (2012).

Amorphous Calcium Carbonate/ Calcium Phosphate Mixtures

Many of the most complex mineral structures found in nature are not from geological origin, but are the result of biological processes. Examples are vertebrate bones or invertebrate exoskeletons, where a mineral phase (calcium phosphate/calcium carbonate) is in close contact with an organic matrix composed of either collagen or chitin. Recent developments show indications that the mineralization of, what is initially a fully organic matrix, is governed by the attachment of spherical, submicron to nm-sized mineral particles that are amorphous and possibly excreted by neighboring matrix-forming cells. In most cases, this amorphous phase crystallizes in the final mineral structure, however, in some cases the amorphous nature is retained. The high stability of such amorphous biominerals against crystallization is remarkable and requires further understanding. From *in vitro* experiments we know that highly charged polymers **[1]**, phosphorylated proteins, small organic molecules or inorganic impurities are able to delay the nucleation of a crystalline phase or even stabilize an amorphous calcium carbonate (ACC) or calcium phosphate (ACP) (**Fig. 1**). However, the more complex the stabilizing agent gets, the less we know about the actual mechanism. Furthermore, the influence of these agents on the local physical conditions of the reaction medium (pH, ionic strength, depletion of ions) is often underestimated, making it a tedious job to extract trustworthy mechanistic data.

To deepen the present understanding on the stability of some biomimetic amorphous minerals, in our research we are focusing on 1 special characteristic of many stable amorphous calcium carbonates, which is the presence of (large amounts of) inorganic phosphate. In line with the proposed influence of Mg^{2+} on calcium carbonate mineralization, next to a possible mismatch in charge (3+ instead of 2+) the large tetrahedral phosphate groups impose a structural mismatch with the planar CO_3^{2-} in the final crystalline calcium carbonate. The procedure we apply is to: 1) prepare amorphous calcium carbonate/calcium phosphate mixtures with various biologically relevant compositions 2) investigate the efficiency of mixing between carbonate/phosphate groups and 3) investigate the stability of the prepared amorphous material. Here, results from step 2) and 3) are used to optimize the synthesis method, thereby providing us detailed information on the conditions necessary to obtain a perfectly mixed ACC/ACP. Furthermore, by varying the ratio between ACC and ACP we can relate the stability of a certain mixture to its chemical composition. Finally, we can compare the specific ACC/ACP mixtures with their biological analogues, telling us more about the origin of their stability. In all steps of the research there is a close cooperation with the Department of Structural Biological of the Weizmann Institute (*Assaf Gal, Lia Addadi*).

Synthesis of Amorphous Minerals

Various synthesis methods for amorphous calcium carbonate and amorphous calcium phosphate have been described. Most of them rely on the formation of an instant high supersaturation with respect to the crystalline phase, thereby provoking the formation of the metastable, amorphous precursor using a simple two-pot synthesis. The extended lifetime of these materials enables the collection of a rather stable dried amorphous phase after carefully extracting the sample from the reaction solution. Using such a preparation method, initial investigation shows that an amorphous phase with chemical and physical properties in Raman-spectroscopy, X-ray diffraction and morphology (SEM), which are intermediate between ACC and ACP, is easily obtained. However, this method doesn't allow us to control the physical conditions in the reaction medium in a great extent and furthermore raises questions whether the sample collection doesn't change the structure or chemistry of the ACC/ACP mixture.

Therefore, to control and monitor crucial parameters during the formation of the ACC/ACP phase like the pH and concentration of Ca^{2+}, a titration setup will be applied **[1,2]**. Furthermore, in addition to analysis performed on extracted samples, analysis of the mineral phase inside the reaction medium will be performed as earlier described for the nucleation of calcium phosphates **[2]**.

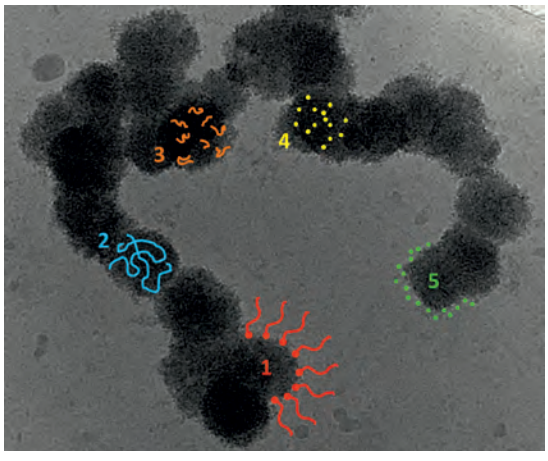

Fig. 1: Different mechanism for stabilization, 1) membrane 2) incorporated polymer/protein, 3) small incorporated molecule, 4) inorganic impurity, 5) surface counter-ion/impurity

W.J.E.M. Habraken
wouter.habraken@mpikg.mpg.de.

