BIOMATERIALS
Department of Biomaterials

The overarching research area of the Department is biological materials science, which connects materials science and biology in a reciprocal way. First, biomedical questions are addressed by methods and approaches borrowed from physics, chemistry or materials science. One such example is the extracellular tissue in the case of skeletal diseases and during regeneration. Second, we tap into the diversity of natural organisms to study naturally evolved solutions of engineering problems encountered by these organisms. Examples are materials combining stiffness and fracture resistance or providing capabilities for sensing, self-healing or shape-change. Many types of natural materials, often based on common classes of natural polymers, such as cellulose, chitin or protein (collagen and others) are addressed in these ways.

This research is carried out by scientifically independent research groups with diverse backgrounds, including mathematics, physics, chemistry, materials science, physical chemistry, biochemistry, wood science, botany, zoology and molecular biology. The group leaders were assembled not only based on their scientific excellence but also on their capability of collaborating – where needed – with each other groups as well as with the director who mainly contributes expertise in x-ray scattering, mechanical modeling as well as general materials science.

Natural Materials Based on Proteins, Lipids, Polysaccharides

Matt Harrington’s group addresses primarily extracellular protein-based materials and is interested in their assembly and in their often exceptional properties.

Yael Politi studies the chitin-spaced cuticle of arthropods, such as spiders. This cuticle supports a variety of tools and sensory devices that are essential for the survival of the animal. The fiber architecture of the cuticle as well as its composite character comprising chitin, protein and water are key for its functionality.

Michaela Edar works with her research group primarily on cellulose-based biological materials, such as wood and certain seed capsules that open with changing air humidity or temperature. These capsules are particularly interesting because they represent models for shape-changing polymeric materials.

Emanuel Schneck runs an Emmy-Noether group (supported by DFG) on the physics of biomolecular interfaces. The research addresses interaction between membranes and with biomolecules. He makes use of x-ray and neutron reflectivity studies as well as numerical modeling.

Mason Dean studies cartilaginous skeletal elements and, in particular, the formation, structure and mechanical performance of tesselated, mineralized tiles covering all skeletal elements.

Reinhard Miller’s research focusses on solution-air interfaces and their dynamics. He retired by the end of 2016.

Biomineralization

Biomineralization is a widely represented topic in the department [1] involving the work of several groups. Damian Faivre heads a group focusing on magnetotactic bacteria and the synthesis and application of magnetic nanoparticles. Wouter Habraken reports on his work studying nucleation and growth of calcium phosphate minerals in-vitro to help our understanding of biomineralization. Very surprisingly, a calcium carbonate phase was discovered in the process that had not previously been described. Wouter Habraken was supported by a 5-year collaborative project with the Weizmann Institute (Lia Addadi and Stephen Weiner, among others), also involving Yael Politi and Luca Bertinetti. Results included the discovery and characterization of mineral precursors, probably transported within vesicles and deposited at the growth front of bone in zebrafish [2,3] and in chicken embryo [4]. Other results of this collaboration are mentioned in Wouter Habraken’s report.

Methodological Approaches

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 as well as spectroscopic imaging to

![Fig. 1: Schematic of research topics in the biomaterials department. Most biological materials of interest are based on protein, cellulose, lipids and minerals (top). They are analyzed by multi-scale, multi-method approaches (pink) and by mathematical modeling (blue) and they lead to multiple collaborations in the contexts of bio-inspired engineering and of regenerative therapies (yellow).]
characterize micro- and nanostructure and many levels of structural hierarchy [5] (see also report by W. Wagenmaier). We use nano-indentation as well as acoustic microscopy to estimate local mechanical properties. Igor Zlotnikov has established modular mapping techniques which push 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 [6].

In a related 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. This approach is now complemented by recent large investments in the Institute that provide new capabilities with high-resolution transmission electron microscopy as well as (cryo)-focused ion beam 3D electron imaging (via slice and view scanning electron imaging).

These characterizations approaches are accompanied 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 (for example the reports by John W.C. Dunlop and Richard Weinkamer). The Humboldt Award Winner F. Dieter Fischer from Montanuniversität Leoben (Austria) is a long-standing collaboration partner in this context.

**Active Materials**

The classical concept that materials are a passive support for the activity of devices is currently challenged by research on active materials which are responsive or adaptive, which regenerate or allow shape changes. The Department takes part in these research activities by focusing on shape-chang-

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**Fig. 2:** Variation of stiffness (Young’s modulus) and of the resulting crack driving force in lamellar bone. The plywood-like structure leads to a periodic modulation which implies larger energy dissipation when a crack propagates. In this way the toughness of bone tissue is increased by a large factor [15].

