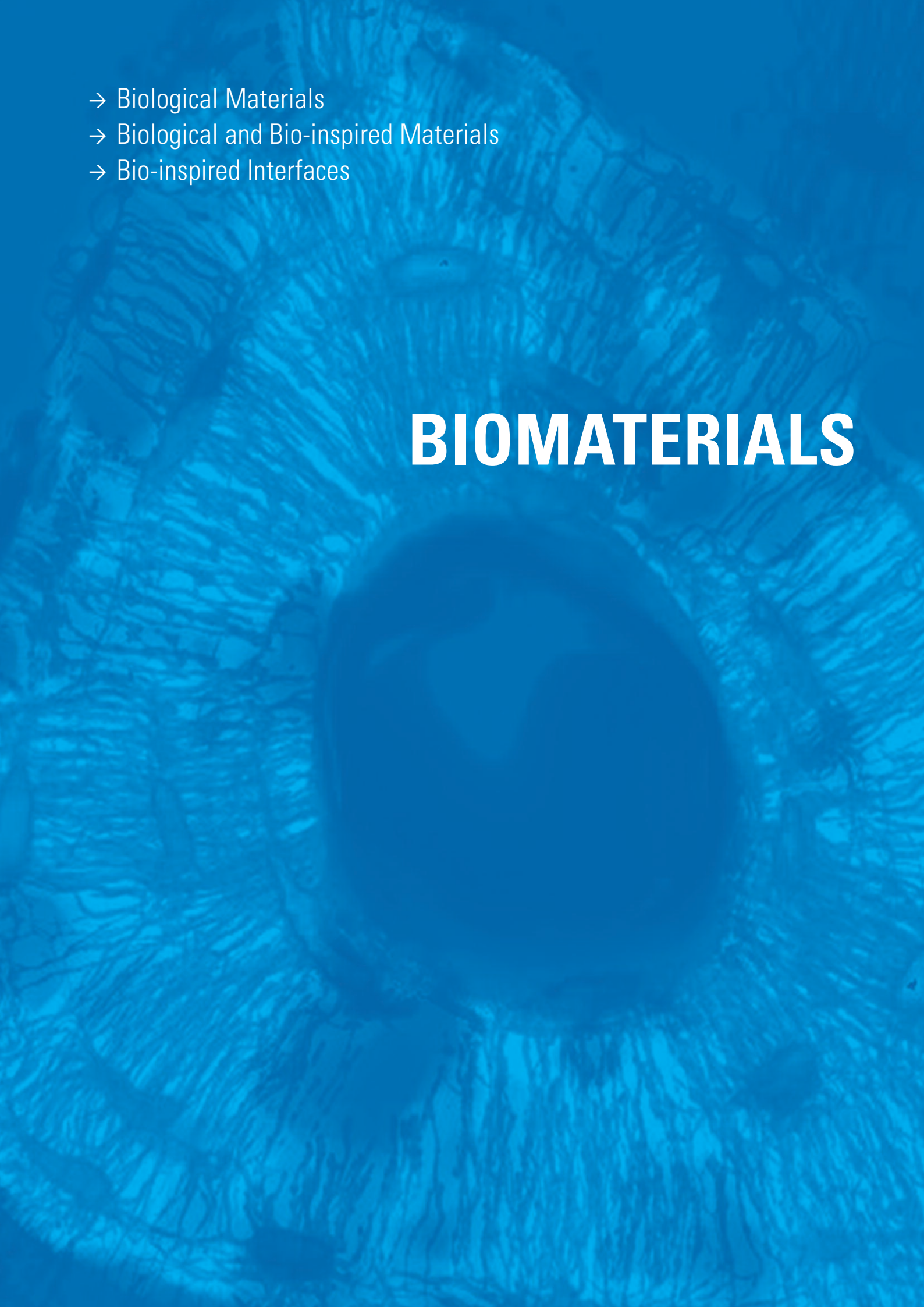

→ Biological Materials
→ Biological and Bio-inspired Materials
→ Bio-inspired Interfaces

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)
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)
Since 1993: Associated member (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,
Fellow of the Materials Research Society, Member of the Austrian Academy of Sciences, the Academy of Science and Literature Mainz, ACATECH as well as the Berlin-Brandenburg Academy of Sciences)

Biological Materials Science is the overarching research area of the Department. As schematically shown in figure 1 below, this research field connects materials science and biology in several interesting ways: First, biological or biomedical questions often require input by methods and approaches borrowed from physics, chemistry or materials science (red arrow on the left). One such example with far-reaching medical importance is the Department's research on bone material quality in osteoporosis and other skeletal diseases associated with bone fragility. Second, the diversity of natural organisms presents a unique opportunity to study naturally evolved solutions to typical materials engineering problems encountered by organisms. Examples are materials combining stiffness and fracture resistance or providing self-healing or self-actuating capabilities to skeletons, shells, hold-fast systems or protective capsules (green arrow on the right). This type of bioinspired research is an important component in the research by most of the groups in the Department. Third, it is essential to understand how cells interact with materials, both biogenic and artificial. Indeed, materials in contact with cells are often carrying (mechanical or chemical) signals for the cells and/or are being modified by them (blue connector in the center of the sketch). Of particular interest in several groups of the Department is the way in which cells interact mechanically with their environment.

To tackle such questions, 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.

The Department addresses Biological Materials Science through all three angles sketched in **Figure 1**. A number of research groups and staff scientists work independently but also in a collaborative way on these topics. **Figure 2** lists these individual research efforts along two lines (red arrows) starting from the analysis of biological materials with the goal to either provide new concepts for the materials sciences (left) or helping the understanding of biological or biomedical problems (right). The position of the various research groups on these arrows is, of course, only a rough attempt of classifying the diverse activities. Since all research group leaders and independent scientists submit their own report, only a brief summary of the research strategies will be given here, with a little more emphasis on the research work done outside these groups (mostly by the director with external partners).

The groups of *Matt Harrington* (a) and *Michaela Eder* (b) are shown on top of the pyramid in Figure 2. Both work primarily in elucidating structure function relations in biological materials, although Matt recently also started some activity in synthesizing bioinspired polymer-based materials. The emphasis in (a) is on protein-based materials with mechanical

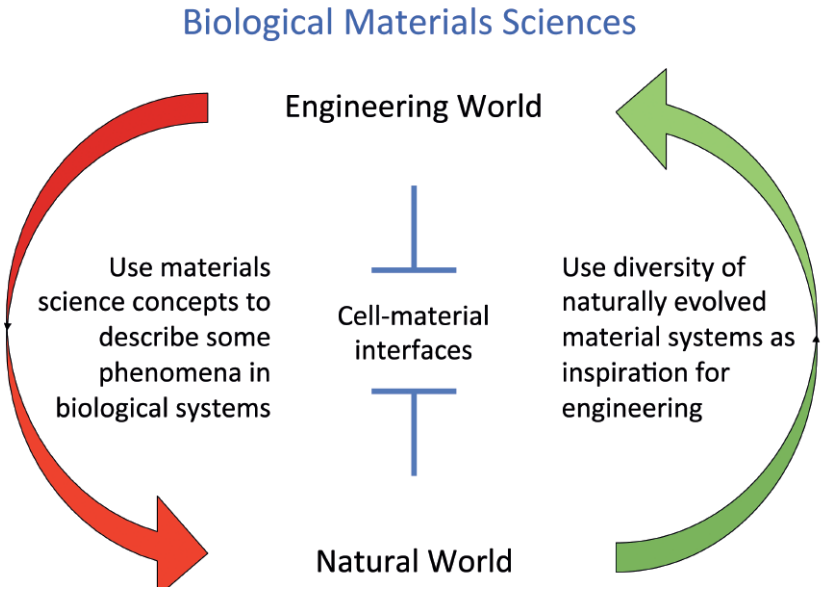

Figure 1

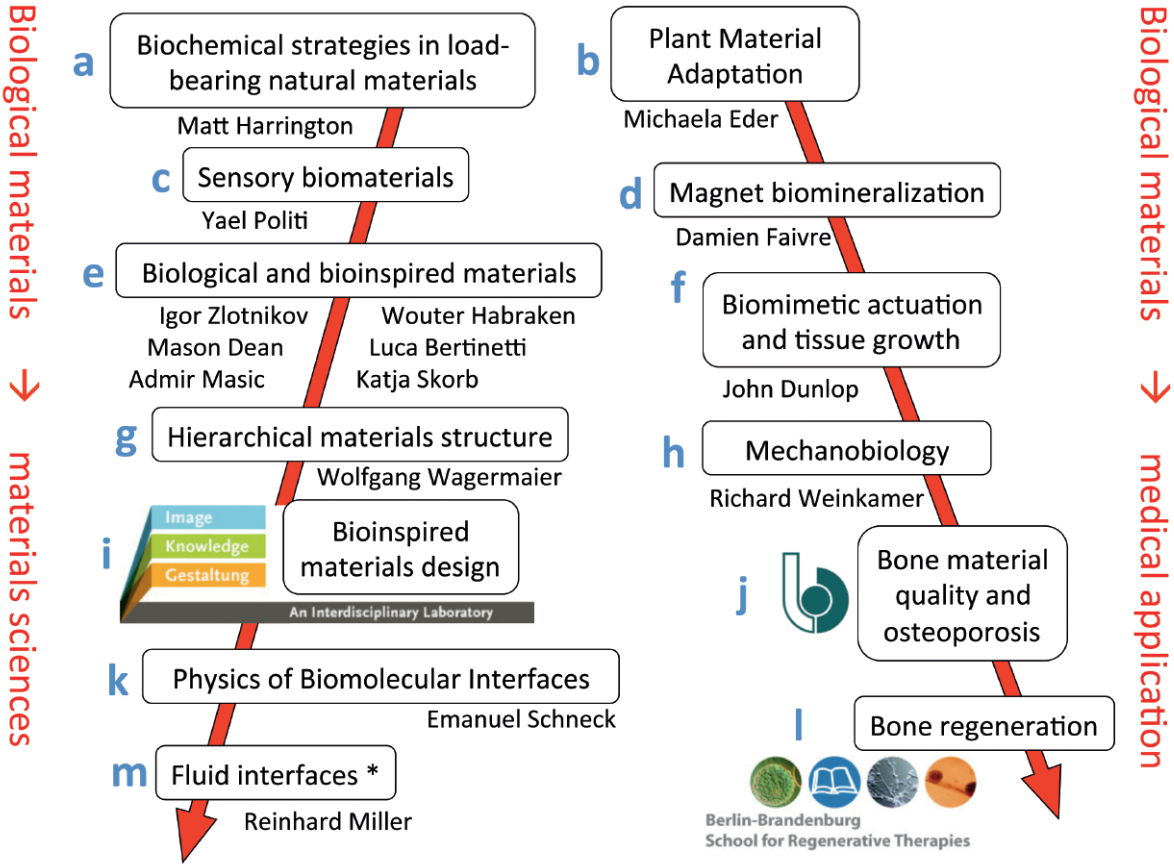

Figure 2: Research group structure, Department of Biomaterials.

function, such as the byssus threads of mussels who show an interesting self-healing behavior based on metal coordination bonds. Michaela's work (b) currently focusses on seed capsule materials. In particular, the follicles of Banksia need a bushfire and subsequent humid weather to release their seeds by a opening mechanism that does not require an active metabolism. Current research is elucidating the intricate material structure and composition that allows such behavior (see their reports).

Sensory biomaterials, especially located in the spider cuticle are at the center of *Yael Politi's* (c) research. Spiders cuticle is a composite material based on chitin and possesses ultrasensitive vibration sensors as well as venom fangs (effectively "injection needles") with very unusual engineering properties. This work both contributes to the better understanding of how arthropods may have evolved these capabilities, but also shows examples of high-performance materials that are interesting from the viewpoint of bioinspired engineering (see her report and **[1]**).

Damien Faivre (d) is heading a research group entirely supported by an ERC-grant to him. The research topic gravitates around magnetic nanoparticles (mostly magnetite), their synthesis in bacteria and in vitro, as well as applications from nanorobotics to medical imaging (see his report).

Several independent researchers work on different problems related to biological or bioinspired materials (e), as described in their individual reports. *Luca Bertinetti* studies the interaction of water with cellulose and collagen; *Admir Masic* develops advanced in situ and in vivo spectroscopic imaging of biological tissues; *Igor Zlotnikov* focusses on structural and nanomechanical characterization of mineralized biomaterials; *Mason Dean* addresses evolutionary perspectives on vertebrate hard tissues; *Wouter Habraken* coordinates a 5-year collaborative project on the physical chemistry of amorphous minerals in living organisms (supported by the DIP-Program of the German Science Foundation), together with partners at the Weizmann Institute (Lia Addadi and colleagues); see his report and **[2-4]**; finally *Katja Skorb* started a program on generating smart systems by surface nanostructuring for bio-applications.

John Dunlop (f) is interested in the autonomous dynamical reconfiguration of materials systems. One line of research is to elucidate how growing tissue is able to sense and react to the curvature of the substrate in its growth behavior. He also studies self-actuating systems based on swelling honeycomb-like structures (see his report). This research may have important repercussions on (soft) robotics and on tissue engineering.

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The mechanobiology group (g) of *Richard Weinkamer* investigates the basis for the capability of bone to adapt to mechanical loads. A network of cells (osteocystes) buried inside the mineralized tissue is thought to control the mechanosensitivity of bone. Current research studies these networks both experimentally and by numerical modeling.

Emanuel Schneck (k) just started an Emmy-Noether group (supported by DFG) on the physics of biomolecular interfaces. The research addresses interaction between membranes and with biomolecules, making use of light and neutron reflectivity studies as well as numerical modeling (see his report).

Reinhard Miller (m), previously member of the Interface Department, moved into the Biomaterials Department after the retirement of Helmuth Möhwald. His research focusses on solution-air interfaces (see his report).

Three further topics are mentioned in Figure 2. First, there is a long-standing collaboration with the Ludwig Boltzmann Institute of Osteology in Vienna, Austria on clinically oriented research on bone diseases (j), such as osteoporosis and osteogenesis imperfecta (brittle bone disease). *Richard Weinkamer* and *Wolfgang Wagermaier* are both involved in this collaboration (see their reports). In addition, methodologies based on Raman imaging **[5,6]** and on acoustic microscopy **[7]** are being established for use in clinical studies. Mineral densities have been studied in a large pre-osteoporotic and osteoporotic patient cohort **[8,9]**. Finally, the behavior of osteoclasts was characterized in in-vitro studies **[10]**.

The same two groups (as well as *John Dunlop*) are also involved in a consortium on bone regeneration (l) with the Berlin Brandenburg School of Regenerative Therapies (supported by the DFG Excellence initiative). The emphasis there is fundamental research on bone healing (see the report by *Wagermaier*), as well as on the interaction of regenerating bone with various types of implants **[11-14]**.