Water Interactions in Complex Biological Materials

Nature shows many examples of nanocomposite tissues constituted of soft materials that are reinforced by complex architectures of stiff components. Besides being tailored at the higher hierarchical levels to bear and distribute loads, these tissues are carefully designed to optimize the interactions of their molecular/macromolecular components with water to obtain desired properties/behaviors on which biological organisms rely to accomplish their vital functions. In many tissues, in fact, the chemistry of the constituents drives water absorption that, thanks to the peculiar arrangement of the macromolecular components or to their structure, results in anisotropic volume changes (swelling). This swelling and deswelling has been shown to generate complex (ametabolic) movements that are exploited in nature for different aims: swimming of the wheat awns**[1]**, opening of pine and spruce cones**[2]** or plant seeds dispersal units during rain in arid regions.**[3]** In a similar way, swelling is responsible for growth stresses in trees to compensate for the load of branches or of side winds.**[4]** Biological materials act then as structure mediated, chemo-mechanical energy converter, as they are able to exploit molecular forces to generate mechanical energy.

Of particular interest for me is:

- to understand and describe molecular interactions in such complex materials,
- to set thermodynamic models able to describe the structure mediated chemomechanical energy conversion,
- to image, at the microscopic level, water sorption and water/tissues interactions in heterogeneous biological materials.

Molecular Interactions

Typically, biological materials are very complex, but they can be described as a collection of primary building blocks regularly arranged at the various hierarchical levels. Their characteristic size lies in the nanometric range and they are usually separated by few nm. Because of these reasons, a variety of interactions occur at the molecular level between the constituents of the tissues or between these latter and the solvent molecules. Typical examples are hydration forces, associated to the particular structure of water when confined to very small spaces (typically sub-nanometric) between two surfaces, Van der Waals interactions, entropic forces, H-Bonds etc… One goal of my research is to describe, starting from a chemical and structural description of the biological nano - compoisites and considering the hierarchical arrangement, which of those interactions play a critical role in the hydration processes and how these forces are varying as a function of the amount of solvent taken up. Mainly my work focuses on plants tissues: in collaboration with prof. Thomas Zemb (ICSM - Marcoule), a model describing the equation of state for wood has been set, and, in collaboration with former groups (I. Burgert) and the group of John Dunlop, a chemical description of the opening of the seed dispersal units of the ice plant has been proposed.

Additionally, I study the hydration of collagen and other protein based fibers**[5,6]** (in collaboration with Dr. Admir Masic) and the interactions between mineral surfaces (biomimetic calcium phosphates) and water.**[7]**

Chemomechanical Energy Conversion

Once the molecular forces driving water sorption are described, the continuum mechanics can be used to express the changes of mechanical energy with the dimensional changes the materials undergoes when taking up solvent. In this way, using the gas/liquid or liquid/liquid phase equilibrium thermodynamics, an ab initio model predicting the equilibrium stresses/strains the structure can produce, under desired geometrical/mechanical constraints and for given changes in the chemical potential of the solvent, can be set.

At the same time, the predictions of the model are compared with *in situ* experimental data.

Water Sorption Imaging

Finally, as the natural tissues are often heterogeneous at the micro/nanometric scale, I lately started to develop, in collaboration with Dr. Breitenstein of the Max Planck Institute of Microstructure Physics in Halle, a technique based on Infrared Lock-In Thermography to spatially resolve and image water sorption sites, water sorption kinetics and possibly water/matrix binding energies in the aforementioned tissues (**Fig. 1**).

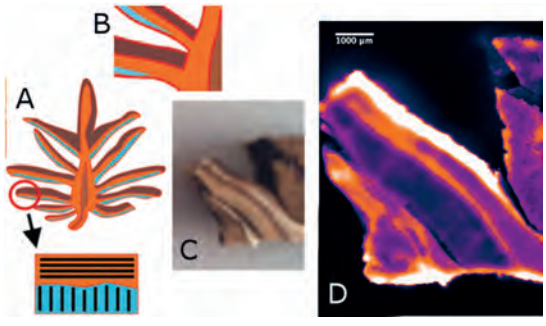

Fig. 1: Water sorption imaging: A) Sketch of a pine cone (After Ref. 2); the bottom (blue) blue layer is swelling more than the upper one. Cellulose fibres are represented in black, B) detail of the scales, C) Optical image of a typical bilayer structure, D) Corresponding water map showing the different water sorption behaviour of the bilayer structure (darker areas indicates higher sorption ability).