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References:


Among many other activities, the Cluster organized a public exhibition on the interaction between science, humanities and design in a Berlin Museum (“+ultra – gestaltung schafft wissen” at Gropiusbau, Berlin, November 2016).

Research on Bone and Tissue Regeneration

In collaboration with partners from the Montanuniversität Leoben and the Austrian Academy of Sciences (F. Dieter Fischer and Otmar Kolečnik) we study by theoretical methods the fracture behavior of multilayered materials and, in particular, of lamellar bone. Recent work shows that the lamellar architecture increases the fracture toughness of bone by more than an order of magnitude [15, 16] (see Fig. 2).

John Dunlop studies micro-tissues in vitro and investigates how they grow as a function of three-dimensional geometry. The approach is primarily biophysical but – since micro-tissues may be composed of bone cells or fibroblasts – also teach fundamental lessons about bone regeneration and wound healing.

Katja Skorb with her group develops methods for nanostucturing metallic surfaces using ultrasound treatment. These surfaces, studied in the context of cell adhesion, proliferation and differentiation; may play an important role for the development of new surface treatments for implant materials.

In a long-standing collaboration with the Ludwig Boltzmann Institute of Osteology in Vienna, Austria, we study bone structure and properties in genetic or metabolic bone diseases, such osteogenesis imperfecta (brittle bone disease) and osteoporosis, see [13, 14] and reports by Richard Weinkamer and Wolfgang Wagemaner. As one example, we could show by a combination of electron microscopic, x-ray scattering and spectroscopic techniques, that one common feature of many forms of OI is a larger number of similarly sized mineral particles in the bone tissue, which implies increased fragility (see Fig. 3). Moreover, we are particularly interested in the dense osteocyte cell network that perfuses all bone tissue and acts both as an endocrine organ and a mechanosensory system. We study its network structure in relation to the architecture of the extracellular matrix. In the context of bone regeneration, the Department is also involved in a consortium on bone regeneration with the Berlin Brandenburg School of Regenerative Therapies (supported by the DFG Excellence initiative).

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 wonderful partnerships.

Peter Fratzl
Director of the Department of Biomaterials
The study of skeletal biology is dominated by work on a few model mammalian species, although mammals represent <10% of living vertebrate species. In contrast, the number of living fish species is staggering, being half of all vertebrates and having an astounding range of morphological, functional and ecological diversity. Fishes are the oldest non-extinct lineages of vertebrates, with the primary taxonomic groups – the cartilaginous fishes and bony fishes – offering two very different primary skeletal materials. Our group exploits the rich diversity and evolutionary history of fishes, using investigations of development, ultrastructure and function to understand skeletal tissue evolution, adaptation, and mechanics.

How do Mineralized Skeletal Tissues Develop? How is “Structure” Regulated?

Vertebrate skeletons vary considerably in microstructure, but a common necessity in their growth is the regulation of the location and timing of mineralization. Shark skeletons are an unappreciated but ideal system for investigating the control and development of mineralization, in that they possess a vast and accessible array of mineralization fronts, arranged in visually striking patterns (Fig. 1). We show, using histological, imaging and material characterization techniques, that mineralized shark cartilage is a curious mosaic of skeletal characteristics, marked by different collagens, cellular processes and mineral density patterns than the bone or cartilage of other fishes or mammals, but exhibiting networks of communicating cells and enzymatic mineralization regulation that mirror those of bone [1–3]. By rooting these investigations in comparisons with other mineralized shark and ray tissues, the skeletal tissues of bony fishes, and well-known mammalian systems [3–5], we begin to characterize the conservation of mineralization and growth mechanisms among vertebrates.

How do the Levels of Structural Hierarchy Mediate Skeletal Mechanical Properties?

All vertebrate mineralized tissues are mixtures of mineral, organic materials like collagen, and water, but few offer morphologies as geometric and amenable to modeling as shark cartilage. By combining techniques for quantifying and imaging the structure of the component parts of shark skeletons and for testing and modeling tissue mechanical properties, we provide the first insights into the high level of performance of shark cartilage [1, 6–8]. Through a Human Frontier Science Program-funded collaboration with computer scientists and engineers in two countries, we define principles of tissue mechanics by incorporating high-resolution structural data (e.g. from synchrotron radiation tomography of biological tissue) into analytical and finite element models and multi-material 3D printed objects for mechanical testing (Fig. 2). This workflow allows us to query relationships between tissue form and function, even for very small structural features (e.g. the ~1-μm wide junctions between the mineralized tiles covering the skeleton), and to begin to abstract design rules of the system for translational science applications (e.g. building of biomimicked low-density, high-stiffness composites).