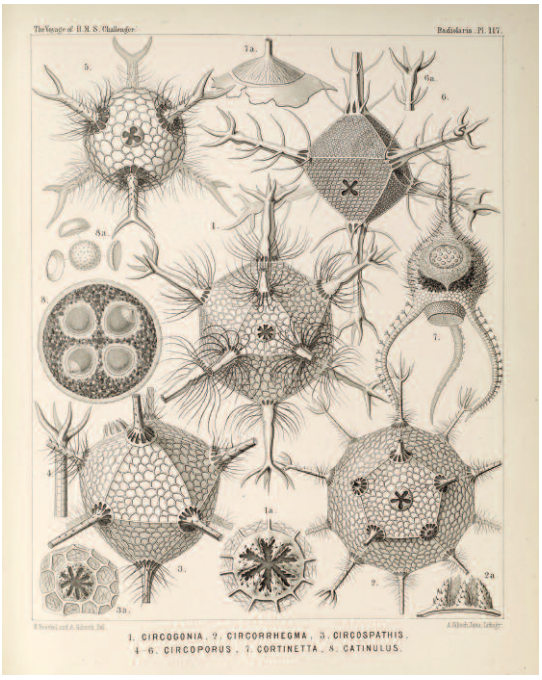

Figure 3: image from the report of the challenger expedition (Radiolaria)

Several researchers of the department of Biomaterials are involved in the Excellence Cluster "Image-Knowledge-Gestaltung" at the Humboldt University Berlin (i). Peter Fratzl is one of the PIs who participated in the definition of the cluster. The goal is highly interdisciplinary work between humanities, natural sciences, and also design and engineering, see *https://www.interdisciplinary-laboratory.hu-berlin.de/en/laboratory*. The Department is involved in several base projects including research on the significance of models in science and humanities, or on historical structures. The latter focusses on establishing a searchable data base collecting descriptions of organisms in historical texts, such as the reports on the challenger expedition 1872-76 **(Fig. 3)** in view of a potential use in bioinspired engineering. The strategy is to generate an ontology connecting engineering problems with natural solutions, as described in modern or historical biological literature.

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. A particular challenge is related to the big amount of data generated in such experiments, which led us to head an effort in developing software for the online analysis of large x-ray scattering datasets **[15].**

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*).

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 **[16]**. *Hartmut Metzger* who arrived in the beginning of 2010 from the European Synchrotron Radiation Facilities (ESRF) brought many years of experience in x-ray diffraction, in particular with grazing incidence and using coherent beams, to our Department and, before retiring in 2013 he was involved in a number of projects utilizing synchrotron radiation such as the study of biological materials. *Emil Zolotoyabko,* professor of materials science at the Technion (Israel Institute of Technology) regularly spends several sabbatical months per year in the Department. He is involved in a wide range of projects by different research groups. *Yves Bréchet,* currently High Commissioner of nuclear and alternative energies for the French government received a Gay Lussac-Humboldt Award and is visiting our Department regularly since 2012. *Scott White,* professor at the University of Illinois at Urbana-Champaign received the Humboldt Research Award and was visiting the Department in 2013. His research is focused on developing self-healing and self-remodeling engineering materials. *Claudia Fischbach-Teschl,* professor at Cornell University spent half a year of her sabbatical in Golm during 2014, supported by the Humboldt Foundation. She is interested in the development of bone metastases from breast cancer and brought this new topic to the department of biomaterials, which lead to currently ongoing collaborative work. 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

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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

We are interested in understanding functionality of plant materials in the context of their environment. Plant material can be defined as any material forming the plant body. The plant body is typically composed of different tissues, formed by cells. The cells - various shapes are possible – are encased by a polymeric cell wall made of pectins, hemicelluloses, lignin and cellulose, the most abundant polymer on earth. The arrangement of the long and stiff cellulose molecules which form so-called fibrils directly influences cell wall mechanics. Furthermore the interactions with the other cell wall substances, including water, play an important role for material performance.

Currently our research activities are focused mainly on two different plant materials: Banksia follicles and wood **(Fig. 1)**.

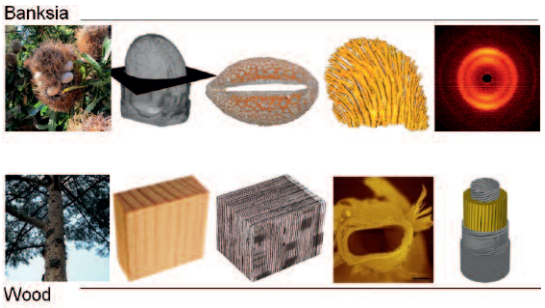

Fig. 1: Banksia follicles, characteristic structures at different length scales, left cm-level, right nm-level. Bottom row shows the structure of softwoods at different length scales.

Banksia follicles are seed capsules that store seeds on the Banksia plants for up to 17 years before releasing them upon an environmental trigger which is in most cases fire. This trait is of particular advantage in environments with nutrient poor soils. The follicles are composed of dead polymeric tissue, and from a material science point of view are of particular interest concerning long-term stability/durability but also functionality when exposed to high temperatures. These properties are highly desirable when thinking about wood, a widely used (construction) material having drawbacks when it comes to fire retardancy, long-term resistance against weathering, microbial and insect attack. In contrast to the almost unknown material properties of Banksia follicles, wood of several tree species is very well studied, however still many open questions related to wood material properties, especially on smaller length scales, eg. at the cell wall level, remain. For these reasons it is a suitable material to establish/apply/verify (new) experimental micromechanical techniques to investigate plant material in detail and at the same time to contribute to a deeper understanding of the material wood.

In many cases micromechanical experiments are the method of choice in answering open questions. During the last years we were able to establish different experimental setups which allow us to test samples with different sizes and shapes as well as fragile and more robust materials **[1].**

In the following both a brief summary about some of our micromechanical testing systems and research projects where we could contribute with our knowledge and techniques are given.

Experimental Micromechanical Testing

During the past years a variety of setups for the micromechanical characterization of biological, bio-inspired and other materials has been developed. **Fig. 2** shows schematically some available systems for tensile tests which allow us to test samples with various shapes and properties ranging from very fragile (eg. primary cell wall systems) to robust (eg. woody tissues). We are able to control temperature and humidity in the sample vicinity to account for the fact that mechanical properties of biological materials are typically humidity dependent. To allow monitoring changes during mechanical loading the systems can be combined with other techniques such as light and electron microscopy, Raman spectroscopy or synchrotron radiation. For tissues and samples that cannot be tested in tension, nanoindentation is often an appropriate alternative to characterize mechanical properties **[2]** and even there humidity control is possible now **[3].**

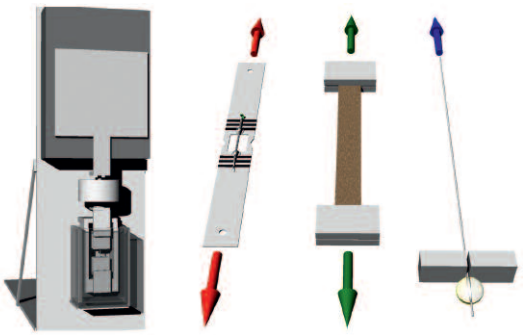

*Fig. 2: Schematic drawing of a tensile tester which allows testing of samples immersed in a liquid in a glass cuvette (left). Foliar frame serving as a support for fragile samples (eg. Arabidopsis hypocotyls or wood cells), red arrows point in loading direction. Green arrows indicate the loading direction of a larger sample clamped between metal plates, alternatively glue can be used for more fragile specimens (eg. hydrogels or membranes **[4]**). Right: glass fibre with a droplet of glue, blue arrow indicates loading direction of fibre, 2 metal plates hold the glue droplet, the fibre can be pulled out of the glue and interfacial shear strength can be determined.*

The Dependence of the Mechanics of Wood Cell Walls on Environmental Conditions

It is well known that the arrangement of cellulose fibrils in wood cell walls significantly affects the mechanical properties of wood, especially the parallel alignment of microfibrils in the predominant S2 layer. By controlling cellulose fibril orientation the tree is able to modulate wood properties in order to react to environmental conditions. A high angle of the fibrils with respect to the longitudinal cell axis (the so-called microfibril angle, black lines in yellow cell wall layer, cartoon Fig. 1, bottom right) results in a more flexible materi-

al, low angles result in stiff material. As long as wood is the material of a living tree, the moisture content is above the so-called fibre saturation point. When wood is used as a material by mankind its moisture content is typically below the fibre saturation point and its properties are then highly dependent on the amount of water in the cell wall. In a recent review **[2]** we collected and summarized literature data on how the mechanical properties of wood cell walls change with microfibril angle and moisture (grey triangles in Fig. 3). To fill some missing data gaps we sampled wood from different locations in a tree (micrographs in Fig. 3) allowing us to investigate single wood fibres with different MFAs. By controlling relative humidity during the tensile test we were able to systematically describe changes in mechanical properties and could show that the influence of moisture on the tensile stiffness becomes larger with higher microfibril angles **[5].** This work is a good example highlighting the mechanical role of the matrix polymers hemicelluloses and lignin in the cell wall, since in cells with higher microfibril angles the matrix substances experience higher stresses.

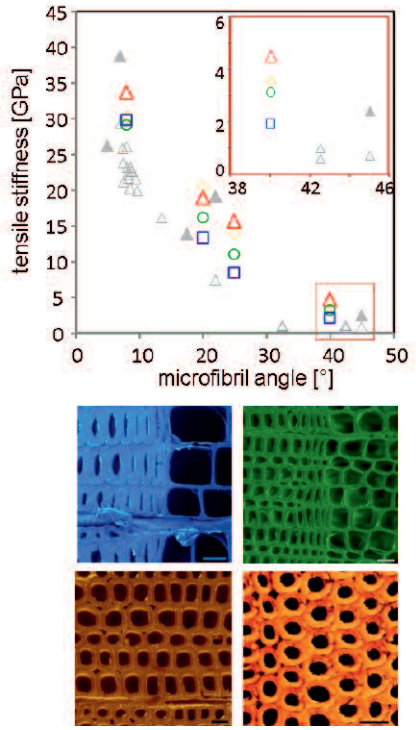

Fig. 3: Diagram showing tensile stiffness of single wood cells plotted against microfibril angle (each data point represents mean values of ~10-20 experiments). Grey symbols show data from literature (details can be found in [2]), white triangles represent data for wet fibres, filled triangles those tested under laboratory conditions (65%rh, 20°C). Coloured symbols represent data from new experiments, red triangles (5%rh), yellow square (50%rh), green circle (75%rh) and blue square (90%rh). SEM micrographs show the selected tissue types: blue adult wood, MFA=8°, green juvenile wood, MFA=20°, brown reaction wood from stem, MFA=25° and orange reaction wood from branch, MFA=40°. Scalebars 20µm.

Unfortunately tensile tests are – so far - only possible for single fibres longer than 0.7 mm. However, nanoindentation is a useful alternative which allows to control environmental conditions **[3].**

Bioinspiration: Interfacial Design of Glass Fibre-Reinforced Composites

Not only cellulose is an important mechanical component of the cell wall. Hemicelluloses are supposed to be the mediators between cellulose and lignin. Even though less stiff and much weaker than cellulose they play a major role in toughening cell walls. Toughness and in addition a fracture behaviour which is comparable to many biological materials are characteristics of particular importance for composite materials such as glass-fibre reinforced polymers. In a project with cooperation partners from the colloids department, the Universities of Bayreuth and Freiburg and the ITV Denkendorf **[6]** surfaces of glass fibres were modified to improve their interactions with the epoxy matrix. The whole process was inspired by the role of hemicelluloses in cell walls and realized by a so-called grafting-from and grafting-onto procedure. It has been shown in pull-out tests (Fig. 2) that the interfacial shear strength (determined in pull-out tests) can be controlled and modified by tailoring the interphase design (eg. grafting density).

Fibre reinforced composites are often used for high-performance applications. Especially for such applications a system which is able to report micro-damages in a reliable way is highly desirable. A group at the University of Basel coated glass fibres with a fluorescent protein-based mechanophore. When protein unfolding occurs upon damage the yellow fluorescence of the protein is lost. This signal can then be used as a damage sensor for microcracks. We were able to show that interfacial shear strength is comparable to native glass fibres and amino glass fibres which indicates that the protein coating does not affect the interfacial shear stress **[7].**

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Biochemical Strategies in Load-Bearing Natural Materials

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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, (Max Planck Institute of Colloids and Interfaces, Potsdam)
Since 2010: Research Group Leader (Max Planck Institute of Colloids and Interfaces, Potsdam)

Biological organisms produce a variety of protein-based polymeric materials under environmentally benign conditions, which achieve an impressive range of industrially desirable properties – e.g., damage tolerance, self-healing, actuation, shape-memory and underwater adhesion. The primary research interest of this research group is to understand the biochemical, biophysical and structural underpinnings of such adaptive material behaviors, with the goal of applying extracted concepts to the design and synthesis of bio-engineered polymers with tailored properties. These aims are achieved through a multi-disciplinary approach consisting of three interdependent strategies: 1. Learn from nature 2. Characterize protein building blocks and 3. Synthesize tailored biopolymers. The self-healing fibers of the mussel byssus provide the primary model system studied in the group **(Fig. 1).**

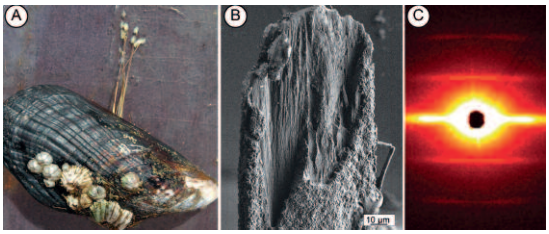

*Fig. 1: In situ structural analysis of mussel byssal threads. A) Mussels attach to substrates with anchoring fibers known as byssal threads. B) Cryo-SEM image of byssal thread revealing a fibrous core surrounded by a thin protective layer. C) Small-angle X-ray pattern of the thread core indicates a highly organized semi-crystalline protein framework **[1].***

Learn from Nature

Learning from nature requires the in-depth characterization of structure-function relationships of protein-based biological materials. Byssal threads are stiff, tough, extensible and self-healing protein-based fibers produced by marine mussels that provide a secure attachment in seashore habitats. X-ray diffraction studies **(Fig. 1)** led by Stefanie Krauss (former postdoc) have revealed the importance of a semi-crystalline protein framework in the deformation and healing behavior of the byssus **[1].** In particular, it was discovered that the elastic and reversible deformation of the framework is vital for re-uniting ruptured sacrificial bonds, whose recovery likely leads to self-healing behavior. Current work by Clemens Schmitt (PhD student) using advanced spectroscopic methods in collaboration with Yael Politi (Dept. of Biomaterials) indicates that the sacrificial bonds likely consist of metal coordination bonds primarily mediated by Histidine residues, whereas further X-ray diffraction work by Antje Reinecke (PhD student) suggests that reversible unfolding of specific folded protein structures contributes to the high extensibility of the threads.