L. Bertinetti and P. Fratzl
bertinetti@mpikg.mpg.de.

Luca Bertinetti 03.09.1975
1994-2001: Master of of Science (110/110 cum Laude) majoring in Materials Science (University of Torino, Italy)
2002-2005: Doctoral Thesis (Chemistry): Nanomaterials for biomedical applications: synthesis and surface characterization. (University of Torino, Italy)
2006-2009: Research Technician in charge of the Electron Microscopy research line of the Structural and Functional Materials Group of the IPM Department of the Torino University, Italy
Since 1/2010: Postdoctoral Scientist Department of Biomaterials, Max Planck Institute of Colloids and Interfaces.

References:

[1] Elbaum, R., Zaltzman, L., Burgert, I. & Fratzl, P. The role of wheat awns in the seed dispersal unit. Science **316**, 884–886 (2007).
[2] Dunlop, J. W. C., Weinkamer, R. & Fratzl, P. Artful interfaces within biological materials. Materials Today **14**, 70–78 (2011).
[3] Harrington, M. J. et al. Origami-like unfolding of hydro-actuated ice plant seed capsules. Nat Commun **2**, 337 (2011).
[4] Fratzl, P. & Barth, F. G. Biomaterial systems for mechanosensing and actuation. Nature **462**, 442–448 (2009).
[5] Masic, A. et al. Observations of Multiscale, Stress-Induced Changes of Collagen Orientation in Tendon by Polarized Raman Spectroscopy. Biomacromolecules (2011).
[6] Bauer, F., Bertinetti, L., Masic, A. & Scheibel, T. Dependence of mechanical properties of lacewing egg stalks on relative humidity. Biomacromolecules (2012).
[7] Bolis, V. et al. Coordination chemistry of Ca sites at the surface of nano-sized hydroxyapatite: interaction with H_2O and CO. Philos Transact A Math Phys Eng Sci **370**, 1313–1336 (2012).

Magnetite Formation and Organization

Damien Faivre 03.10.1977
2001: Master, fundamental and applied geochemistry (Institute of Earth Physics and University Denis Diderot, Paris) Thesis: Effect of formation conditions on the geochemical properties of magnetite nanocrystals
2004: PhD, fundamental and applied geochemistry (University Denis Diderot, Paris)Thesis: Kinetics, mineralogy, and isotopic properties of magnetite nanoparticles formed at low temperature: Implication for the determination of biogenicity criterion
2005-2007: PostDoc (MagnetoLab, Max Planck Institute of Marine Microbiology, Bremen, Germany)
Since 2007: Group Leader Biomaterials Department, (Max Planck Institute of Colloids and Interfaces, Potsdam)
Since 2011: ERC Group Leader

Systematic studies of biologically formed materials have showed that they have remarkable properties. Nature thus not only provides us with inspiration for designing new materials but also teaches us how to use soft molecules such as proteins to tune interparticle and external forces to structure and assemble simple building blocks into functional entities.

Magnetotactic bacteria and their chain of magnetosomes (**Fig. 1**) represent a striking example of such an accomplishment where a very simple living organism precisely controls the properties of inorganics via organics and at the nanometer-scale to form a single magnetic dipole that passively orients the cell in the Earth magnetic field lines **[1, 2]**. In my group, we have thus developed a bio-inspired research based on magnetotactic bacteria. This research combines the recent developments of nanoscale engineering in the chemical science, the latest advances in molecular biology together with modern progresses in physical analysis. My research thus focuses at the interface between chemistry, materials science, physics, and biology to understand how biological systems synthesize, organize and use minerals, and to apply the design principles to sustainably form hierarchical materials with controlled properties that can be used e.g. as magnetically directed nanodevices towards applications in sensing, actuating, and transport.

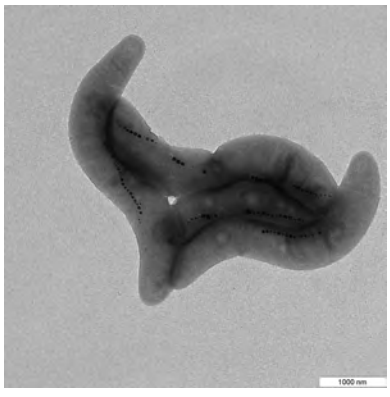

Fig. 1: a typical TEM image from magnetotactic bacteria (strain AMB-1). The magnetosomes are the electron-dense particles that are aligned and form chain in the cells. Image by A. Körnig and M. Widdrat.