Fig. 1: Morphology-to-modeling workflow. MicroCT scanned skeletal elements are semi-automatically segmented and 3d physical models printed with different optical and material properties to quantify mechanics-shape relationships.

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The functionality of plant materials in given environments is the main interest of our research group. We define plant material as any material forming the plant body: cellular components form cells which agglomerate to tissues, which in turn are the building blocks of organs of whole plants. The plant cell wall, its chemical composition and structure as well as its proportion in a given volume plays a major role for the material performance at larger length scales such as at the organ level. A deeper understanding of plant materials implies therefore investigation at multiple length scales. The groups’ activities can be assigned to both living and dead tissues. As long as cells are growing they are surrounded by the primary cell wall, rigid enough to withstand internal and external forces but at the same time pliable to allow for growth. This is achieved by the spatial orientation and interplay between the primary cell wall components cellulose, hemicelluloses and pectins. Dependent on the orientation of stiff and softer cell wall components different cell geometries emerge. After cells have reached their final form, thick, lignified secondary cell walls are synthesized in many cells. If one compares tissues with mainly primary cell walls to tissues with secondary cell walls the material properties are highly different. E.g., both hypocotyls and wood consist of elongated, cylindrical cells, however, the stiffness of hypocotyls in the longitudinal direction is – 25 MPa [1] whereas in plant based fibrous materials with secondary cell walls 200–1000 fold higher tensile stiffness can be reached [2,3,4]). Both primary and secondary cell wall systems, with and without active metabolism (living and dead) are interesting systems to study adaptations/adjustments of plant materials to given environments. We think this is equally interesting for a basic understanding of biological systems, for a deep understanding of the renewable resource plant material which is a prerequisite for targeted use and for the development of new (bioinspired) materials [5].

In the following some examples of recent research activities are given.

**Primary Plant Cell Walls: The Effect of Age on Dark Grown Arabidopsis Hypocotyls**

Dark grown Arabidopsis hypocotyls are a primary cell wall model system for a large scientific community. Numerous studies on the 200–400 µm thick and up to several mm long hypocotyls are performed to better understand the influence of molecular processes and genetic modifications on cell wall structure and growth. However, only few studies include experimental micromechanical data that give insights how such processes and modifications relate to Arabidopsis primary cell wall mechanics. Furthermore it is still unclear how hypocotyl age influences mechanical properties. We studied effects of age on hypocotyls of different seed batches with two tensile-testing-setups designed in our lab [1, 6]. They offer complementary possibilities of studying mechanical properties of Arabidopsis hypocotyls. Data were evaluated and discussed by considering age, geometrical parameters, hypocotyl density and cellulose content. Tensile stiffness and breaking stress of 4 day old hypocotyls were significantly lower than of 5–7 day old hypocotyls where no clear differences could be found (Fig. 1). Naturally the hypocotyls got longer with age. Furthermore the dataset allows estimations concerning biological variability of this model system. With this study we were able to establish both experimental protocols and reference values for the mechanical parameters tensile stiffness and fracture stress, useful for future studies on both wild-type and modified hypocotyls grown under various conditions.

**Secondary Plant Cell Walls: Important Components in Many Seed Pods**

Fruits and seed pods are plant structures essential for long-term species survival. Hence, it is not surprising that functionalities are incorporated in the material. Frequently, seed pods are composed of dead tissue which still provides possibilities for mobility and movements to allow for seed dispersal and distribution. E.g. the devils claws (Martyniaceae fruits) interlock with hooves and ankles of large animals to disperse seeds [7]. This is possible since the flexibility of the structures allow for attachment during dynamic locomotion and the high strength and stability prevent premature failure due to heavy loads which has been described in detail in the article “A materials perspective of Martyniaceae fruits” [7]. In contrast to seed dispersal based on attaching to animals and
the forces created by the animals, seed dispersal often relies on the movement of the seed pod itself. This is particularly interesting when initiated by an environmental trigger:

**Serotiny – Elevated Temperatures Trigger Seed Release**

A so-called serotinous plant does not release seeds upon maturity (such as wheat or pine cones in temperate regions) but it rather needs an environmental trigger for release and dispersal. Often the triggers are rain or drastic changes in temperature. In the last years we got interested in the seed pods of Banksia species which require heat – typically caused by bush fires – for seed release. The plant genus *Banksia* is native to Australia and received its name from the British botanist Joseph Banks who collected the first Banksia samples in 1770. Banksias are also considered being iconic Australian plants, they are frequent motives in indigenous and other arts, probably because of their impressive flower spikes (inflorescence) which can contain up to 6000 single flowers.