In collaboration with several other groups, we have harnessed numerical and computer-based modeling in an attempt to understand the observed damage-tolerant mechanical behaviors of the byssus as well as similar biopolymers. In collaboration with the group of Markus Hartmann (Montanuniversität Leoben), Monte Carlo simulations of polymer chains with sacrificial bonding sites modeled after mussel byssal proteins provided several important insights into the intricacies of bond rupture and reformation under load, as well as the importance of bond topology in controlling the effective strength of sacrificial bonds **[2, 3].** Additionally, a numerical model developed in collaboration with Peter Fratzl (Dept. of Biomaterials) and Dieter Fischer (Montanuniversität Leoben) was able to describe the pseudoelastic mechanical behavior of whelk egg capsules in terms of a classical phase transformation – leading to important mechanistic insights into the origin of the characteristic mechanical hysteresis of the material **[4].**

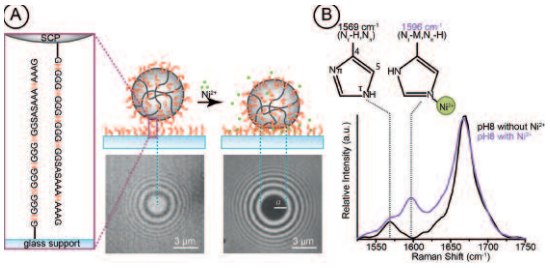

*Fig. 2: Characterization of peptides based on His-rich domains (HRDs) of the preCols. A) Soft colloidal probe spectroscopy revealed a significant interaction between HRD peptide layers in the presence of metal ions, but not in their absence. B) Raman spectroscopic analysis revealed that the interaction is largely mediated through His-metal coordination bonding. Adapted from reference **[5].***

Characterize Protein Building Blocks

The primary building blocks contributing to the tensile mechanical behavior of mussel byssal threads are collagen-like proteins, known as the preCols. As mentioned, histidine amino acid residues concentrated in the terminal domains of preCols are believed to contribute to deformation and healing behavior by forming reversibly breakable metal coordination bonds. To test this hypothesis, Stephan Schmidt (former postdoc) and Antje Reinecke (PhD student), in collaboration with the group of Laura Hartmann (Dept. of Biomolecular Systems), investigated the metal-dependent mechanical behavior of peptide sequences derived from the His-rich domains of the mussel byssal preCols. Soft-colloidal probe force spectroscopy combined with Raman spectroscopy demonstrated the propensity of these peptides to form stable, yet reversibly breakable cross-links mediated by interactions by metal ions and histidine **[5].** Notably, the PEG-based colloidal probes also exhibited increased stiffness in the presence of metal ions indicating the potential for such a strategy in reinforcing polymeric networks reversibly.

Synthesize Tailored Biopolymers

The principles extracted from studying the byssus and characterizing its building blocks – namely, the use of metal coordination interactions as robust and reversible cross-links – were integrated into a recombinant biopolymer through rational design of protein sequence. Specifically, in a project led by Elena Degtyar (Postdoc), metal-binding histidine residues were genetically engineered into the sequence of insect resilin, which was recombinantly expressed, purified and photo-cross-linked into biopolymeric thin films. Mechanical characterization of the thin films with AFM-based indentation indicated a nearly 800-fold increase in stiffness in the presence of Zn^{2+} ions compared with wild-type resilin, which was shown by spectroscopic means to arise at least in part from histidine-mediated metal coordination cross-links **[6].**

Mussel-Inspired Biomimetic Polymers

In continued collaboration with the group of Niels Holten-Andersen (MIT), we investigated mussel-inspired PEG-based hydrogels stabilized by metal coordination cross-links between various metal ions (e.g. Fe, V, Al) and 3,4-dihydroxyphenylalanine (DOPA), a post-translational modification of tyrosine found enriched in many byssus proteins **[7].** Metal- and pH-dependent variations in the affinity of the ions for DOPA led to tunable mechanical properties of the hydrogels, which also display self-healing properties.

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Biological Chitin-Based Tools and Sensors

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After cellulose chitin is the second most abundant natural bio-macromolecule as it forms the main building block of all arthropod cuticles – the richest phyla in nature. 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 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 α-chitin crystallites tightly coated by a protein shell form the fibrous phase and the matrix is composed of a wide range of proteins **[2].** The main goals of our group are to obtain basic understanding of the cuticular material and to gain insight into the structure-properties-function relations in specific organs such as cuticular tools (e.g. fangs, claws) and sensors. 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. Emil Zolotoyabco from the Technion Institute of technology (Haifa, Israel).

The current members of the group are Ms. Ana Licuco, Dr. Hanna Leemreize, Ms. Inga Hettrich, Dr. Marie Albéric, Dr. Osnat Younes-Metzler and Ms. Birgit Schonert.

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 background 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 investigated the direct spatial correlation among cuticle morphology, hierarchical structural organization and micromechanical properties in spider metatarsal slit-sensor and the cuticlar pad just in-front of it.

The metatarsal lyriform organ of the Central American wandering spider *Cupiennius salei* is its most sensitive vibration detector. It is able to sense a wide range of vibration stimuli over four orders of magnitude in frequency between at least as low as 0.1 Hz and several kHz. Transmission of the vibrations to the slit organ is controlled by the cuticular pad. While the mechanism of high frequency stimulus transfer (above ca.40 Hz) is well understood and related to the viscoelastic properties of the pad's epicuticle **[3],** it was not yet clear how low frequency stimuli (<40 Hz) are transmitted. We recently **[4]** addressed this question using a variety of experimental techniques, such as, *in-situ* x-ray micro-computer tomography (μCT) for 3D imaging (Fig. 1), x-ray scattering for structural analysis, and atomic force microscopy (AFM) and scanning electron microscopy (SEM) for surface imaging. We showed that large tarsal deflections (necessary for low frequency signal transmission) cause large deformation in the distal highly hydrated part of the pad. Beyond this region, an unusual sclerotized region serves as a supporting frame which resists the deformation and is displaced to push against the slits, with the displacement values considerably scaled down to only few micrometers. Importantly, we have shown how the organization of the chitin fibrils in 3D contributes to the mechanical properties and the performance of the pad under biologically relevant loads **[4].**

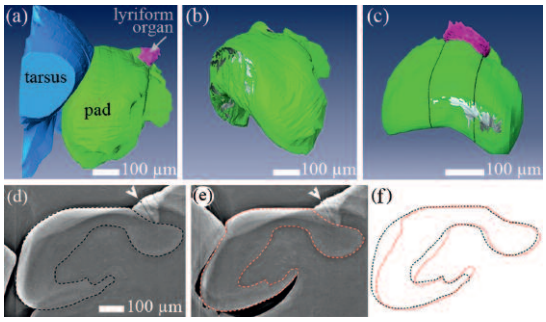

Fig. 1: (a) Surface rendering of the reconstructed µCT data of the pad and tarsus during in-situ compression study. tarsus (blue), pad (green), slit-sensilla (pink). (b) 3D shape of the cuticular pad during compression extracted from (a). Grey regions at the distal side of the pad indicate the contact area with the tarsus. (c) 3D shape of the cuticular pad under load with a slight lateral component. Grey regions at the distal side of the pad indicate the contact area with the tarsus. (d-f) µCT virtual slices of the sample in a-b sectioned in the sagittal plane in the center of the pad in relaxed (d) state (< 0°), and deflected by 9° (e). The dashed lines indicate the outline of the cuticular material of the pad. The white arrows indicate one slit of the metatarsal lyriform organ. (f) An overlay of the pad shape from (d) and (e).

Further research is focused now on the structure-properties of the slits organ it-self. In fact the exact mechanism of how the mechanical signal is transferred to the slits and from the slits to the nerve cells is still poorly understood. A better understanding of the slits structure, mechanical performance and how they behave under biological relevant loads is a key for deciphering their functional mechanism. We analyse slit compression during load from similar in-situ µCT measurements and describe the 3D fiber orientation along the slits walls using nano-focused x-ray beam **(Fig. 2).** Unravelling the structural arrangement in such specialized structures may provide conceptual ideas for the design of new materials capable of controlling a technical sensor's specificity and selectivity, which is so typical of biological sensors.

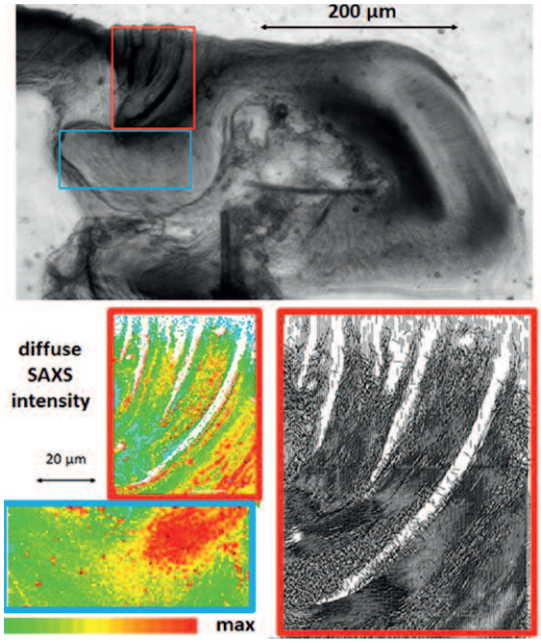

Fig. 2: left: light microscope image of a sagittal section (10 µm thickness) of the distal end of the metatarsus showing the slits and the pad. Red and Blue frames mark the measured regions. Middle: the scattering signal intensity from (110) peak reflection of chitin crystal and diffuse SAXS signal intensity at lowest measured scattering angles. Right: vector graphics showing the fiber orientation as extracted form azimuthal integration of the scattering data.

Multi-Scale Structural Gradients Analysis of the Spider Fang

The spider fang is a natural injection needle, hierarchically built from a complex composite material comprising multi-scale architectural gradients **[5].** Considering its biomechanical function, the spider fang has to sustain significant mechanical loads. We analyzed **[6]** the macroscopic fang stiffness and damage resilience in view of its multi-scale architectural motifs using mechanical modeling based on experimental observations from previous work **[5].** We first studied the macroscopic architecture of the fang and then proceeded to the material level. We applied experiment-based structural modelling of the fang, followed by analytical mechanical description and Finite-Element simulations and showed that the naturally evolved fang architecture results in highly adapted effective structural stiffness and damage resilience.

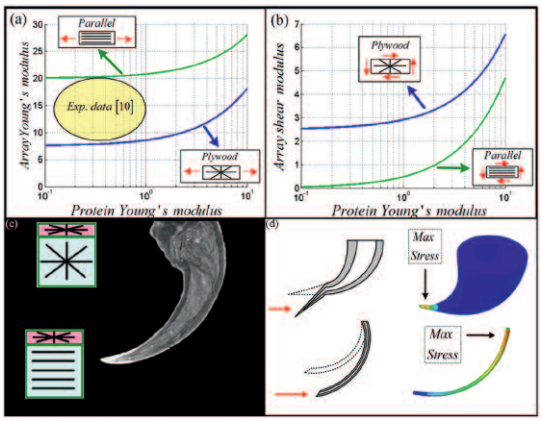

Fig. 3: Effect of the proteins' Young's modulus on the composite moduli. (a) Young's modulus and (b) shear modulus of parallel-fibred (green) and rotated-plywood (blue) fibril arrays, made from chitin fibrils (modulus ~100 GPa, volume fraction 0.2) and a protein matrix (range of moduli). The circle in (a) indicates the typical range of the experimental values (afM).(c) distribution of fiber architecture along the fang: the external layer is always composed of rotated plywood (pink), while the internal layer is composed mostly of parallel fiber at the tip of the fang and rotated plywood at its base. (d) The effect of the fang macroscopic shape on the Von-Mises stress (kPa) distributions in the fang model.

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Evolutionary Perspectives on Vertebrate Hard Tissues

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Natural selection can act on multiple size scales in the evolution of skeletons, altering material, structure, and/or gross anatomy to affect how tissues respond to the demands of their environment. Yet, there is often a disconnect between our understandings of finescale material performance (e.g. how structure and composition relate to tissue mechanics), and the larger scale relationships between species-level anatomical variation and ecology (e.g. how skeletal structure relates to diet and behavior). The bridging of these hierarchical size scales and disciplines represents a grand challenge for biomaterials science, one we tackle through study of 'lower vertebrate' skeletal systems (particularly in fishes), using a rich network of interdisciplinary approaches that incorporate material science perspectives, as well as species' ecologies and evolutionary relationships.

How can Cartilage Perform like Bone?

Fish skeletal tissues are extremely diverse, with many species possessing cartilage and bone (similar to our skeletons), but also tissues that represent hybrids between these. These allow us to better understand form-function relationships in skeletal tissues in general, as well as the evolutionary pressures that shaped animal anatomy. In particular, we investigate the materials, structure and mechanics of mineralized shark cartilage, supported by a Human Frontier Science Program Young Investigator's grant between our department, the Wyss Institute for Biologically Inspired Engineering (Harvard University), and the Zuse Institute Berlin (ZIB). This interdisciplinary collaboration, supporting a variety of students and post docs, brings together high-resolution material property and ultrastructure data (MPIKG) with quantitative analyses of skeletal form (ZIB) to build bio-realistic and idealized 3d-printed models (Wyss) for hypothesis testing and comparison with mechanical testing data of native tissues **[1-2].** By combining these structure-function studies with investigations into muscular anatomy, skeletal development and patterning **[e.g. 3],** we are learning fundamental design rules for this unique tissue, and layered, low-density composite materials in general.

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

Mammalian bone, unlike cartilage, is capable of repairing the microdamage it accumulates from daily use. The longevity of our skeletons depends on this 'remodelling', which has long been thought to rely on osteocytes (cells in bone) to sense when and where to repair. This paradigm is called into question by the skeletons of most bony fishes, which lack osteocytes (and therefore should be irresponsive to load and damage), and yet exhibit mechanical performance similar to mammalian bone **[4].** We examine fish bone ultrastructure and mechanical properties in broad contexts, linking bone structure and mechanics to ecology, and comparing with bone from other taxa **[4-6] (Fig. 1)** to ask whether "bone" exhibits only a limited range of properties or is functionally and structurally diverse. Our results indicate a range of mechanical properties across vertebrate bone types and evidence for remodelling even in 'anosteocytic' fish bone, suggesting that there are unexplored principle regulators in bone mechanobiology beyond osteocytes, and that fishes represent ideal systems for bringing these to light.

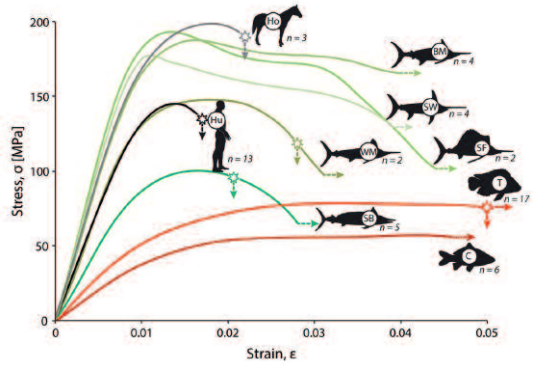

Fig. 1: Bending tests for bone beams from two mammals and seven fish species of different lineages, ecologies and tissue types. Stiffness is calculated from the initial, linear slope of the curve - whereas only some fish bone is as stiff as mammal bone (i.e. has a similar initial slope), all fish bone reaches considerably larger post-yield strains (i.e. deforms considerably before breaking). ***[6]***

The mineralized skeletal tissues of fishes (e.g. bone, mineralized cartilage) are largely similar in composition to mammals' – a mix of water, apatitic mineral and collagen – yet our data show that they exhibit distinct structure and mechanics. Our studies help to clarify the origins, constraints and distribution of tissue types among taxa, also allowing deep understanding of form-function interactions in biological and manmade structural materials.