Biological Materials

Magnetosomes: Hierarchy at the Structural Level

The biomineralization of the mineral magnetite inside the magnetosome organelle together with the chain formation in magnetotactic bacteria are two processes that are highly controlled at the cellular level in order to form cellular magnetic dipoles. The smallest building block in this hierarchical structure is the magnetosome crystal. However, only controversial results about its micro-structure were obtained so far, partly because of the very limited amount of materials available. Thereby, the influence of the ultrastructure in the formation of the magnetic dipole i.e. on the function of the assembly is also to be specified.

We have thus investigated the structure of the magnetosomes using high-resolution synchrotron X-ray diffraction at the microspot beamline of the BESSY II synchrotron of the Helmholtz-Zentrum Berlin **[3]**. Significant differences in lattice parameter were identified between intracellular magnetosomes from cultured magnetotactic bacteria and isolated ones (**Fig. 2**). Through comparison with synthetic nanoparticles (abiotic control materials) of similar size, we showed that this difference could be associated with different oxidation states and that the biogenic magnetite was stoichiometric, i.e. structurally pure. However, as soon as the magnetosomes were isolated from the cells, oxidation took place.

We thus proposed that the hierarchical structuring of the magnetosome chain starts with the formation of structurally pure magnetite nanoparticles. In addition, this property can be directly connected with the magnetic property of the magnetosome chains where it is of advantage for the cell to form structurally pure magnetite crystals for optimal magnetic response.

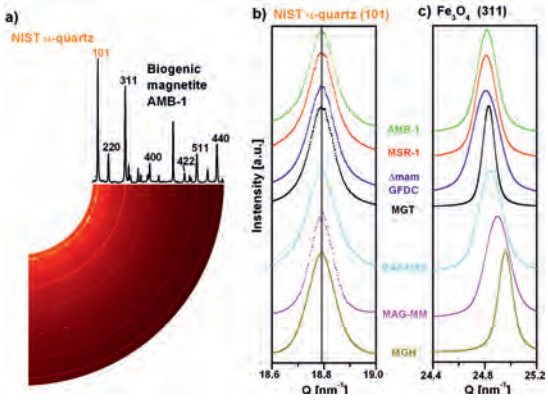

Fig. 2: a) Exemplary 90° sector of AMB-1 diffraction pattern to visualize the azimuthal integration. Analyzed magnetite peaks and calibration peak (NIST α-quartz) indexed b) α-quartz (101) calibration peaks of different biogenic and abiotic magnetite/ maghemite samples. All peaks calibrated to Q = 18.7910nm^{-1} c) most intensive (311) reflex of all analyzed samples. Remarkable peak shift bet-ween biogenic magnetite in cell solution samples (AMB-1, MSR-1 and ΔmamGFDC) compared to isolated magnetosomal magnetite with and without membrane (MAG+MM and MAG-MM) and inorganic magnetite (MGT) or even more pronounced with maghemite (MGH).

Magnetosomes Chains: Hierarchy at the Chain Level

Magnetotactic bacteria benefit from their ability to form cellular magnetic dipoles by assembling stable single-domain ferromagnetic particles in chains as a means to navigate along Earth's magnetic field lines on their way to favourable habitats. After studying the smallest building-blocks, i.e. the magnetosomes and their ultrastructure, we studied their assembly by a combined experimental and theoretical approach **[4, 5]**. A number of genetic factors involved in the controlled assembly of these magnetosome chains have been identified in recent years, but we have addressed how the specific biological regulation is coordinated with general physical processes.

The simulations indicate that physical processes of magnetosome diffusion, guided by their magnetic interactions, are not sufficient for the chain formation observed experimentally. In turn, they suggest that biologically encoded active movements of magnetosomes may be required. Not surprisingly, the chain pattern is most resembling experimental results when both magnetic interactions and active movement are coordinated (**Fig. 3**).

In addition, we estimate that the force such active transport has to generate is compatible with forces generated by the polymerization or depolymerization of cytoskeletal filaments. The simulations suggest that the pleiotropic phenotypes of mamK deletion strains may be due to a defect in active motility of magnetosomes and that crystal formation in magnetosome vesicles is coupled to the activation of their active motility in *M. gryphiswaldense*, but not in *M. magneticum*.