Some of the flowers get pollinated, often by mammals, such as nectar-sucking opossums and develop into follicles, the seed containing structures (Fig. 2B). Many species accumulate cones (without active metabolism) in the canopy for up to 17 years until a bush fire. The fire initiates follicle opening (Fig 2C). The gap between the follicle halves is large enough to expose the inner surface to the environment but too small for immediate seed release. Wetting and drying cycles are necessary for further follicle opening (Fig. 2D).

From a materials science perspective an understanding of the long-term dimensional stability and functionality of the woody and polymeric *Banksia* follicles during fire is interesting and might contribute to a better use of renewable resources and polymers since many plant based materials such as wood are not suitable for various applications due to swelling and shrinkage upon humidity changes. On the other hand many polymers lack stability at elevated temperatures causes problems for applications.

Together with the Botanical Garden in Perth we collected cones of *Banksia attenuata* at 5 sampling sites from Perth to Wannaroo, following a climatic gradient. By exposing the collected samples to stepwise heating we found that the opening temperatures gradually changed along the climatic gradient [8]: the southernmost follicles opened at 54 °C, the northernmost at 72 °C. These temperatures were much lower than the ones found in literature. When the follicles open they separate along the junction zone which is characterized by interdigitating cells and a substance gluing the follicles halves together (Fig. 3).

![Image](image_url)

**Fig. 2**: (A) Flower spike of *B. attenuata*, (B) follicles developed out of pollinated flowers (C) initial follicle opening caused by fire and (D) completely open follicles, seeds and separator have fallen out

Investigations on the melting behavior of the substance between the two follicle halves, a wax, revealed no differences in melting temperatures between the sampling sites (−45−50 °C) and were below the opening temperatures. This indicates that the wax might act as a sealing agent for microcracks since 45–50 °C can be reached in the field. Keeping the follicle tight is essential for avoiding any microbial attack, such as degradation caused by bacteria or fungi. Furthermore in the outer layer – the exocarp – sclerenchymatous fibres without preferential orientation form globules which are in contact to each other similar to puzzle pieces. Probably small movements in the plane of the follicle surface are possible; at the same time outward bending of the follicles valves seems to be prevented by the geometry and arrangement of the globules [9]. *Banksia* follicles are an excellent example for long-term stability of a purely polymeric material, properties highly desirable for plant-based materials for buildings and/or constructions

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Organisms such as mussels, spiders and hagfish, are able to rapidly fabricate remarkable biopolymeric fibers from bottom-up assembly of protein building blocks possessing properties that rival those of the best man-made polymers. As our fundamental knowledge of these materials improves, they are emerging important archetypes for inspiring the development of high performance synthetic polymers [1]. However, this requires a deep, holistic understanding of the underlying biogenic design principles. Along these lines, the overarching goals of our research group are to:

1) Elucidate structure-function relationships and bio-fabrication processes from biogenic materials.
2) Adapt natural design principles for development of advanced bio-inspired materials.

Structure-Function Relationships of the Bysus

Learning from nature requires the in-depth characterization of structure-function relationships of biological materials. X-ray diffraction studies coupled with in situ mechanical testing led by Antje Reinecke have identified the critical role of cross β-sheet protein conformation in the characteristic large extensibility and elastic recoil of mussel byssal threads [2]. A complementary project led by Clemens Schmitt in collaboration with Yael Politi (Dept. of Biomaterials) employed X-ray absorption spectroscopy (XAS) coupled to mechanical testing in order to probe the hypothesis that protein-metal coordination is essential for thread mechanical properties. These results reveal the presence of a strong, yet reversible sacrificial network of histidine-Zn2+ cross-links that contribute to the high stiffness, toughness and self-healing behavior of the threads [3]. Taken together, these studies greatly improve our picture of the underlying mechanism of thread performance, which can adapted for developing new polymers as described below [1].

In addition to work on the thread core, another project led by Clemens Schmitt established a clear connection between the mechanical behavior of the hard, yet extensible outer cuticle of byssal threads (Fig. 1) and the presence of metal coordination bonds mediated by 3,4-dihydroxyphenylalanine (DOPA) – a post-translational modification of the amino acid tyrosine. Particularly surprising was the observation that the DOPA-metal complexes provided over 80% of the stiffness and hardness of the protective outer coating, but that the metal center could be replaced by iron, vanadium or aluminum without a major influence on mechanical properties [4]. This strongly suggests that the mussel byssus has evolved to be opportunistic to changing metal conditions in the seawater.
**Fig. 2. Mussel byssus biofabrication.** Byssal threads are produced as a secretion of protein building blocks stored in secretory vesicles in the byssus secretory glands. Using a combination of histological staining and confocal Raman spectroscopic imaging, the dynamic byssus assembly process was investigated.