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Advanced *in situ* and *in vivo* Spectroscopic Imaging of Biological Tissues

Research at the interface between biology and materials science is leading to new discoveries that draw on the unique methodologies from each of these disciplines with potential applications in fields as diverse as bio-medicine, mechanical engineering, and energy conversion and storage. Complex biological materials, such as bone, silk or collagen fibers, often exhibit outstanding mechanical properties, a feature that can be directly related to their functional adaptations and interactions at multiple hierarchical length scales. Our research is focused on development of novel high performance *in situ* and *in vivo* characterization techniques that are able to overcome current research bottlenecks in the investigation of living tissues and complex hierarchically organized biological materials. These objectives are realized by implementing innovative techniques such as *in situ* multi-scale, simultaneous X-rays-Raman scattering (integrated at the µ-spot beamline at Helmholtz-Zentrum Berlin synchrotron facility), or *in vivo* simultaneous fluorescence-Raman chemical imaging platform (developed in collaboration with Mathieu Bennet and Damian Faivre (MPIKG)). The latter setup, for example, allowed for the unprecedented *in vivo* chemical characterization of the earliest stages of bone formation in genetically modified zebrafish larvae (in collaboration with Anat Akiva, Weizmann Institute, Israel, **(Fig.1)**), **[1, 2].** In collaboration with A. Skirtach and H. Möhwald (MPIKG) we also developed a Surface Enhanced Raman Spectroscopy (SERS) platform, based on silica probes coated with single wall carbon nanotubes and gold nanoparticle aggregates, for sensing biomolecules inside living cells **[3].**

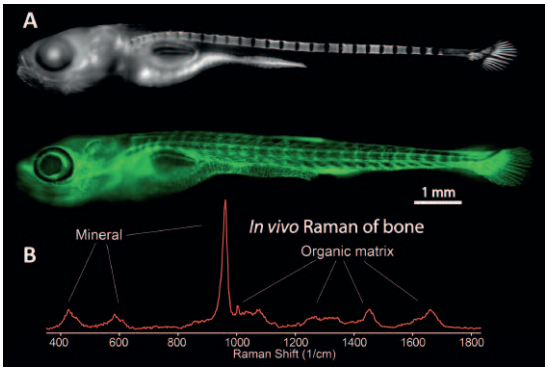

Fig. 1: Simultaneous fluorescence-Raman imaging of the early stages of bone formation in zebrafish larvae. (A) In vivo fluorescence image of the transgenic tg(fli1:EGFP)y1 zebrafish skeletal (top) and circulatory (bottom) systems. (B) In vivo Raman spectrum of newly formed bone tissue.

One of the goals of our work is to elucidate precise structure-property relationships in collagen – a protein that is main component of tendons, bones, skin and other structural tissues in the body. In this context we developed methodologies to assess collagen 3D orientation in tissues **[4, 5]** (in collaboration with Kay Raum, Charité Hospital Berlin), water associated changes of the molecular and nanoscopic structural features in tendons **[6, 7]** (in collaboration with Markus Buehler, MIT, USA), as well as processes connected with the deterioration of the Dead Sea Scrolls **[8]** (in collaboration with Ira Rabin, BAM, Berlin). Recently, for example, we discovered the mechanisms of hydration-driven force generation in tendon collagen, revealing an unexpected and still unexplored active function of collagen fibrils **[6].**

The ultimate aim of our work is to collect complementary information regarding structural complexity and chemical composition in biological and biomimetic materials **[9-14].** One such example is collaboration with James Weaver (Harvard University, USA), where, using sea urchin *(Strongylocentrotus franciscanus)* as a research model, we demonstrate a new set of high throughput, multi-spectral methods for the large scale characterization of mineralized biological materials **(Fig. 2)**, **[15].**

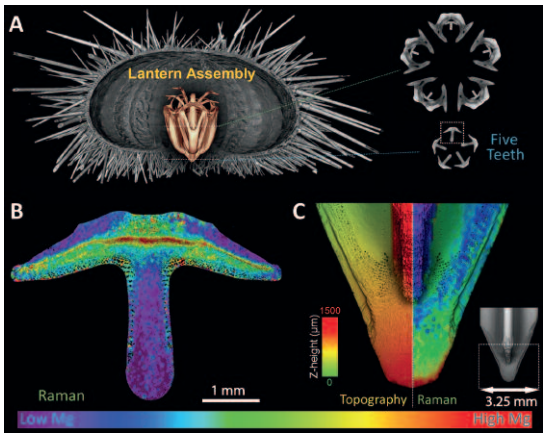

Fig. 2: Large area chemical imaging of magnesium content in the sea urchin tooth. (A) micro-CT reconstruction of an entire sea urchin with the highlighted feeding apparatus and the support ossicles for the 5 radially organized T-shaped teeth. Large area (B) and True Surface® (C) Raman chemical imaging of magnesium content in this multiphasic calcite composite. For details see ref. [15].

Using these approaches, in conjunction with whole animal micro-computed tomography studies, we have been able to spatially resolve micron and sub-micron structural features across macroscopic length scales on entire urchin tooth and correlate these complex morphological features with local variability in elemental composition.

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Mechanobiology

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In mechanobiology the structural changes of biological materials **[1]** as response to mechanical stimulation are studied. An important example is living bone with its many mechano-regulated processes. The mechanical performance of bone is the result of an intricate interaction between the cellular component of bone and the extracellular matrix. One cell type – the osteocyte – is embedded in the mineralized bone material. Osteocytes make use of a network of small channels – so-called canaliculi – to connect with each other. The network of osteocytes is thought to sense the mechanical stimulation thereby controlling bone remodeling, a process which results in a continuous renewal of the bone material and allows for a structural adaptation of bone **[2].** Evidence is accumulating that osteocytes also contribute to mineral homeostasis by dissolving mineral in their vicinity **[3].**

The aim of the research group is to describe quantitatively how mechanical stimulation influences the process of bone remodeling, healing and mineralization. Since the mechanical stimulation depends on the mechanical properties of the bone itself, measurements of the bone quality are performed. The aim is pursued by an interplay between experimental characterization techniques and computational simulations, where quantification of experimental images often serves as a link.

Mechanobiology of Bone Remodeling

With the perspective to improve our understanding of the function of the osteocyte network, in a first step its topology was characterized. The osteocyte lacuno-canalicular network (OLCN) is imaged by staining with rhodamine, which enters into the porous network, followed by confocal laser scanning microscopy **[3].** The 3-dimensional image data of the OLCN was skeletonized rendering the network topology **(Fig.1).** The investigations focused on human osteons, the cylindrical building blocks of cortical bone formed during remodeling **(Fig. 2).** The density of the network in osteons was determined to be 0.071 ± 0.013 $\mu m/\mu m^3$ **[4].** Within osteons the network density showed large variations, with extensive regions without network at all **(Fig. 2).** Most of the network is oriented radially towards the center of the osteon. More quantitatively, 64±1% of the canalicular length has an angle smaller than 30° to the direction towards the osteon center, while the lateral network - defined by an orientation angle larger than 60° - comprises 16±1%. The orientation of these lateral canaliculi twists when moving along the direction of bone deposition towards the center of the osteon **[4].** The lateral network can, therefore, be described by a twisted plywood model being coaligned with the orientation of the collagen matrix. The results of our investigation agree with the hypothesis that early osteocytes are involved in the alignment of the collagen matrix during bone formation. The regions without network raise the question, whether parts of the network get lost with time thereby reducing the mechano-sensitivity of bone.

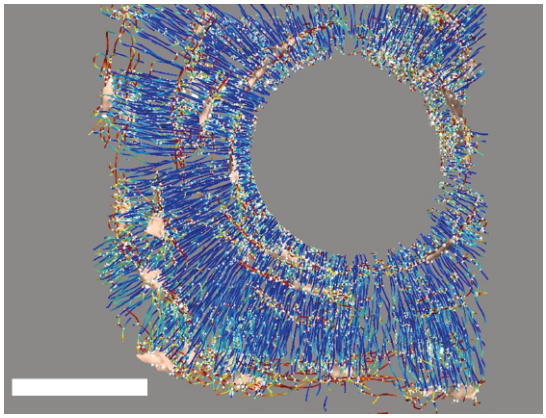

Fig. 1: Network of canaliculi in a human osteon obtained by confocal microscopy and advanced 3-dimensional image analysis. The color of canaliculi denotes their orientation (blue towards the center, red tangential to it). Pinkish objects mark the lacunae of the osteocytes and white balls locations where canaliculi meet; scale bar 50 µm.

Recent advances in experimental methodology allow monitoring bone remodeling in living animals. Using *in vivo* microcomputed tomography the amount and specific site of remodeled bone can be determined **[5].** In animal experiments performed at the Julius Wolff Institute, Charité, (B. Willie, G. Duda) a controlled mechanical stimulation is applied to one leg of the mice, while the other serves as control. Evaluation of experimental data showed that mechanical stimulation acted stronger on enhancing bone formation than suppressing resorption. Comparison of animals of different age demonstrated that only the amount of bone forming surface could be increased by mechanical stimulation in old animals **[6].** The spatial correlation between the local probabilities for bone formation/resorption and the local mechanical strains calculated using the Finite Element method **[7]** provided quantitative information of how the mechanical control of remodeling changes with age. A study performed with ETH Zürich indicated that mechanical stimulation can also speed up the mineralization process **[8],** i.e. the incorporation of mineral in the collagen matrix after bone formation.

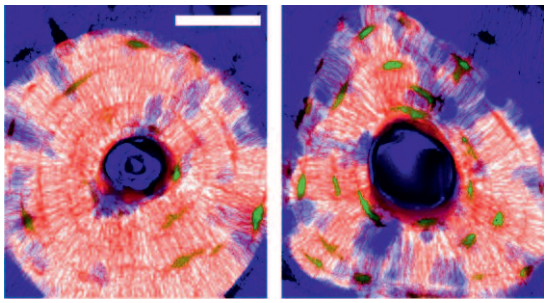

Fig. 2: The network of canaliculi in two different osteons. Osteocyte lacunae are shown in green. Areas without an accessible network are marked in blue; scale bar 50 µm. [4]

In a recent simulation study we questioned the common belief that mechanics helps to conserve the integrity of the network formed by trabecular bone **(Fig. 3).** The line of argumentation is that an "accidental" thinning of a trabecula due to a resorption event would result in a local increase of load, thereby activating bone deposition. Simulating a dynamic network structure undergoing remodeling, we could demonstrate that - in contrast to the argumentation above – mechano-regulated remodeling within a network-like architecture leads to local concentrations of thin trabeculae **[9].**

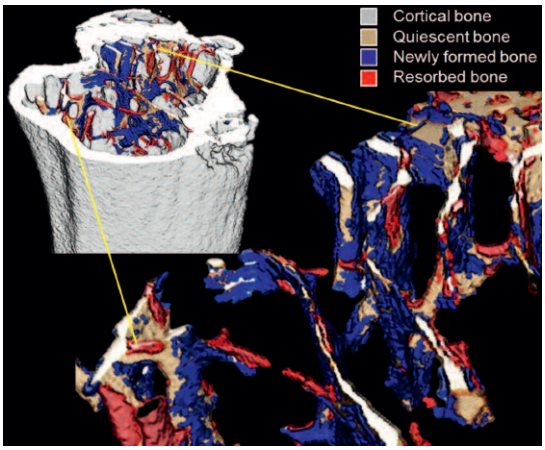

Fig. 3: Trabecular bone in the upper part of a mouse tibia, which was mechanically stimulated with a loading device. The alignment of two micro-CT scans taken at a time interval of 2 weeks allows the identification of regions on the surface of the bone, where bone was deposited (blue) or resorbed (red). ***[5]***

Bone Material Quality

In a project performed together with LION corporation, Japan, the influence of type 2 diabetes on bone structure and properties have been studied. In two different mouse models of diabetes and healthy control mice, the investigations focused on the femur and the jaw bone since diabetes favors oral diseases. The quantitative analysis of the bone porosity showed that while in control mice the sizes of the osteocyte lacunae became smaller when comparing young to older animals, such a reduction in microporosity was not observed in the diabetic mice. This increased microporosity has to be considered as a contributing factor towards the reduced bone material quality with diabetes. The characterization of the size and alignment of mineral particles in the jaw bone of the mice were performed together with Wolfgang Wagermaier using synchrotron small-angle X-ray scattering (SAXS) **(Fig. 4).** On both, the buccal and lingual side, the particle thickness and length were decreasing in alveolar bone towards the tooth. In the animals with diabetes a trend towards smaller particle thicknesses was observed. Interesting was the detected structural asymmetry between the buccal and lingual side with often more than one preferred direction of the mineral particle orientation on the lingual side. This nanoarchitectural asymmetry of alveolar bone can be interpreted as the result of an asymmetric loading during mastication.

An efficient way to functionally characterize biological materials on the micrometer scale is by scanning acoustic microscopy (SAM). With this non-destructive method the spatial variation of bone stiffness in human osteons was estimated taking into account the full opening of the acoustic lens of the microscope. The additional information of the mass density allowed to separate the variation of the stiffness due to differences in mineral content from variations due to orientation effects of the fibrous collagen matrix **[10].**

Bone Regeneration and Healing

The formation of different tissues in the callus during secondary bone healing is at least partly influenced by mechanical stimuli. We used computer simulations to test the consequences of different hypotheses of the mechanobiological regulation at the cellular level on the tissue patterns formed during healing. The computational results were compared with an experiment on sheep. Our simulations showed that the amount and location of the cartilage formed at intermediate phases of healing are least robust with respect to the mechanobiological regulation **[11].** Using a generic model of healing it was studied how the two pieces of a broken mechano-responsive material reconnect depending on the response of the material to mechanical stimulation **[12].** These insights are important for the design of self-healing materials. Simulations of bone healing were also discussed in the Excellence Cluster "Image Knowledge Gestaltung" at Humboldt University as an example of how biological complexity compels model simplifications to perform predictive simulations **[13].**

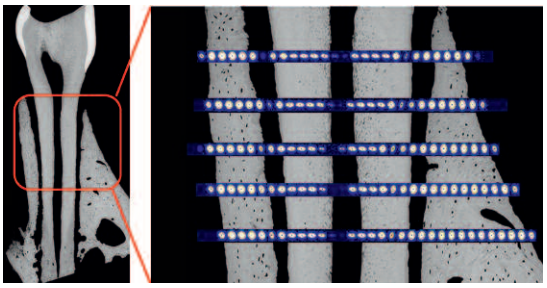

Fig. 4: Left, longitudinal cross-section through the first molar of a mouse showing the tooth anchored in alveolar bone with the buccal (lingual) side left (right). Right, synchrotron small angle X-ray scattering (SAXS) spectra were taken at every 30 µm. The more or less circular shape of the spectra provides information about the preferred orientation of the mineral particles in bone and tooth.