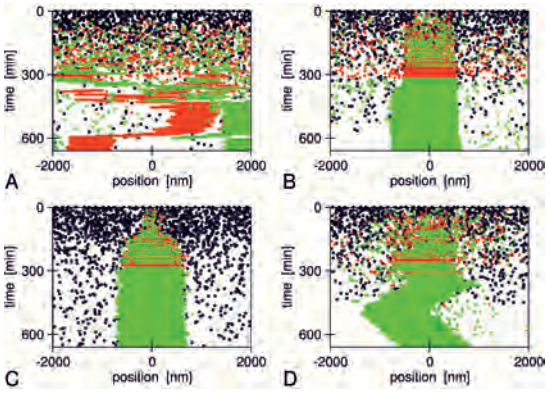

Fig. 3: Example time traces of magnetosome formation in our simulations. (A) Magnetic interactions and diffusion only, (B) binding zone in the cell center (Lb = 1000 nm), (C) and (D) active transport to the cell center with an active force Fact = 1 pN (C) and 0.01 pN (D). In all panels, black dots indicate empty magnetosome vesicles, green and red points indicate magnetosomes containing a crystal with plus or minus orientation of its magnetic moment. In all panels, the magnetosome mobility is given by D=10^5 nm^2/s. (Fig. from Klumpp and Faivre, 2012)

Biomimetic Materials

Synthetic Magnetite Nanoparticles: Studying the Nucleation and Growth of Nanoparticles

The formation of crystalline materials from solution is typically described by the nucleation and growth theory, where atoms or molecules assemble directly in and from solution. For various systems however, the formation of the thermodynamically stable mineral is preceded by intermediate phase(s). More complex pathways have recently been proposed, such as aggregational processes of nanoparticle precursors or pre-nucleation clusters, which seem to contradict the classical theory.

Multiple synthetic routes for the production of magnetite nanoparticles have been reported in the literature. Indeed, the ferrimagnetic properties of such particles are increasingly exploited in bio- and nanotechnological applications. However, the formation mechanism has remained unclear. We have developed a set-up for the controlled growth of magnetite particle *in vitro* **[6]**. We can reach average particle dimension of 50 nm (**Fig. 4**), and thereby control the magnetic properties of the particles, changing from superparamagnetic for particles smaller than 25 nm to stable single domain for particles larger than 25 nm. We are thus able to synthetically reach particle size so far only attainable by biological synthesis.

We further have studied the mechanism of such formation by cryogenic transmission electron microscopy **[7]**. We found out that the nucleation and the growth of magnetite proceeds through rapid agglomeration of nanometric primary particles and that no intermediate amorphous bulk precursor phase is involved. We also demonstrate that these observations can be described within the framework of classical nucleation theory.

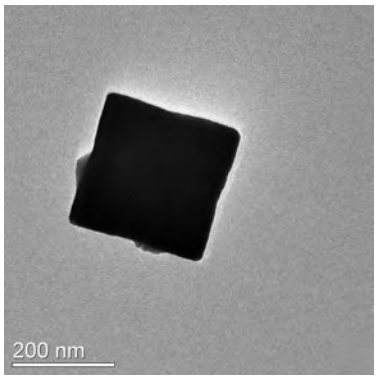

Fig. 4: TEM image of a large synthetic magnetite nanoparticle.

Biomimetic Chains: Towards Hierarchy in a Semi-Synthetic System

Hierarchical structuring of single particles can lead to the formation of multifunctional materials. We are thus are interested in the biomimetic arrangement of the magnetic particles we form *in vitro*. MamK is a filamentous Actin-like magnetosomal protein sharing significant homology with bacterial cytoskeletal proteins such as MreB and ParM. With little or no information on the structural and behavioural characteristics of MamK outside the cell, the mamK gene from *Magnetospirillium gryphiswaldense* was cloned and expressed to better understand the differences in the cytoskeletal properties with its bacterial homologues **[8]**.

Despite the low sequence identity shared between MamK and MreB (22%) and actin (18%), the behavior of MamK monitored by light scattering broadly mirrored that of its bacterial cousin MreB. The broad size variability of MamK filaments revealed by light scattering studies was supported by transmission electron microscopy imaging. Filament morphology however, indicated that MamK conformed to linearly orientated filaments that appeared to be distinctly dissimilar compared to MreB suggesting functional differences between these homologues.

D. Faivre, J. Andert, J. Baumgartner, M. Bennet, M. A. Carillo, A. Fischer, K. Halbmair, A. Körnig, P. Lesevic, A. Olszewska, T. Perez Gonzalez, M. Schmitz, P. Vach, M. Widdrat
faivre@mpikg.mpg.de.