**Processing and Assembly of Mussel Byssus**

In a remarkable feat of material processing, each byssal thread is produced in just three minutes as an external secretion of over ten different protein building blocks under ambient conditions, which self-organize into the complex nano- and micro-architectured structure observed in the native thread. A recently published study led by Elena Degtyar and Tobias Priemel have used a unique combination of traditional histology and cutting edge confocal Raman spectroscopic imaging to follow the processing steps of the byssal proteins in mussel tissue as a thread is formed [5]. Among other things, this study revealed that a large part of the impressive structure of the byssus is acquired via spontaneous self-assembly of proteins, rather than through biologically regulated steps. Additionally, a separate project led by Antje Reinecke in collaboration with Gerald Brezesinski (Dept. of Biomolecular Systems) examined how histidine-rich protein sequences in the byssal proteins responsible for the self-healing behavior also function as pH-sensitive triggers during thread assembly [6]. These projects provide a foundation for a number of future characterization studies and have clear implications for the development of sustainable materials fabrication.

**Mussel-Inspired Materials**

Our group maintains an ongoing DFG-funded collaboration with the group of Prof. Ulrich Schubert (Friedrich Schiller University) through priority program SPP-1568 with the goal of developing and characterizing mussel-inspired self-healing polymers based on metal coordination. This has culminated in two joint publications describing metalpolymeric coatings containing His-Zn\(^{2+}\) complexes, which are able to heal scratches following mild heating [7,8]. Furthermore, two projects led by Franziska Jehle and Ana Trapaidze, respectively, are attempting to harness histidine-rich peptides based on byssal thread protein sequences to fabricate new materials exhibiting higher order organizational structure and tunable viscoelastic or self-healing properties.

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The cuticle of arthropods, a fascinating multipurpose functional material, is made up primarily of chitin and proteins. The cuticle is the arthropod exo-skeleton so that it also serves as skin and holds sense organs and functional tools [1]. These structures are all built of similar material however their underlying architectures and compositions are locally modified yielding fine-tuned materials properties. The study of chitin and chitin-based materials therefore holds a promise for clever bio-inspired materials design.

Despite many years of studies, there is still much to discover regarding the manner in which the cuticle components are assembled and how they interact within the final material. One of the goals of our group is to obtain basic understanding of the cuticular material, to gain insight into the structure-properties-function relations in specific structures such as cuticular tools (e.g. fangs, claws) and sensors and to investigate the manner in which they are formed. We work in close collaboration with Prof. Friedrich Barth, from the University of Vienna (Vienna, Austria) Prof. Bernad Moussian from the Technical University in Dresden (Germany), Prof Jan-Henning Dirks from Hochschule Bremen (Germany), Prof. Emil Zolotoyabco from the Technion Institute of technology (Haifa, Israel), Prof. Benny Bar-On from Ben-Gurion University (Beer Sheba, Israel), James Weaver from the WYSS institute, (Cambridge, USA) and others.

Chitin-protein-water Interactions

The cuticle can be described as a fiber reinforced composite material, where α-chitin crystallites are tightly coated by a protein shell. We studied the tarsal tendon of the spider Cupiennius salei, in which the chitin-protein fibers are highly aligned, using synchrotron-based X-ray diffraction and Raman spectroscopy in its intact, deproteinized, hydrated and dried states in order to shed light on the chitin-protein-water interactions in this system.

We observed high degree of order within the protein matrix and we identified protein β-sheet signature with highly defined orientation: the direction orthogonal to the β-strand long axis is slightly inclined with respect to the chitin c-axis.

In addition we observed variations in the position of the (020) diffraction peaks in pristine and treated chitin samples (Fig. 1C). Such that the lattice parameter b, extracted directly from the (020)-reflection, showed a large apparent increase relative to the bleached dry state – up to about 9% wet and 6.5% for dry samples. Although to a smaller extent (1.5%), such an increase was also found in ‘wet’ deproteinized and bleached samples. These findings were best explained by modeling the protein- and water-induced shifts in the diffraction profiles resulting from the tight interactions between them and the chitin crystallites. In this model X-ray interference effect is caused by the electron density modulation at the interface of chitin and surrounding protein coat and water due to their coherent ordering with the chitin crystalline organization. Based on this analysis, we predict protein ordering up to 2 nm and water ordering up to about 0.5 nm with protein sub-layers, spaced by 1.13 nm, and water molecules spaced by 0.25 nm. Such protein spacing is typical to β-sheets inter-sheet stacking in amyloids (see also report by Matt Harrington), whereas the distance found for water spacing is only slightly smaller than the mean distance between molecules in liquid water (0.29 nm). Moreover, we observed that the hydration caused swelling of the protein-coat and brought about larger order both in terms of the smallest dispersion in protein spacing and in chitin mis-orientation.