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Biomimetic Actuation and Tissue Growth

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Biological materials have remarkable property combinations arising through the exquisite control of their microstructures at multiple length scales **[1, 2].** Perhaps one of their most interesting features is their ability to change their shape, form and internal structure through the processes of tissue growth, remodelling and swelling. As highlighted in the classic work of D'Arcy Thompson, these shape changes are often mediated by the physical environment of the tissues. The presence of external boundaries for example may constrain a swelling or growing tissue resulting in the development of internal stresses. On one hand such stresses may be sufficient to deform the boundaries, causing macroscopic shape changes, but on the other hand they may act as mechanical signals that are sensed by the cells further modifying their growth behaviour. Such mechanical signalling can act at large distances with respect to the size of a cell, and is thought to be a potential mechanism that allows tissues to organise in complex ways. An understanding of the physics of shape change in biology is thus fundamental to understand the genesis of complex multi-scale architectures in biological materials **[1]** with obvious applications in tissue engineering and medicine, and may also provide inspiration for the development of synthetic actuator systems. In this research group we focus on investigating the role of the geometry of external boundaries on the behaviour of growing and actuating (swelling) tissues, using combined experimental and theoretical approaches.

The Role of Geometry on Actuation

Many examples abound in the plant kingdom of natural actuators that change shape with changing humidity. Actuation arise due to differences in the swellabilities and the geometric arrangement of the constituent tissues. In the seed capsules of the ice-plant, which open to release seeds upon wetting, actuation is directed by the shape of the cells making up the active tissue **[3]** (with I. Burgert, ETH Zurich and L. Bertinetti). The flattened diamond-like cross sections of the cells converts isotropic swelling of the cell lumens to a strongly anisotropic swelling at the macroscopic tissue scale. Using finite element simulations and simple "ball-spring" models we could simulate the swelling behaviour of the diamond honeycomb-like structures found in the natural system **[4].** Another outcome of these simulations is the realisation that by changing pore shape and tiling it is possible to control and modify macroscopic swelling behaviour giving expansion in arbitrary directions. Fig. 1 (top) shows the results of two finite element simulations of the expansion of a honeycomb made of "step-like" pores with two different arrangements: one expands only uniaxially, the other in almost pure shear (with Y. Bréchet, CEA). State of the art rapid prototyping methods allows physical models of these structures to be printed in 3D (**Fig. 1** bottom) allowing for experimental validation of our theoretical approaches (with J. Weaver, Wyss Institute).

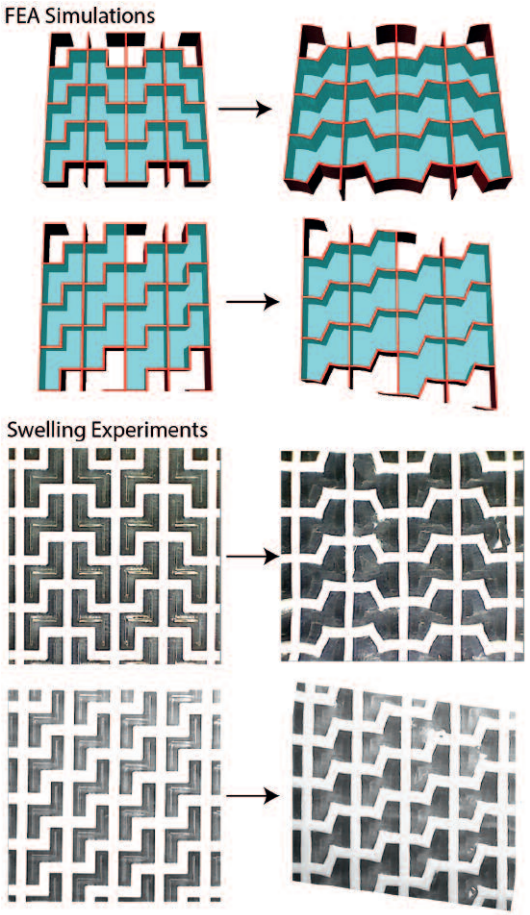

Fig. 1: Finite element simulations (above) and swelling experiments (below) to explore the role of cell shape and arrangement on the actuation of honeycombs

The group also collaborates with materials chemists to help understand the physics of polymer actuator systems. Even simple bilayers can reveal surprising results. The group of L. Ionov (Leibniz Institute of Polymer Research, Dresden) demonstrated that by controlling the time at which swelling occurs in different parts of a bilayer it is possible to fold them into complex 3D structures **[5].** The group of J. Yuan (Colloid Department) have produced poly-ionic liquid membranes with gradients in cross-linking and porosity, which give rise to ultrafast bending responses to the presence of solvents **[6].** By investigating the physics of these well defined synthetic systems we also hope to provide a useful basis in supporting the research of more complex natural actuators such as those studied in the group of M. Eder.

Geometry is also fundamental in controlling the unfolding of thin biological membranes. Together with T Stach (Humboldt Uni., Bild Wissen Gestaltung) we are exploring the 3D shape and function of the "filter house" of the tunicate, *Oikopleura dioica* **(Fig. 2).** This structure consists of a polysaccharide containing membrane produced around the animal's head and inflated by the action of the animals tail. The house's shape controls internal fluid flow, important for inflation, as well as concentration of the food particles for the animal.

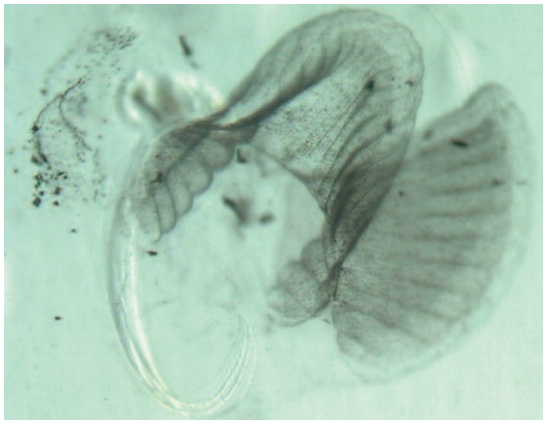

Fig 2: Image of the house of the tunicate O. dioica

The Role of Geometry on Tissue Growth

From previous research done in the group e.g. **[7, 8]** we have demonstrated the importance of substrate curvature on the rate of tissue formation in scaffolds. Within scaffolds with straight sided pores we observe that tissue grows on concave surfaces at a rate proportional to the local curvature. These pores only differ in their convex cross sections and show no significant difference (experimental and theoretical) between the total tissue growth rates. However when we test the model on non-convex cross sections for example cross-shaped pores we can accelerate the rate of tissue formation by a factor of two as confirmed experimentally **[7].** Further extensions to the model now enable us to predict tissue growth in 3D **[9].** We are now focussing on optimising the experimental conditions in order to observe the rate of tissue formation in 3D for arbitrarily curved and re-entrant surfaces. In order to understand the role of mechanics on growth we have also developed, more detailed continuum models for growth together with F. D. Fischer and co-workers at the Uni. Leoben **[10].** These models demonstrate the importance of surface stress on the curvature response of tissue growth, and are now being extended to more realistic 3D geometries.

The majority of our work till now e.g. **[7, 8]** has focussed on observing the tissue produced by a bone-like cell line (MC3T3-E1). We have observed such response to curvature with fibroblasts, and in a collaboration with C. Werner (Max Bergmann Institute Dresden) it has been possible to show that human mesenchymal stem cells, are also able to sense and respond to large scale geometries as a function of differentiation state. Together with K. Skorb, we are also investigating the role of surface nanostructuring on tissue growth in 3D titanium scaffolds, being more realistic materials for load bearing tissue engineering applications.

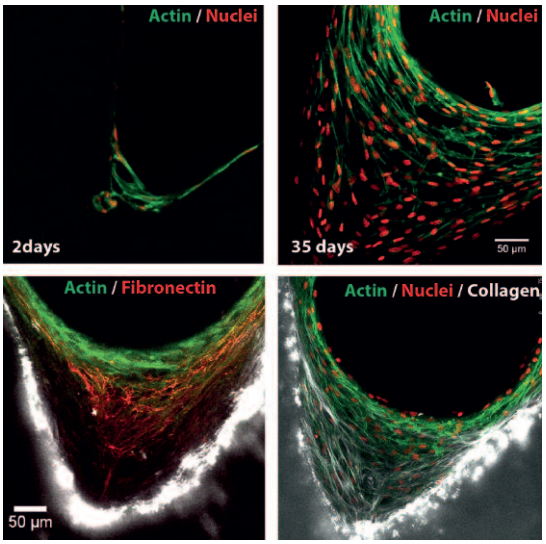

Fig. 3: Confocal microscopy images of tissues formed in the corners of triangular pores showing the role of geometric features on the organisation of tissue as it grows.

In addition to controlling the rate of growth, geometric constraints also influence the microstructure of tissues formed in the pores. This is illustrated in **Fig. 3,** which shows the orientation and distribution of actin stress fibres, nuclei and the extracellular matrix (ECM) proteins fibronectin and collagen within a pore. These experiments indicate that cells align with external geometric features, which in turn has a strong influence on ECM organisation (with A. Petersen (Julius Wolff Institute, Berlin), P. Kollmannsberger (ETH Zurich), and C. Bidan, (UJF Grenoble)).

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Magnetite Mineralization, Organization and Function

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Since 2011: ERC Group Leader
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In the conventional scientific classification, biology deals with the study of Life and living organisms whereas chemistry and physics deals with the constituents of matter and their dynamics. Materials science in turn combines engineering aspects to chemistry and physics and focuses on the structure-function relationship of materials. My group is putting genes on the menu of materials science: we perform interdisciplinary studies of biological materials.

Biological materials, the combination of biological components with inorganic parts such as bone and shells have indeed been used by humans for tens of thousands of years. These materials with remarkable properties are even more outstanding when we realize that they are formed under physiological conditions i.e. at ambient temperatures and pressures, and with commonplace constituents, which is not the case of typical engineered man-made materials. Nature thus not only provides inspiration for designing novel materials but also teaches us how to use soft molecules to structure and assemble simple building blocks into functional entities.

Biological Materials

Magnetotactic bacteria and their chain of magnetosomes represent a striking example of a simple organism that precisely controls the properties of individual building blocks together with their assembly at the nanometer-scale in order to form a functional entity **(Fig. 1), [1].**

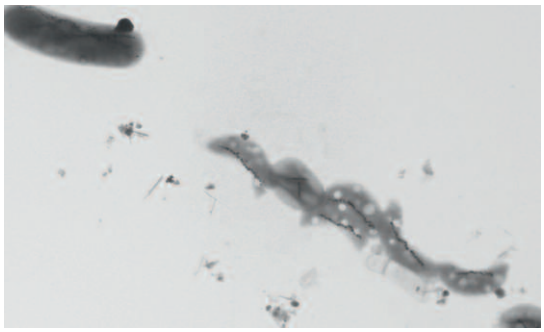

Fig. 1: a typical TEM image from magnetotactic bacteria extracted from a sediment. The magnetosomes are the electron-dense particles that are aligned and form chain(s) in the cells. Three different types of microorganisms are observed here. Image by C. Lefèvre.

Magnetosome's Magnetite Forms from Poorly Organized Minerals

The biomineralization of the mineral magnetite inside the magnetosome organelle is a process that is controlled at the cellular level **[2].** The chemical route by which magnetite is formed intracellularly has been debated. We used X-ray absorption spectroscopy at cryogenic temperatures and transmission electron microscopy to characterize and spatially resolve the mechanism of biomineralization in magnetotactic bacteria **[3].** We showed that magnetite forms through phase transformation from a highly disordered phosphate-rich ferric hydroxide phase, consistent with prokaryotic ferritins, which is found outside the organelle. Then, a transient nanometric ferric (oxyhydr)oxide intermediate is observed within the magnetosome vesicles. This pathway remarkably resembles our results obtained on synthetic magnetite formation.

Magnetosome Chains are Mechanically Stabile

Magnetotactic bacteria do not simply assemble magnetosomes in chain but also control the crystal orientation to form their cellular compass. We performed a texture analysis of aligned bacteria to show that the magnetite particles in the organelles are aligned along their easy axis of magnetization **[4].** This axis is the [111] for isotropic magnetite **(Fig. 2).** However, some strains showed a texture along the [100] axis, which is associated with the hard axis of magnetization. We showed that the magnetosome produced by this strains are elongated in such a way that the easy axis also switch to this direction in this strain.

MamK is in particular a protein from the bacteria that forms a filament to which magnetosomes are attached by the MamJ protein. We showed that MamJ and MamK indeed interact in a host organism in vivo **[5].** In addition, when fixing the cells in a gel and rotating strong magnetic field around them, we revealed that the MamK filament is mechanically stabile and that it is certainly the interaction between MamJ and MamK that is first disrupted **[6].**

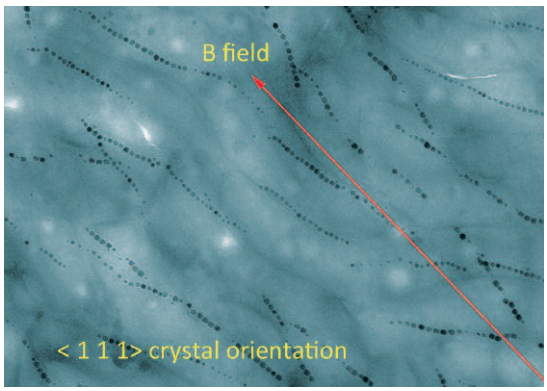

Fig. 2: false colour transmission electron microscopy image of aligned magnetotactic bacteria. The bacteria are aligned on the TEM grid by the application of a strong external magnetic field. For Magnetospirillum gryphiswaldense, the magnetosome crystals are oriented along the <1 1 1> crystallographic direction.

Microorganisms Swim with a Compass

Magnetotactic bacteria perform so-called magnetotaxis. They use the Earth's magnetic field together with chemical sensing to move towards favored habitats. We developed a multi-modal microscopy platform that permitted simultaneous fluorescence and high-speed imaging to map the physiological environment and record the cellular position. Combining this with aerotactic models, we characterized the magneto-aerotaxis of *Magnetospirillum gryphiswaldense* as a function of the magnetic field **[7].** We found that neither a ten-fold increase of the field strength nor a tilt of 45° results in a sig-

nificant change of the aerotaxis. However, when the field is zeroed or when its angle is tilted to 90°, the magneto-aerotaxis efficiency is drastically reduced. Our experimental evidence thus shows that this behavior is more complex than assumed in previous models.

We then studied the behavior of 12 magnetotactic strains when confronted to an inversion of the magnetic field direction **[8].** We report six different behaviors that can be described as a combination of three distinct mechanisms, including the reported (di-)polar, axial, and a previously undescribed mechanism we named unipolar. We implement a model suggesting that the three magneto-aerotactic mechanisms are related to distinct oxygen sensing mechanisms that regulate the direction of cells.

Biomimetic Magnetite

Synthetic Magnetite Forms from Particulate Intermediate

The formation of crystalline materials is typically described by the nucleation and growth theory, where atoms or molecules assemble directly in and from solution. For many systems however, the formation of the stable mineral is preceded by intermediate phase(s), which seem to contradict the classical theory. Magnetite is a ferrimagnetic mineral with multiple applications for which the formation mechanism has remained unclear. We have developed a set-up for the controlled growth of magnetite particle *in vitro* **[9].** We can reach average particle dimension of 50 nm, and thereby control the magnetic properties of the particles. We are able to synthetically reach particle size so far only attainable by biological synthesis.