References:

[1] Perez-Gonzalez T., Bennet M., Wood D. and Faivre D., Magnetosomes, in Biological self-assembly and its applications (ed. B. H. A. Rehm), 241-272, Horizon Scientific Press, Hethersett (2012).
[2] Körnig A., and Faivre D., Nanoparticles in magnetotactic bacteria, in Nature's Nanostructures (ed. A.S. Barnard and H. Guo), 249-271, Pan Stanford Publishing, Singapore (2012).
[3] Fischer A., Schmitz M., Aichmayer B., Fratzl P., and Faivre D., Structural purity of magnetite nanoparticles in magnetotactic bacteria, Journal of the Royal Society Interface **8**, 1011-1018 (2011).
[4] Charilaou M., Sahu K. K., Faivre D., Fischer A., Garcia-Rubio I., and Gehring A., Evolution of magnetic anisotropy and thermal stability during nanocrystal-chain growth, Applied Physics Letters **99**, 182504 (2012).
[5] Klumpp S., and Faivre D., Interplay of magnetic interactions and active movements in the formation of magnetosome chains, PLoS ONE **7** (3), e33562 (2012).
[6] Baumgartner, J., Bertinetti, L., Widdrat, M., Hirt, A. M. & Faivre, D. Formation of Magnetite Nanoparticles at Low Temperature: From Superparamagnetic to Stable Single Domain Particles. PLoS One **8**, e57070 (2013).
[7] Baumgartner, J. et al. Nucleation and growth of magnetite from solution. Nat. Mater. **12**, 310-314 (2013).
[8] Sonkaria S., Fuentes G., Verma C., Narang R., Khare V., Fischer A., and Faivre D., Insight into the assembly properties and functional organisation of the magnetotactic bacterial actin-like homolog MamK, PLoS ONE **7** (5), e34189 (2012).

Biomimetic Actuation and Tissue Growth

John Dunlop 06.04.1978
1996-2001:
Bachelor of Science (1st Class Honours) majoring in Chemistry Bachelor of Engineering (1st Class Honours) majoring in Materials Engineering University of Western Australia (Perth, Australia)
2002-2005: Doctoral Thesis: Internal variable modelling of creep and recrystallisation in zirconium alloys Institut National Polytechnique de Grenoble, Laboratoire Thermodynamique et de Physico-chimie des Matériaux. Grenoble (France)
Since 02/2006:
Postdoctoral Scientist Department of Biomaterials, Max Planck Institute of Colloid and Interfaces, Potsdam (Germany)
2007: Alexander von Humboldt Fellow
Since 11/2008: Research Group Leader Department of Biomaterials, Max Planck Institute of Colloids and Interfaces

Biological materials, in addition to having remarkable physical property combinations such as high toughness and stiffness, can also change shape and volume. These shape and volume changes allow organisms to form new tissue during growth and morphogenesis, as well as to repair and remodel old tissues. In addition shape or volume changes in an existing tissue can lead to useful motion or force generation (actuation) that may even still function in the dead organism. Both growth and actuation of tissues are mediated, in addition to biochemical factors, by the physical constraints of the surrounding environment and the architecture of the underlying tissue.

This research group combines experimental and theoretical methods to understand how tissue architecture and external physical constraints interact to control firstly tissue growth and secondly tissue actuation.

The work on tissue growth was done in collaboration with: *M. Rumpler*, Ludwig Boltzmann Institute for Osteology, Vienna, *F. D. Fischer*, and *E. Gamsjäger*, Uni. Leoben, *C. Werner* and co-workers at the Max Bergmann Institute, Dresden, and *A. Petersen* and co-workers at the Julius Wolff Institute, Berlin. The work on actuation was done in collaboration with *I. Burgert*, now at the ETH - Zurich, *R. Elbaum* and *Y. Abraham*, Hebrew Uni. Jerusalem, *Y. Bréchet*, INP-Grenoble, *T Antretter* and *G. Zickler,* Uni. Leoben, *L. Ionov* and co-workers at the Leibniz Institute of Polymer Research, Dresden.

Using Geometry to Direct Tissue Growth

Previous research in the group has shown that the shape of the surrounding environment can have a surprising influence on the rate of tissue formation **[1]**. 3D-printing techniques allow the production of pores with controlled surface geometries which can then be tested in tissue culture. The experimentally measured growth rates were shown to be proportional to the local surface curvature, meaning that despite the cells small size, collectively cells can measure geometries at length scales much larger than themselves. These observations can be readily implemented in a simple 2D computer model for curvature driven growth **[2,3]**, and give excellent predictions for the position of the tissue interface as a function of time (**Fig. 1**). Furthermore this model was also used to determine optimal pore shapes for tissue engineering applications **[3]**.

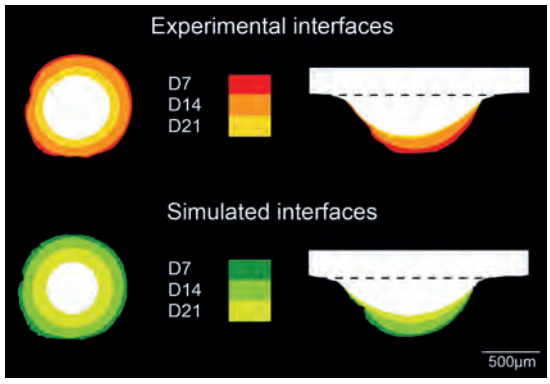

Fig. 1: Comparison between experimentally measured interfaces and predicted interfaces as a function of time in circular pores and semi-circular channels [2].