Our results highlight the importance of hydration to the structural integrity of chitin based materials and may bare significance to the understating of fiber ordering during cuticle formation with implications for material’s synthesis and design.

Nano-channels at the Tip of Spider Fangs and Cuticle Fortification

Metal ion cross-linking is used by many invertebrates for fortifying their hard parts [3], however, not much is known about the chemical nature and the mechanisms of this incorporation. We studied the spider fang, which is a natural injection needle comprising multi-scale architectural gradients, including high levels of Zn incorporation at its tip [3,4] to gain insight into these questions. We used high-resolution transmission electron microscopy (HR-TEM), spectroscopic methods and amino-acid analysis of fangs from adult spider as well as from spiders shortly after ecdysis - the shedding of the old cuticle during molting.

We found an array of multiple vascular Nano-channels, which seem to attend the transport of zinc to the tip of the fang. The channels are filled with Zn precipitate in adult spiders, but not during and right after ecdysis. On the other
hand, amino-acid analysis of the same samples shows that the protein matrix composition is similar during ecdysis and in adult spider fangs, including a high content of histidine residues at the tip of the fangs. Thus His-rich proteins, which are the expected to bind Zn, are deposited before Zn is incorporated into the cuticle.

Using Electron Energy Loss Spectroscopy (EELS) at the N K-edge we demonstrated that His-Zn cross-links indeed occur within the protein matrix of adult spider fangs, but not in fangs of spiders during ecdysis.

We believe that our observations of the nano-channel array serving the Zn-transport within the pre-deposited His-rich protein matrix may also contribute to recent bio-inspired efforts to design artificial vascular materials for self-healing and in-situ curing.

Amorphous calcium carbonate, the precursor phase in sea urchins, is hydrated, whereas the crystalline form into which it transform, calcite is anhydrous. Most of the dehydration is thought to take place before the crystallization [9]. However the details of this mechanism and the manner in which access water and the organic molecules are incorporated in the final biomineral are still unknown. We are currently investigating the structure and properties of sea urchin spine using high-resolution x-ray powder diffraction (HR-XRPD) and in-situ heating small angle scattering (SAXS) in order to gain understating of this process, which appear to be fundamental for various biomineralizing systems.

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References:
Biomineralization is the process by which organisms form materials. These materials are as different as the function they fulfill. Examples encompass calcium phosphate in bones for mechanical support, calcium carbonate in sea shells for protection against predators and magnetic minerals for orientation. Over the years, it has become evident that the biological materials not only have outstanding properties when compared to man-made materials of similar composition but also that they are also formed under physiological conditions. Accordingly, materials scientists can learn from the design principle to produce engineered materials with reduced ecological footprints when compared to current state of the art techniques. In my group, we thus study the formation of these biological materials and their unmatched properties as well as we test extracted principles to form similar materials synthetically.

Biological Materials

Biomineralization is typically of primary importance for the biomineralizing organisms as stated above. It may even be vital and as such, it may be impossible for example to completely turn off bone formation to study the associated mineralization pathway. In contrast, biomineralization in unicellular organisms can be turn on and off. This is in particular the case in coccolithophorid algae or magnetotactic bacteria. In particular discovered that magnetosomes in the early stages of their formation when they are derived from the main constituent building the mineral (Calcium for the former and Iron for the later). In addition, these organisms play strategic geological roles for CO₂ fixation or in the Fe-cycle. In the last years, we have studied the mineral formation in these organisms by a variety of analytical approaches as described below.

Calcite Biominnralization in Coccolithophores

Coccoliths (Fig. 1) are calcite crystals produced by coccolithophores, which are a group of unicellular algae representing a major part of the marine phytoplankton with potential effect on the geological sequestration of carbon dioxide. Each cell is surrounded by several coccoliths [1]. The biological function of coccoliths is currently unclear.

The mechanism leading to the formation of the coccoliths has remained unclear, in particular the exact pathway followed by Calcium has remained elusive. We have thus developed an approach minimizing potential artefacts to follow the dynamics of the process. We studied cryo-preserved cells by X-ray absorption spectroscopy, X-ray imaging, focused ion beam sectioning coupled with scanning electron microscopy imaging, analytical transmission electron microscopy and optical microscopy. Thereby, we identified a compartment that is distinct from the coccolith-producing compartment, and which is filled with high concentrations of a disordered form of calcium [2]. Surprisingly, we did not observe carbonate co-localized Calcium but rather phosphorus. We will continue to analyze the role of this intermediate in the future.