We then studied the mechanism of such formation by cryogenic transmission electron microscopy **[10].** We found that the nucleation and the growth of magnetite proceed through rapid agglomeration of nanometric primary particles and that no intermediate amorphous bulk precursor phase is involved. We demonstrate that these observations can be described within the framework of classical nucleation theory.

Finally, we studied the role of particular additive on the mechanism of magnetite formation. In particular, we showed that MamP, a protein from the magnetotactic bacteria can control the redox state of the iron and thereby enable the formation of magnetite from the sole Fe(II) under reducing condition **[11].**

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*. While studying the role of several additives, we found that a dedicated polypeptide was enabling the formation of a chain of magnetite nanoparticles **(Fig. 3),** certainly by controlling the particles size and thereby the magnetic interactions between particles **[12].**

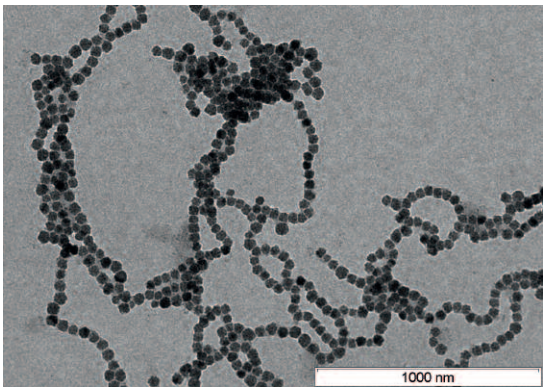

Fig. 3: Typical image of magnetite chain as observed when the synthesis is performed in the presence of polyarginine. Image by V. Reichel.

Random Synthetic Magnetic Swimmers

We finally used magnetic nanoparticles as building blocks to form carbon-coated magnetic aggregates. We show that we can select magnetically steerable nanopropellers from a set of these randomly shaped materials using weak homogeneous rotating magnetic fields **[13].** Despite their arbitrary shape, all nanostructures propel parallel to the vector of rotation of the magnetic field. We use a simple theoretical model to find experimental conditions to select nanopropellers which are predominantly smaller than previously published ones.

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Water Interactions in Complex Biological Materials

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Natural materials are constituted by molecular/supramolecular building blocks, assembled at several hierarchical levels, which in most cases interact molecularly with water. From the point of view of a living organism, in an evolutionary perspective, a choice has to be made about how to tailor a material with respect to this interaction. In nature, a high variety of material's responses to water and changes in moisture content or environmental relative humidity can be observed. My main goal is to describe, from the molecular level upward, the effect of water "crowding" around the molecular components of some selected natural materials and to understand what molecular mechanisms are responsible for the observed responses. This understanding allows to extract biomimetic principles to be applied in several different fields, in particular for energy harvesting and conversion. To describe those interactions at the various hierarchical levels I use a multi-technique approach, developing environmental setups (in collaboration with many groups of the department, as for instance in **[1]**) allowing the control of temperature and water chemical potential (either from the gas phase or with osmotic stress techniques) and following the changes occurring in the materials from the molecular level (vibrational spectroscopies), to higher supramolecular levels (X-Ray scattering, electron microscopy), to the macroscopic size (micromechanical testing) possibly measuring molar free energies and enthalpy changes (through microgravimetric and calorimetric techniques) at the same time. The experimental data are then compared with thermodynamic and mechanical modeling of the considered material.

Energy Conversion in Plants Tissues

Many plants developed organs that, by controlling the organisation of their underlying tissues, can move or generate stresses in complex ways, which are powered by water sorption. Using a force balance approach, one can describe how chemical energy can be used to overcome the work of swelling for fibre reinforced polymeric composites and be used to accomplish mechanical work. This approach allows to establish the full thermodynamics of the actuation for non-living plant tissues **[2].** For example, from mechanical testing experiments we could extract the free energy of water within the wood material **(Fig. 1)** that is lower than that of the bulk liquid water by about a seventh of a H-bond. This relatively large binding energy represents the "energy source" used by the tissues to generate large stresses.

On the other hand, other systems profit from very small entropic forces to generate large strains. This is the case for instance of the seed capsules of the ice plant that, thanks to a sophisticated design at various hierarchical level, can accomplish a full opening cycle by exploiting the entropy of dilution of a hydrophilic polymer that fills the keels cell compartments **[3].**

As actuation in these systems relies on solvent-materials interactions, in collaboration with prof. Thomas Zemb (ICSM Marcoule, France), we aim to quantitatively describe the thermodynamics of solvent related molecular forces existing between natural tissues' building blocks. Because of the structural and chemical complexity of the systems, it is crucial to take into consideration their geometry and the composition and account for the presence of electrolytes in solution as well.

Molecular Changes in Collagen-Based Tissues

Another system very sensitive to differences in water content is collagen. In this case, in collaboration with A. Masic, we aim to describe from the molecular to the macroscopic level the changes the collagen undergoes when its hydration state changes. In fact, applying osmotic pressure changes comparable to those occurring in vivo, the triple helix undergoes heterogeneous conformational changes and can generate a macroscopic tensile stress which comparable to that of the peak stress of human muscles **[4].** This effect can be of extreme importance for processes occurring *in vivo*.

Development of Data Analysis Tools

Finally, I develop data analysis techniques to extract structural features of nanometric/molecular moieties from spectroscopic, scattering and imaging data **[5-7].**

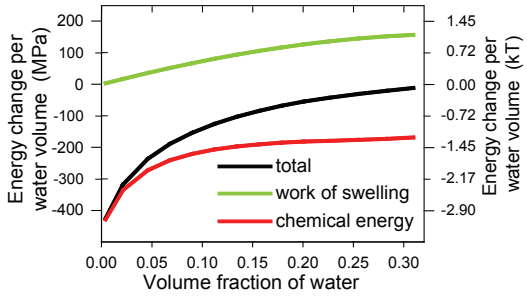

Fig. 1: Balance of energy densities for compression wood in Piceas Abies.

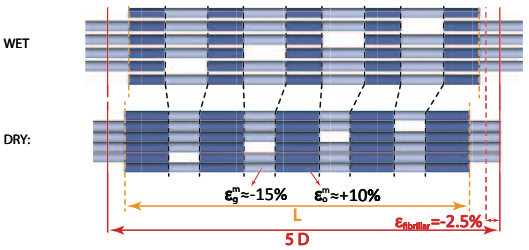

Fig. 2: Heterogeneous structural changes in collagen due to dehydration

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Synthesis and Thermodynamic Stability of Amorphous Minerals: Deeper Understanding of the Amorphous Precursor Route

Although the formation of crystals from solution is a well-described process for soluble salts, especially in the case of poorly to non-soluble salts like carbonates, phosphates and sulphates formation processes are complex and often involve an amorphous intermediate stage **[1].** Interestingly, biology uses the same materials to form its complex mineralized structures, which are often composites of inorganic and organic origin with improved mechanical properties compared to the abiotic mineral. The amorphous precursor is prerequisite for the formation of these biominerals, as its properties can be easily manipulated by the presence of additives or by changing the physicochemical conditions of the local environment. By doing so, biology can choose where the amorphous precursor crystallizes and also in which type of mineral preferably it crystallizes into. However, also examples of stable amorphous minerals are known in biological records **[2, 3].**

As biological mineralization is complex, to retrieve more insight into the so-called amorphous precursor route, comparative laboratory studies are prerequisite where the influence of specific actuators on the formation, stability and transformation behavior of amorphous precursors is investigated. Until now mechanistic insights into these processes, however, are limited as the introduction of one specific actuator often changes more than one parameter. Additionally, to mimic biological mineralization also the interplay between different actuators need to be understood.

To enable a mechanistic evaluation, in our studies we directly focus on the intrinsic chemical, physical and morphological properties of the amorphous mineral itself as a translation step between external actuators and final outcome. As for this a high control over the synthesis is prerequisite **[4],** use is made of a state-of-the art titration equipment as well as numerous *in-situ* and *ex-situ* analysis techniques. Furthermore, in all steps of the research there is a close cooperation with Luca Bertinetti and Yael Politi (both MPI, Biomaterials) as well as the Department of Structural Biology of the Weizmann Institute of Science.

Particle Size Effect

Synthetic amorphous calcium carbonate (ACC) is always present as nanometer-sized spherical particles, and also in biology this morphology can be found **[2, 3].** The size of these particles is variable, however, little is known about the consequences of the particle size. As one of the intrinsic properties of ACC, in our studies we investigate the effect of ACC particle size on its stability against crystallization and polymorph selection.

Additionally, we perform destabilization experiments using changing environments. By doing so, we retrieve additional insights into the effects of different kinetics and water on the crystallization mechanism. In these experiments use is made of ion-selective electrodes (in solution), an online synchrotron SAXS/WAXS setup and TGA/DSC analysis.

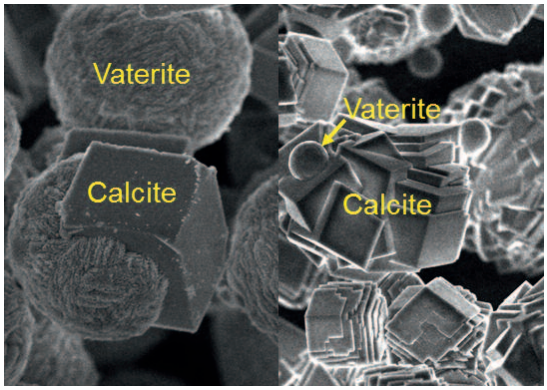

Fig. 1: SEM-images of crystallized products from ACC transformation in solution at 34°C (left) and 7°C (right) using exactly the same 200 nm sized ACC particles. Note especially the difference in crystal habit as well as in polymorph abundance of the rhombohedrical calcite crystals and spherical aggregates of hexagonal vaterite crystals.

Effects of Mg^{2+} and PO_4^{3-}

Next to organic molecules, foreign ions like Mg^{2+} and PO_4^{3-} are commonly found inside biological calcium carbonate minerals **[2],** and have been observed to influence calcium carbonate mineralization *in vitro*. Similar as Mn^{2+} and Sr^{2+}, Mg^{2+} hereby enters the lattice of the final crystalline calcium carbonate polymorph (calcite), whereas PO_4^{3-} can be observed inside the amorphous precursor phase, but is expelled the moment the mineral crystallizes. Comparison of the effects of both commonly found impurities gives us insights into general mechanisms of additive controlled calcium carbonate mineralization.

Phase Behaviour

The mechanism at which amorphous calcium carbonate is formed is a hot topic of discussion, where lately numerous possible pathways have been described, but little experimental evidence is presented. By systematic synthesis of calcium carbonate mineral at controlled conditions, and analysis of the physicochemical properties of extracted material, in our studies we try to retrieve empirical information on the phase behaviour of amorphous calcium carbonate. Additionally, the role of previously described additives on the phase behaviour is investigated.

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Hierarchical Structure of Biological and Biomimetic Materials

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Biological materials are often an inspiring source for materials scientists developing new materials with specific functions and properties. In our group, we use combinations of materials science approaches to answer (i) biologically driven questions in natural materials and (ii) to understand structure-function relations in biological and synthetic materials. By this approach we aim to elucidate biological processes and to transfer knowledge from natural materials to the design of man-made materials, such as polymer-based hybrid materials and nanostructured mineral-based materials.

In our research, bone serves as a prototypical system for a hierarchically structured material with extraordinary mechanical properties **[1].** Bone as a living organ has the capability to adapt to environmental conditions and to regenerate after injury. These processes are closely related with changes in the material structure at all size levels and can therefore be assessed indirectly by materials science methods. 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.

Our central experimental methods are X-ray scattering (SAXS, WAXS) **[1,2],** X-ray fluorescence (XRF), polarized light microscopy (PLM), confocal laser scanning microscopy (CLSM), electron microscopy, micro-computed tomography (µCT) and nanoindentation (NI). 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). To evaluate large data sets from synchrotron sources, we also develop tailored plug-in based software **[3].**

The Role of Osteocytes in Bone

Osteocytes are bone cells coordinating bone remodeling. We found that osteocytes are involved in mineral homeostasis and explored their impact on the bone material **[4].** The osteocyte network in bone was visualized with CLSM and the nanoscopic bone mineral particle properties relative to the cell network were characterized using high resolution SAXS/WAXS techniques. Most of the mineral particles reside within less than a micrometer from the nearest cell network channel and mineral particle characteristics depend on the distance from the cell network.

Together with cooperation partners from the FU Berlin, we have been working on a synthesis of new staining molecules and explored their capacity to effectively stain and consequently visualize bones with varying porosity **[5].** In a study on mouse bone we found that regions labeled with a commonly used calcein fluorochrome have lower mean mineral thickness and degree of mineral alignment **[6].** Surprisingly, fluorochrome seems not only binding to mineralizing surfaces, but also alters mineral properties, stunting their growth.

Bone Healing

A fracture in bone results in a strong change of mechanical loading conditions at the site of injury, where a bony callus is formed. In fractured bone we found that primary bone formation was followed by secondary bone deposition with mineral particle sizes changing from on average short and thick to long and thin particles **[7] (Fig. 1).** Comparing healing in samples with a small and a large fracture gap, we found that the difference of geometry of the initial condition led to completely different mechanical situations. In the case of successful healing, a bony connection in the marrow space enabled a load transfer across the fracture gap promoting further healing. This is considered the essential step compared to critical healing (large gap size), which resulted in the formation of a bony closure at each bone end without a reunion **(Fig. 1c and d).**

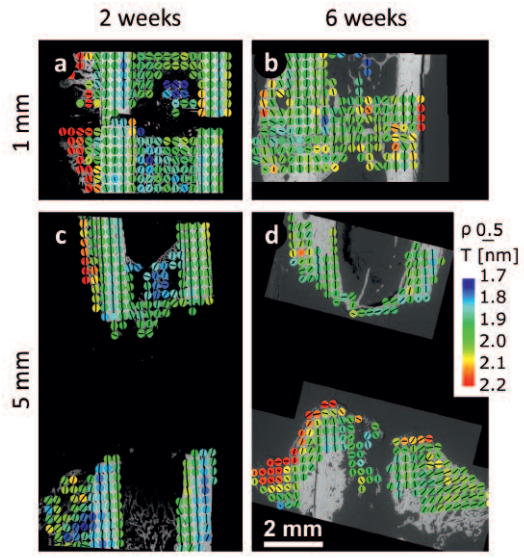

Fig. 1: Bone healing ***[7].*** *Results of SAXS measurements of normal (a and b) and critical (large gap size) (c and d) healing samples at two and six weeks after fracture. Color-coded measurement points represent the mean mineral particle thickness (T). The degree of orientation (ρ) and the predominant particle orientation are denoted by the length and orientation of the bar.*

In addition, we investigated bone during healing by means of µCT and different two-dimensional methods **[8].**Together with visualization experts from Zuse Institute Berlin we developed an approach to assemble 2D data in a 3D µCT reference frame.

With our multi-method approach we also studied osseointegration of zirconium and titanium implants by characterizing mineral particle characteristics **[9].** We found that the bone material quality around zirconium implants is at least as good as for titanium.