Despite the success of this simple geometric model in describing growth, it is difficult to directly link to it the mechanisms responsible for growth at the cellular scale. It seems likely that mechanical stresses developed by the cells themselves are responsible for the tissue patterning observed **[1]**. Inspired by the observation of high contractile stresses in the tissue surface, we have also been developing, together with *E. Gamsjäger* and *F. D. Fischer* (Uni. Leoben), a more complex model for tissue growth. This model takes into account both the stresses induced by confined growth as well as the stress induced by a contractile layer of cells on the surface **[4]**, and is successful in describing the asymmetric response of cells to the sign of curvature.

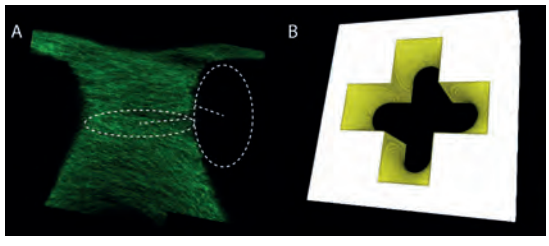

Fig. 2: A) An oblique view of the 3D actin architecture of tissue formed in the corner of a pore. The circles highlight the double curvature produced during growth. B) Result of the 3D curvature driven growth simulation in a cross-shaped pore.

The 3D nature of the tissue formed in the scaffolds has up till now been neglected, as the pores studied up till now have straight sides (they are prismatic), meaning one of the principle curvatures of the starting interface is always zero. 3D imaging methods, as illustrated in (**Fig. 2A**), show that due to growth the tissue develops a double curvature, with positive and negative mean curvatures, which may in turn influence

the overall curvature driven growth. As such the 2D geometric model has been extended to 3D (**Fig. 2B**). In this model, much akin to the Laplace law, the mean surface curvature is taken as the driving force for growth.

Using Geometry to Direct Actuation

Plants move their organs during their lifetime via active biological processes such as differential growth, or active changes in osmotic pressure exemplified by the fast closing of the Venus-fly trap. In addition to this some organs may also move after death due to the swelling of tissues upon hydration/dehydration. Such hygroscopic actuation is controlled solely by the clever arrangement of swellable and non-swellable tissues, and in principle can be readily copied by the Engineer. Many examples of such pre-programmed shape changes can be found in structures related to seed-propagation, such as in the awns of many seeds, or in the opening mechanisms of a variety of seed capsules (See also the work being done on Banksia in the Plant Adaptation group of *M. Eder*). The twisting/untwisting movement of the awns of *Erodium gruinum* for example **[5]** propel the seeds along and into the ground. This is controlled by the complex arrangement of tilted spirals cellulose microfibrils inside the cell walls. Similarly the awns of wheat also move upon humidity changes, with the rapid response to humidity changes thought to be accelerated by swelling induced pore opening **[6]**. The opening of the ice-plant seed capsule was studied by *M. Harrington* and *I. Burgert* **[7]**, and was shown to be controlled by hygroscopic keels consisting of diamond-honeycombs filled with a swellable cellulose-like gel (**Fig. 3A**). Such a honeycomb like structure converts the isotropic swelling into a strongly anisotropic response, which may be interesting in the design of artificial actuators.

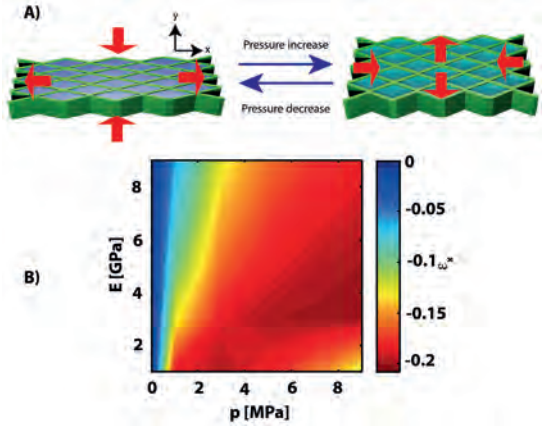

Fig. 3: A) Schematic of swelling of a diamond-honeycomb upon pressure changes in the cells, akin to what is observed in the ice-plant [7]. B) Simulated actuation strains in a diamond honeycomb as a function of pressure and cell-wall modulus.