In parallel, we studied the role of the so-called baseplate, which is an organic template onto which calcite nucleate in vivo. This template was expected to spatially direct the mineralization of calcite to its outermost region (Fig. 1). We showed that a specific interaction between soluble biological determinants and this baseplate indeed directed Calcium-based compounds to this region, but that these compounds were at least initially non-crystalline [3]. The macromolecules therefore here do not control mineralization, but already play a critical role prior to it.

References:

Magnetite Biominalrization in Magnetotactic Bacteria

Magnetotactic bacteria (Fig. 2) are a group of microorganisms that synthesize and organize magnetic nanoparticles called magnetosomes [4]. The magnetosomes are membrane-enveloped magnetite (Fe₃O₄) or greigite (Fe₃S₄) nanoparticles that are supposed to help the cells navigating along the magnetic field lines of the Earth’s magnetic field to reach their preferred conditions at the bottom of lakes / seas [5].

One of our long-standing works has been to elucidate the chemical route by which magnetite is intracellularly formed. In our most recent study, we investigated the early stages of magnetosome formation by utilizing advanced analytical electron microscopy techniques. We correlated the size and emergent crystallinity of magnetosome nanoparticles with the changes in chemical environment of iron and oxygen. We in particular discovered that magnetosomes in the early stages of biominalrization with the sizes of 5–10 nm were amorphous, with a majority of iron present as Fe³⁺, indicative of ferric hydroxide [6]. In turn, the magnetosomes with intermediate sizes showed partially crystalline structure with a majority of iron present as Fe²⁺ and trace amounts of Fe³⁺. These findings corroborate the results obtained on other species.
Magnetotactic bacteria do not simply form magnetosomes, they also arrange them in chain [7][8]. This feature, however, drastically complicates their division when they have only one flagellum or one bundle of flagella. In this case, the bacteria indeed have to pass on to their daughter cells two types of cellular polarities simultaneously, their magnetic polarity and the polarity of their motility apparatus. The specific magnetotactic bacteria magnetotactic bacteria solve this problem by synthesizing the new flagellum at the division site, a division scheme never observed so far in bacteria [9]. Even though the molecular mechanisms behind this scheme cannot be resolved at the moment due to the lack of genetic tools, this discovery provides a new window into the organizational complexity of simple organisms.

**Biomimetic Systems**

**Random Synthetic Magnetic Swimmers**

In the previous years, we have studied the formation of magnetite nanoparticles in a synthetic process but with a particular focus towards low-temperature process [10]. We also tried out several strategies to form 1D magnetic materials [11][12]. We now profit from our expertise to assemble nanoparticles and use these aggregate as steerable micro- to nanoswimmers.

In contrast to previously designed magnetically actuated devices that all are of helicoidally-shaped, ours are of random morphology [Fig. 3]. This enable the same materials not only to be used with different actuation strategies such as rollers, swimmers or propellers [13], but also to profit from their different properties to be able to actuate them concomitantly along independent trajectories [14]. We finally showed that out of a pool of such propellers, we could select particularly fast devices outperforming any state-of-the-art materials [15].

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[Fig. 2: TEM image of typical magnetotactic bacteria and their characteristic magnetosomes chains.](image)

[Fig. 3: TEM images of selected microswimmers (image from Vach et al., Nano Letters, 2013).](image)


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BIOLOGICAL AND BIOINSPIRED MATERIALS

Biomimetic Actuation and Tissue Growth

The ability of biological tissues to change both their external shape and internal structure, allows organisms to grow, to adapt to their environment, and even to heal damage. Shape changes may even perform a function in tissues without an active metabolism, such as found in seed dispersal units in the plant world (see the group Plant Material Adaptation of M. Eder). These processes of morphogenesis are mediated by biochemical and genetic signals operating within the physical constraints of their surroundings. The importance of these physical constraints has become clearer in recent years with the observation that cells and growing tissues indeed respond to mechanical signals. An important feature of mechanical signalling is that it can act at long range, meaning the shape of external constraints can be “felt” deep inside the tissue itself. Such mechanical signals may arise due to loads acting on the external boundary or even be created by active stress-generating processes occurring inside the tissue itself (see the Mechanobiology group of R. Weinkamer).

In our research group we explore how mechanical boundary constraints influence growing and swelling tissues. The research uses a combination of theoretical and experimental techniques and is performed in collaboration with several groups both within and without the department.