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Mineralization in Healthy and Diseased Bone

The course of bone mineralization is a crucial determinant influencing properties of healthy and diseased bone. The detailed mechanism by which calcium is deposited during mineralization and removed during resorption is largely unknown.

We studied medullary bone (bone in the central cavity of long bones in egg-laying birds) as a model system for rapid bone turnover rates as it is a calcium source for egg shell formation in hens **(Fig. 2a) [10].** The microscopic and nanoscopic architecture of avian medullary bone material is rapidly changing during the daily egg-laying cycle. Additionally to the two known bone types (cortical and medullary bone) a third type (represented by a calcium halo) has been discovered, which may represent an intermediate phase during mineralization **(Fig. 2b and c).**

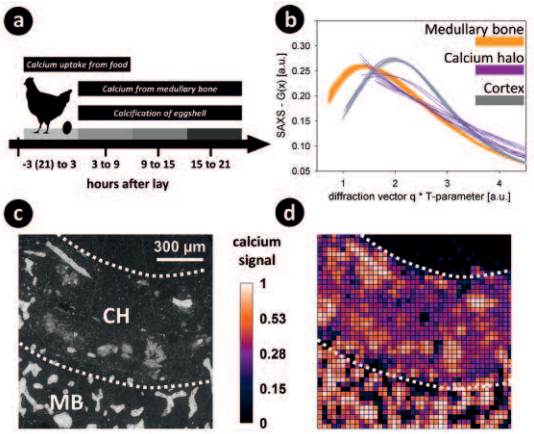

Fig. 2: Characterization of different bone types with SAXS and XRF: (a) processes during the 24h egg-laying cycle. (b) BSE micrograph showing medullary bone (MB) and a calcium halo (CH). (c) XRF mapping of the calcium concentration. High Ca concentrations are present adjacent to MB trabeculae ***[10].***

Osteogenesis imperfecta (OI), also known as brittle bone disease, relates to a group of connective tissue disorders characterized by mutation in genes involved in collagen synthesis. Beside increased bone fragility, OI leads to low bone mass, impaired bone material properties and abnormally high bone matrix mineralization. We investigated mineral particle properties in human bone of children with OI type I and compared it with a control group. We found that the increase in mineral density in OI type I was not due to an increase in particle size, but due to an increase in the number of particles **[11].**

Hybrid Materials

Hybrid materials consist -like bone- at the nanoscale of an inorganic phase embedded in an organic matrix. With the aim of understanding structure-function relations and consequently tuning materials properties we elucidate deformation mechanisms in a material synthesized by cooperation partners at the HU Berlin. This hybrid material with nanometer-sized metal fluoride particles embedded in poly(ethylene oxide) is currently being investigated by a combination of SAXS/WAXS techniques and tensile testing experiments. The second hybrid material of interest is based on natural collagen extracted from turkey leg tendons as organic part infiltrated with different transition metals (Zn, Al and Ti) as inorganic part. In this study, we investigate the usability of turkey leg tendons as matrices for nanoparticle infiltration to modify materials properties.

Crystallization Patterns in Calcium Carbonate Microlens Arrays

Exploring fundamental formation and crystallization processes in tailored mineral-based materials can contribute to a deeper understanding of complicated biomineralization processes. We produced thermodynamically stable, transparent calcium carbonate-based microlens arrays (MLA) by transforming an amorphous $CaCO_3$ phase into nano-crystalline calcite **(Fig. 3a) [12].** Structure and properties of crystallized MLA have been visualized by WAXS, polarized light and electron microscopy **(Fig. 3b and c).** The nano-crystallinity of the formed calcite minimized structural anisotropy and resulted in greatly reduced birefringent effects.

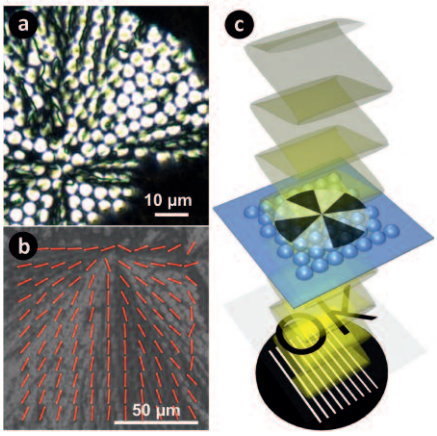

Fig. 3: Morphology and optical properties of crystallized $CaCO_3$ microlens arrays. (a) PLM image of the $CaCO_3$ microlens array showing spherulite-like patterns. (b) SEM image of crystallized microlens array with results from scanning WAXS: red bars indicate the crystallization direction. (c) Schematic illustration of the optical microscope setup to test birefringence and other optical properties: the incident light (yellow) passes the optical microscope polarizer, the glass slide with an "OK" symbol, the MLA and finally the analyzer ***[12].***

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Structural and Nanomechanical Characterization

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Living organisms form complex mineralized biocomposites that perform a variety of essential functions. These biomaterials are often multifunctional, being responsible for not only mechanical strength, but also provide optical, magnetic or sensing capabilities. Many studies have emphasized the complexity of biochemical mechanisms in charge of the delicate equilibrium and interaction chemistry between inorganic precursors and macromolecular components leading to nucleation, assembly and growth of different biominerals. In contrast, mechanical and thermodynamic constraints, governing the microstructure formation, growth kinetics, morphology and mechanical properties of the mineralized tissue are much less understood. Therefore, we aim to address the fundamental question of how nature takes advantage of mechanical and thermodynamic principles to generate complex functional structures.

Thermodynamically Driven Mesostructure Formation in the Shell of Pinna nobilis:

We studied the structural evolution of the calcitic prismatic layer in *P. nobilis* by analogy to classical grain growth theories **[1].** The microstructure of the layer was reproduced using high-resolution synchrotron-based microtomography, beamline ID19 in ESRF. Mainly, we focused on mean field considerations, where the growth kinetics of a single prism was described by an average behaviour of the entire prismatic layer, and topological considerations of space filling. As a result, we showed that the classical theories of normal grain growth and coarsening completely describe the growth process of the prismatic layer of *P. nobilis,* **Fig. 1.**

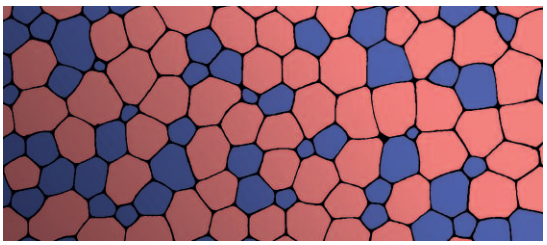

Fig. 1: 2D microtomography section perpendicular to the growth direction of the prismatic layer. Growing prisms are color-coded pink, shrinking prisms are color-coded blue.

This outcome supports the idea that the biological organism which regulates calcite growth is not controlling the shape evolution of the prisms beyond setting the thermodynamic boundary conditions. In addition to providing new insights into the way biogenic minerals are built, these results demonstrated that the prismatic layer of the mollusc shell is actually a textbook example for grain growth.

Environmentally-Controlled Static and Dynamic Mechanical Characterization on the Nanoscale:

Understanding the structure-to-function relationship in biological materials at the macroscopic level requires studies of all the hierarchical levels at many different length scales. Recent progress in applications of the nanoindentation equipment includes the nanoscale modulus mapping technique enabling to probe static and dynamic mechanical properties with high spatial resolution, **Fig. 2.**

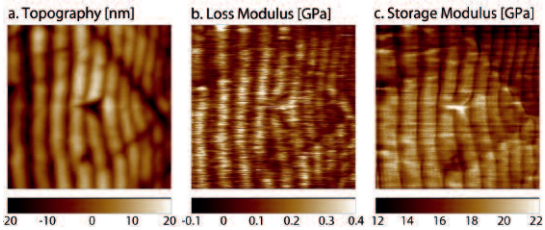

Fig. 2: Nanoscale modulus mapping of a chitin plywood structure in the exocuticle area of a tibia of the wandering spider, Cupiennius salei, produced on a cut parallel to tibia long axis: (a) – topography map; (b) – loss modulus map; (c) – storage modulus map. The maps size is 3x3 micron^2.

Nanoindentation based instrumentation was initially developed for mechanical characterization of stiff and hard composite structures. Therefore, for correct evaluation of mechanical properties, its application on compliant and soft biocomposites requires adaptation of the experimental set-up and modification of the theory behind it. Our research was focused on implementing this technique in biomaterials research. We adapted the technique and combined it with reverse finite element analysis in order to determine the elastic moduli of nanometric inclusions even when embedded in a matrix which is 50 times stiffer **[2].** Furthermore, we adjusted the theoretical backbone of this technique to fit to the analysis of relatively soft tissues **[3].** Finally, because biological materials typically reside in humid environments in their natural condition and perform under a variety of relative humidities and temperatures, we successfully designed and realized experimental set-up allowing moisture and temperature dependent mechanical properties of the S2 layer of *Picea abies* wood cell walls to be exclusively and independently determined **[4].** Currently, we are the only laboratory in the world able to routinely perform static and dynamic nanoindentation in controlled environment.

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Surface Nanostructuring for Bioapplications: Intelligent Smart Systems

Our focus is the surface nanoarchitecture which provides spatially and temporally defined control over the behaviour of biomolecules and cells at the solid-liquid interface **[1].**

Metal Surface Nanostructuring

As a fast and versatile methodology which provides controllable variation of surface topography and roughness by tuning the numerous synthetic parameters we use high intensity ultrasonic treatment for the formation of mesoporous surfaces **[2].** Mesoporous surfaces are believed to be the most promising for the formation of surface encapsulation systems **[3].** We also use titanium nanotubes slides obtained by electrochemical oxidation. By titanium surface nanostructuring we (J. Dunlop, Biomaterials, and P. Knaus, FU Berlin) aim to control the adhesion of cells to surface, as well as their behavior in terms of proliferation, migration and differentiation.

Surface Drug Depot

Methods for encapsulation, prolonged storage and controllable release were developed **[3-4]** and are in focus (with H. Möhwald, Emeritus Group Interfaces) **[1].** Formation of stimuli responsive encapsulated systems are suggested via layer-by-layer assembly, mobile chemical bonding (hydrogen bonds, chemisorptions) and formation of special dynamic stoppers. The most essential advances of the systems presented are multifunctionality and responsiveness to a multitude of stimuli **(Fig. 1).**

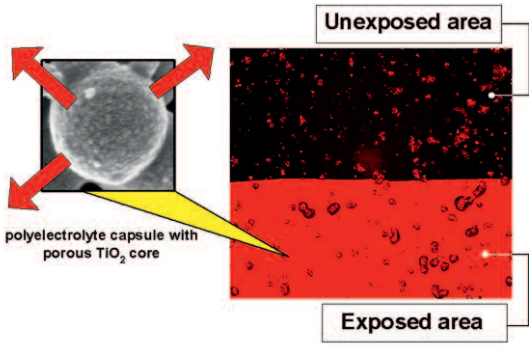

Fig. 1: Example of pH and light responsive surface capsules: micrograph of the edge of the laser beam trace at the surface containing polyelectrolyte capsules with titania core. The red area corresponds to spatial coatrolled release of Rhodamine 6G from the capsules.

Stimuli Sensitive Response

Stimuli responsive behavior, which is intrinsic to natural systems, is becoming a key requirement for advanced artificial materials and devices. Intelligent surfaces which are able to control the behavior of biomolecules and cells in both space and time are in focus in our group (with D.V. Andreeva, Univ. Bayreuth) **[1-6].** External stimuli or internal stimuli can be used to alter surface properties. In particular, we decorate the surfaces with stimuli responsive layers. Thus, for example, we use as a pH-sensitive polymer layer commercial or sensitized by our partners (R. Haag, FU Berlin; M. Karg, Univ. Bayreuth) polymers, e.g. polyelectrolytes, biopolymers and bioinspired polymers, microgels, etc. For etch particular applications the system is require the individual nanostructuring. It is shown in **Fig. 2** the nanostructuring of mesoporous metal sponge layer with pH responsive micelles **[6]** for self-regulation of *Lactic* bacteria adhesion. *Lactic* bacteria change pH via generation of lactic acid in their life cycle. The pH responsive micelles change their corona size and push of the bacteria from the surface.

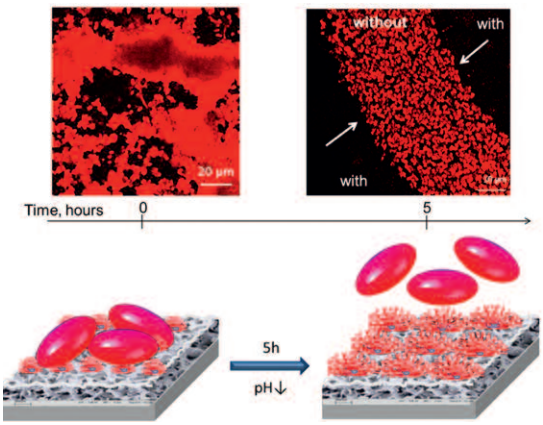

Fig. 2: Example of self-controllable responsive antifouling surface: confocal images of spatial controlled release of Lactic bacteria on patterned with pH responsive micelles mesoporous sponge surface.

The developed nanoengineered systems represent a generic technological tool, which opens numerous applications in chemical technology, biotechnology and bioanalytical chemistry, among them: self- and light-healing dynamic surfaces; anti-fouling surface; 'smart' supports for growing cells and tissues; controlled implant coatings; drug delivery systems; (bio-)sensors.

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Physics of Biomolecular Interfaces

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Biological tissues and cells are composed of diverse functional units such as organelle membranes, protein complexes, and carbohydrate assemblies. The structural organization of these cellular constituents on the sub-micrometer scale is essential for their proper function and in the congested biological environment largely depends on the physical interactions between their surfaces.

Molecular Interactions at Membrane Surfaces

In our Emmy-Noether research group, supported by the German Research Foundation (DFG), we study the physical mechanisms that govern the interaction of biological interfaces with their aqueous environment and also their mutual interaction in the aqueous milieu, with a specific focus on interactions involving biological membranes (see Fig. 1). Without regulation of these interactions by the organism essential cellular processes such as material transport or cell division would not be possible. One of our main goals is to understand the relation between membrane interactions and the molecular composition of membrane surfaces. In this context we are also interested in Nature's strategies to control the interactions by adjusting membrane compositions. To investigate interactions at biological interfaces we carry out experiments with model systems of well-defined biomolecular composition. Our primary tools are various x-ray and neutron scattering techniques, however we also employ complementary methods, such as ellipsometry, calorimetry, and spectroscopy techniques. In addition, computer simulations carried out in collaborations provide a means to interpret the experimental results on an atomic scale level.