Surprisingly despite the simplicity of such a pressurised honeycomb, very little work has been done on modelling it's mechanical properties. By combining Finite Element (FE) methods with micromechanical modelling it is possible to develop maps of actuation response as a function of actuation pressure, material properties and architecture (**Fig. 3B**). Further work is underway together with *J. Weaver* (Wyss Institute, Boston) to produce working mechanical prototypes of these systems using the latest generation multi-material 3D printer. Initial testing has begun on linear structures with extrude-able cross-sections simulated previously **[8]**.

Fig. 4: Simulated rolling and folding of three actuating bilayers with different aspect ratios attached to a partially adherent substrate [9].

Despite their apparent simplicity, bilayer structures can produce quite complex motion **[9,10]**, depending on their shape. In a collaboration with the experimental group of *L. Ionov* (Leibnitz Institute, Dresden) we have been using FE simulations to understand the role of external shape on how active polymeric bilayers attached to a substrate unpeel and fold. (**Fig. 4**) illustrates some examples of simulations carried out on layers with different aspect ratios. This illustrate the competition in all-side rolling observed in low aspect ratio systems compared to the one side rolling seen in more elongated structures. One surprising output of the model was the prediction of wrinkling in early stages of rolling that was subsequently confirmed in the experiments. More recent experiments by the Dresden group on star-like shapes led to the development of a set of simple design rules for folding **[10]**, supported by our mechanical simulations. Further work needs to be done to address the problems of kinetics, or the rate of shape change and to include these effects in our models.

J. Dunlop, C. Bidan, A. Bornmüller, L. Galvis, V. Gering, L. Guiducci, P. Kollmannsberger, K. Kommarreddy, P. Leibner, J. Panichpakdee, S. Turcaud, F. Wang.
dunlop@mpikg.mpg.de

References:

[1] Kollmannsberger, P., Bidan, C. M., Dunlop, J. W. C., Fratzl, P. The physics of tissue patterning and extracellular matrix organisation: How cells join forces, Soft Matter, **7**, 9549-9560, (2011).
[2] Bidan C.M., Kommareddy K.P., Rumpler M., Kollmannsberger P., Bréchet Y., Fratzl P., Dunlop J.W.C., How Linear Tension Converts to Curvature: Geometric Control of Bone Tissue Growth, PLoS ONE, 7(5): e36336, (2012).
[3] Bidan C.M., Kommareddy K.P., Rumpler M., Kollmannsberger P., Fratzl P., Dunlop J.W.C. Geometry as a factor for tissue growth: Towards shape optimization of tissue engineering scaffolds, Adv. Healthcare Mater., **2**, 186-194, (2013).
[4] Gamsjäger, E., Bidan, C. M., Fischer, F.D., Fratzl P., Dunlop, J.W.C., Modelling the role of surface stress on the kinetics of tissue growth in confined geometries, Acta Biomater., DOI: **10.1016**/ j.actbio. 2012.10.020, (2012)
[5] Abraham, Y., Tamburu, C., Klein, E., Dunlop, J. W. C., Fratzl, P., Raviv, U., Elbaum, R., Hygroscopic coiling movement from a single layer is created by a tilted cellulose arrangement in stork's bill awns, J. Roy. Soc. Int., **9** 640-647, (2012).
[6] Zickler, G. A., Ruffoni, D., Dunlop, J. W. C., Elbaum, R., Weinkamer, R., Fratzl, P., Antretter, T., Finite element modeling of the cyclic wetting mechanism in the active part of wheat awns, Biointerphases, **7**, 42, (2012).
[7] Harrington, M. J., Razghandi, K., Ditsch, F., Guiducci, L., Rueggeberg, M., Dunlop J. W. C., Fratzl, P., Neinhuis, C., and Burgert, I., Origami-like unfolding of hydro-actuated ice plant seed capsules, Nature Comm., **2011**, 2, 337, (2011).
[8] Turcaud, S., Guiducci, L., Fratzl, P., Bréchet, Y. J. M., Dunlop, J. W. C., An excursion into the design space of biomimetic architectured biphasic actuators, Int. J Mater Res. **102**, 607-612 (2011).
[9] Stoychev, G., Zakharchenko, S., Turcaud, S., Dunlop, J. W. C., Ionov, L., Shape programmed folding of stimuli-responsive polymer bilayers, ACS Nano, **6**, 3925-2934, (2012).
[10] Stoychev, G., Turcaud, S., Dunlop, J.W.C., Ionov, L., Hierarchical multi-step folding of polymer bilayers, Adv. Func. Mater., DOI: **10.1002**/ adfm. 201203245, (2012).