X-Ray & Neutron Scattering Techniques and Complementary Computer Simulations

The research group *Physics of Biomolecular Interfaces* is the most recent research group in the Biomaterials department and was installed only in autumn 2014. Within the group leader's PhD project at Heidelberg University and a postdoctoral research project at the Institut Laue-Langevin (Grenoble France), funded by a Marie-Curie research grant by the European Commission, we have established a number of experimental strategies to create planar models of biological and biotechnologically relevant surfaces and to structurally investigate them by means of scattering techniques **[1-4].** During a post-doctoral research project in soft-matter theory at Technical University of Munich and Free University of Berlin we have developed computer simulation methods that allow reproducing and mechanistically interpreting experimental results on surface interactions **[5-6].** These simulations accurately account for the chemical potential of water between the surfaces and have lead to a better understanding of the long-debated "hydration repulsion" between membranes **[6].**

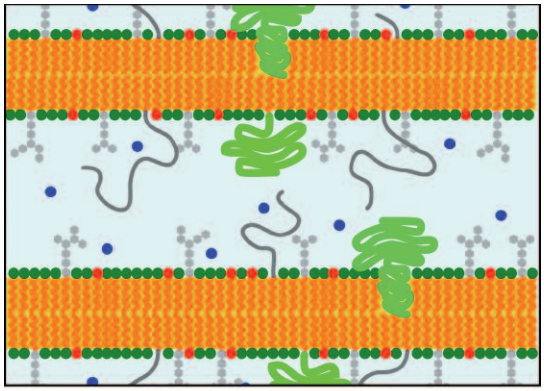

Fig. 1: Cartoon of two interacting biological membranes. Their surfaces display a variety of hydrophilic lipid moieties and membrane-bound macromolecules. The mutual interaction of membranes is governed by this molecular composition.

Protein Adsorption to Material Surfaces with Biocompatible Functionalization

In 2013/2014 we studied interactions between proteins and polymer brushes at solid/liquid interfaces. Protein adsorption to material surfaces causes problems in medical applications such as implanted biomedical devices (e.g., catheters or stents), as it can promote foreign-body reaction. A common approach to prevent undesired protein adsorption is to functionalize surfaces with hydrophilic polymer brushes, most frequently of poly[ethylene glycol] (PEG). However, the interaction of polymer brushes with proteins is not well understood. In particular, little is known about the mechanisms responsible for regularly observed „brush failure", where protein adsorption arises despite brush functionalization. We have fabricated PEG brushes of well-defined grafting layer chemistry, polymer length, and polymer grafting density, and structurally investigated different modes of undesired protein adsorption using neutron reflectometry with contrast variation. This experimental technique yields matter density profiles perpendicular to the interface with sub-nanometer resolution. The brushes were created from amphiphilic lipo-polymers with PEG portions of defined lengths. They were first prepared as water-insoluble (so-called Langmuir-type) monolayers at an air/water interface and then transferred onto hydrophobically functionalized surfaces of planar silicon blocks at controlled lateral densities. Our results obtained after incubation with different types of proteins highlight the importance of the brush parameters **[3]** and the implications of PEG's reported but often neglected antigenicity **[4].**

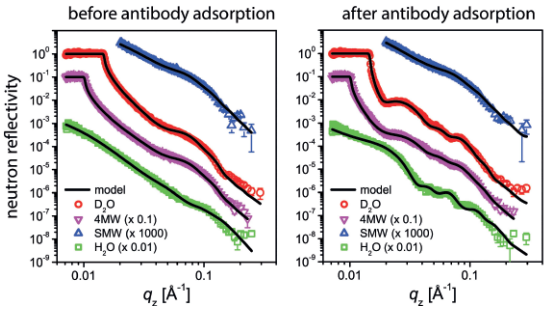

Fig. 2: Neutron reflectivity curves from a PEG brush in H_2O and D_2O as well as in H_2O/D_2O mixtures termed 4MW and SMW, before (left) and after (right) incubation with antiPEG IgG antibodies. Solid lines indicate the reflectivity model used to reconstruct the protein density profiles.

Fig. 2 shows a set of reflectivity curves from a PEG brush in aqueous solution before (left) and after (right) incubation with solutions of antiPEG IgG antibodies (Fig. 3 top), as are sometimes found in the human blood. The four curves in each panel correspond to four different "water contrasts" in neutron reflectometry, which are realized by mixing H_2O and D_2O in defined ratios. The adsorption of proteins leads to a number of additional features (in particular minima and maxima) in the reflectivity curves, from which the density profiles of the polymer brushes and adsorbed antibodies were reconstructed with the help of a suitable reflectivity model (solid lines in Fig. 2). The reconstructed protein density profiles (Fig. 3 middle) distinctly showed that the adsorption of antibodies occurred onto the brush itself, an adsorption mode termed "ternary adsorption" in the theoretical literature. Closer inspection revealed that the antibodies form dense layers and assume an inverted "Y" configuration (Fig. 3 bottom), indicating strong and specific protein/polymer interactions involving the binding regions on the F_{AB} segments **[4].** In this configuration the antibodies display their F_C segment to the aqueous phase suggesting that foreign body reaction is promoted.

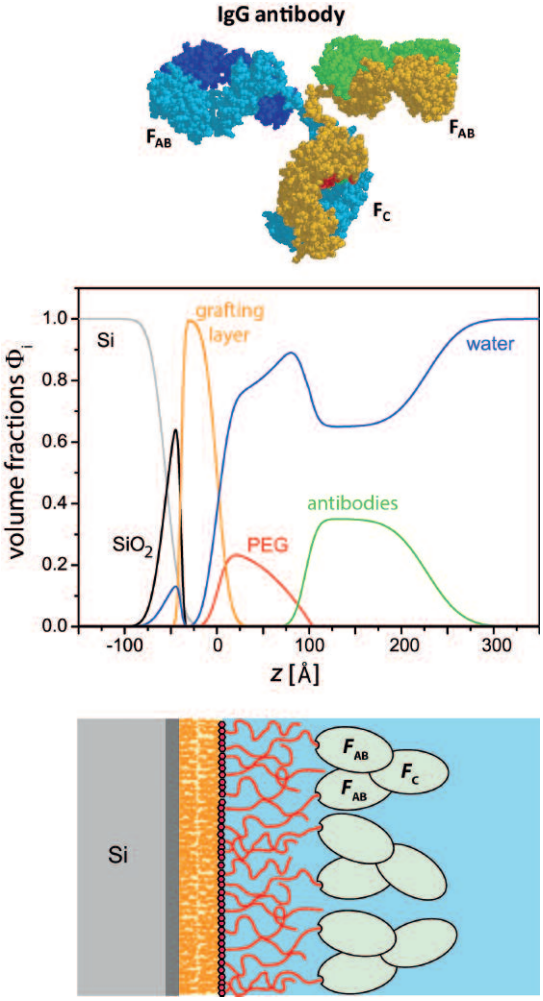

Fig. 3: (top) Structure of an IgG antibody. (middle) Density profiles of antiPEG IgG antibodies (Abs), PEG, and other compounds in the vicinity of the silicon/water interface as reconstructed from the reflectivity curves in Fig. 2. (bottom) Cartoon illustrating the interpretation of the density profiles.

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Mixed Protein-Surfactant Adsorption Layers

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Proteins are used in many applications due to the particular interfacial properties of their adsorption layers. Even more, mixtures of protein with low molecular weight surfactants allow tailoring the interfacial behavior such that optimum conditions can be provided for many industrial applications in food processing, pharmacology or cosmetics. The adsorption of surfactants influences the equilibrium and dynamic properties of liquid interfaces. This modified behavior depends on the nature of the surfactant. Proteins mixed with ionic surfactants form complexes with a higher surface activity due to the compensation of the charged groups in the protein and the addition of hydrophobicity by the surfactant's alkyl chains. In contrast, the addition of non-ionic surfactants to protein solutions leads only to weak hydrophobic interactions. The formation of such mixed adsorption layers was described so far mainly by a competitive adsorption mechanism. The non-ionic surfactants adsorb in competition to the proteins and at sufficiently high surfactant concentrations a replacement of the protein molecules from the interface can be observed.

Aggregate formation in the bulk of mixed solutions takes obviously place via hydrophobic interaction when the amount of added non-ionic surfactants is sufficiently high. In literature a number of studies show that this is true for protein concentrations above 10^{-4} mmol/l, and the results were discussed mainly in terms of dipole interactions of weakly charged hydrophilic groups in the protein molecules and the hydrophilic groups of the non-ionic surfactants.

In recent investigations we studied the dynamic surface tension and dilational surface rheology of protein solutions (β-lactoglobulin – BLG, β-casein - BCS) at very low concentrations mixed with very small amounts of non-ionic surfactants (dodecyl and tetradecyl dimethyl phosphine oxide - C_{12}DMPO, C_{14}DMPO, dodecanol - C_{10}OH and pentaoxyethylene decyl ether - $C_{10}EO_5$). The investigations were performed at surfactant concentrations between 10^{-8} and 10^{-4} mol/l, a range in which the used surfactants alone do not show any measurable adsorption effects. The protein concentrations were in the range between 10^{-9} and 10^{-7} mol/l.

Fig. 1 shows the dynamic surface tensions of an individual BCS solution (10^{-8} mol/l) in absence (curve 1) and in presence of different amounts of C_{12}DMPO. The measurements were done with the profile analysis tensiometer PAT using the buoyant bubble configuration. Even at very low C_{12}DMPO concentrations the dynamic and equilibrium surface tensions of the mixtures are significantly lower than those for the individual protein solution. Note, for concentrations below 10^{-6} mol/l the surfactant C_{12}DMPO alone does not show remarkable surface tension changes. For comparison, the same figure presents results of a 100 times higher BCS concentration (10^{-6} mol/l) with similar admixtures of C_{12}DMPO (see the three lower curves 9-11). At this BCS concentration the addition of the non-ionic surfactant does not affect the tension remarkably.

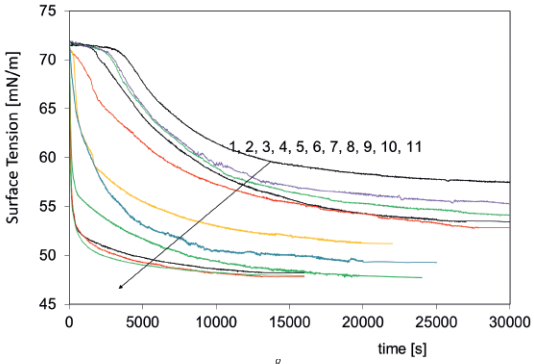

Fig. 1: Dynamic surface tension of 10^{-8} mol/l BCS solutions at different C_{12}DMPO concentrations: curve 1 - 0.0, curve 2 – 4×10^{-8}, curve 3 - 10^{-7}, curve 4 - 3×10^{-7}, curve 5 - 10^{-6}, curve 6 - 3×10^{-6}, curve 7 - 5×10^{-6}, curve 8 – 10^{-5} mol/l)); and 10^{-6} mol/l BCS solutions at different amounts of added C_{12}DMPO (the concentrations are: curve 9 -0.0, curve10 – 10^{-7} mol/l, curve 11 - 10^{-5} mol/l); according to ***[1].***

The equilibrium surface tension of pure BCS solution at the concentration of 10^{-5} mmol/l (horizontal dotted line) as well as the isotherms of mixtures with C_{12}DMPO and C_{14}DMPO as a function of the surfactant concentrations, are shown in **Fig. 2.** The results for the pure C_{12}DMPO and C_{14}DMPO solutions, also shown in this figure, can be well described by the Frumkin adsorption model (thin solid lines). Note, for the surfactants an intrinsic compressibility coefficient of the adsorption layer of $\epsilon = 0.003$ m/mN was considered.

The Fig. 2 contains also calculated surface tension isotherms for the mixed systems BCS/C_{12}DMPO and BCS/C_{14}DMPO at a fixed BCS concentration of 10^{-5} mmol/l using a classical Frumkin adsorption model for mixed adsorption layers (dashed lines, red for C_{12}DMPO and blue for C_{14}DMPO). As one can see, the calculated data are inconsistent with the experiments. In [2] a new approach was proposed to consider the presence of traces of non-ionic surfactants as a reason for the increase of the surface activity of the protein. For this a coefficient $k = 1 + a^* \cdot c_s$ being a linear function of the surfactant concentration c_s was introduced to modify the adsorption activity constant for the protein (details see **[2]**). The solid lines in Fig. 2 confirm that such a model reflects the changes in the protein's effective surface activity very well.

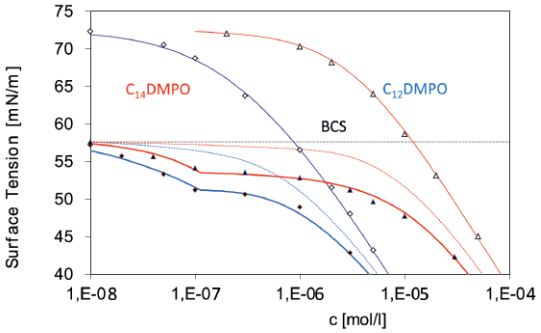

Fig. 2: Equilibrium surface tension isotherms for BCS+C_{12}DMPO (▲) and BCS+C_{14}DMPO (◆) mixtures at a BCS bulk concentration of 10^{-8} mol/l; (△) and (◇) are the data for individual C_{12}DMPO and C_{14}DMPO solutions; the equilibrium surface tension of pure 10^{-8} mol/l BSC solution is given by the dotted horizontal line; the dashed lines were calculated with a Frumkin type adsorption model, the bold solid lines are calculated with the new thermodynamic approach; according to ***[2].***

The variation of the 'effective' adsorption activity on the surfactant concentration in a certain concentration range could probably depend on the structure of the protein as well as on the kind of surfactant. The efficiency of the surfactant, expressed by the parameter a*, is governed by the interaction between the polar groups of the surfactant molecules with the polar groups of the amino acids located in the protein structure. In **[2]** these effects for four non-ionic surfactants was discussed: C_{12}DMPO, C_{14}DMPO, C_{10}OH and $C_{10}EO_5$.

For a deeper understanding of the effect of non-ionic surfactants on the adsorption activity of proteins at very small amounts of added non-ionic surfactants, dilational viscoelasticity studies were performed. These properties are most sensitive to the composition of mixed adsorption layers and can reflect best the interactions between the components adsorbed at a liquid interface. The dependencies of the viscoelasticity modulus on the surfactant concentration at an oscillation frequency of 0.1 Hz for mixtures of BCS (again at a fixed concentration of 10^{-8} mol/l) with C_{12}DMPO are shown in **Figs. 3** as example.

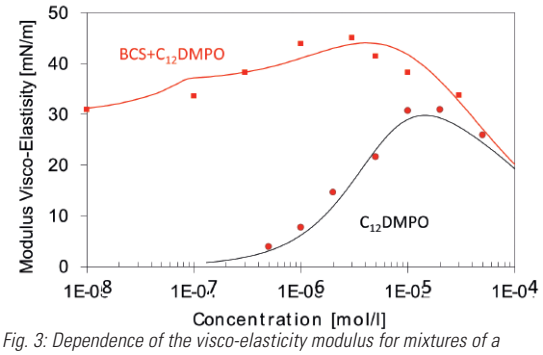

Fig. 3: Dependence of the visco-elasticity modulus for mixtures of a 10^{-8} mol/l BCS solution with C_{12}DMPO (■) and for the individual C_{12}DMPO solutions (●) on the surfactant concentration for an oscillation frequency of 0.1 Hz; the lines refer to the thermodynamic model discussed in the text; according to ***[3].***

The results obtained for this mixture are similar to those for mixtures of the other three studied surfactants and also for the equivalent mixtures with the protein BLG. The obtained data can be interpreted very well and further confirm the quality of the new proposed model.

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