Tissue Growth

In previous work we have shown that cells respond to the curvature of the substrate to which they are adhered to (See refs [1-4] and earlier references contained therein). We use 3D printing techniques to produce scaffolds of controlled geometry and have shown using cell culture experiments on these surfaces, together with theoretical modelling, that surface curvature influences the microstructure of tissue as well as its local growth rate. Due to experimental restrictions, in our early work we explored the response of growing tissues to rather simple geometries consisting of prismatic pores. As these pore have straight sides, one of their (starting) surface curvatures is zero, which allowed us to model the role of curvature in 2D. To explore more thoroughly the role of curvature in 3D, we have extended our modelling approach to 3D [1]. In a collaboration with D. Fischer (Montan University Leoben) we have used these 3D models to highlight that when growing tissues are considered as viscous fluids then a simple pressure based model for growth can explain many features observed in the process of bone healing (Fig 1).

From an experimental perspective we have also explored how stem cells respond to curvature [2]. Our results indicated that the controlled response we observed previously in osteoblasts can be generalised to other similar cell types (collaboration C. Werner, Dresden). The role of surface geometry on extra-cellular matrix (ECM) organisation was further explored in a collaboration with two former group members (C. Bilden, UJF Grenoble, and P. Kollmannsberger, P. ETH Zurich). In this study we used novel ECM labelling method to explore the temporal sequence of ECM deposition in pores of controlled geometries [3] and showed that not only cells but also ECM components are influenced by shape.

**Fig. 1:** Three example configurations of simulated “bone-healing” after osteotomy [1]. Tissue is shown in red and bone is shown in grey.

**Fig. 2:** A maximum projection of tissue grown on a constant mean curvature surface (MC3T3 pre-osteoblasts). Tissue is stained for actin and imaged using a light-sheet fluorescence microscope. Scale bar 400 µm.
culture experiments. Interestingly tissues growing on these surfaces spontaneously form chiral patterns of cell alignment (Fig. 2). This seems to be a collective response of cells to geometric constraints, and highlights how cells in 3D can self-organise in ways much akin to liquid crystals. These ideas are supported by work we have done in developing active particle models confined to surfaces in 3D [7], where we see a strong coupling between surface curvature and particle ordering on curved surfaces (Fig. 3). It is hoped that these observations and models of pattern formation in 3D will help in the understanding of complex 3D architectures observed in many tissues e.g. [8].

Fig. 2. Active particle simulations on ellipsoidal surfaces reveal an intrinsic coupling between particular geometric features (a) umbilic points (in red) and the dynamics of vortices highlighted in yellow in (b) that appear due to the collective movements of interacting particles [7].

Actuation
Macroscopic shape changes also occur in tissues due to differential swelling of spatially separated regions inside a tissue or organ. This actuation behaviour is well illustrated by the example of the ice-plant [9], whose seed-dispersal unit is powered by the swelling induced by liquid water. The unique diamond lattice cell structure of this tissue converts isotropic expansion into anisotropic tissue motion thus opening the capsule. We used advanced multi-material 3D printing of swellable and non-swellable polymers, combined with finite element simulations to investigate the role of cell shape on the actuation behaviour [9,10] (see Fig. 4 for an example).

Fig. 4. Swelling of a 3D printed honeycomb structure containing of transparent swellable and white non-swellable materials [9].

We are also working with the plant biomechanics group of M. Eder, to model the actuation behaviour of other plant tissues. For this we mainly focus on developing theoretical tools to simulate plant organ actuation based on segmented micro CT mages. In addition to investigating natural actuators, together with polymer chemists, we have explored how shape and structure can influence actuation of artificial polymeric materials. In two separate collaborations with the groups of J. Yuan (Colloid Department) and L. Ionov (Georgia University, USA) we have investigated the role of internal structure and external geometry on the actuation of planar polymeric films [11-14]. The group of J. Yuan have developed porous poly-ionic liquid (PIL) actuators that respond to multiple solvents extremely rapidly [11]. Actuation is achieved via a chemical gradient across the membrane. By modifying the internal microstructure of these membrane actuators using carbon nanotubes [12], or cloth [13], it is possible to control and direct actuation in particular directions with respect to the principle fibre orientation. An alternative way to control actuation is to control when actuation occurs in a particular location. These concepts were explored together with L. Ionov who produced actuating polymeric bi-layers with a variety of different geometries [14]. As these bi-layers start swelling from the edges, different geometries (i.e. the presence of holes) gives rise to different rates of swelling which in turn force the membranes to roll or fold into non-equilibrium configurations. This opens up the possibility of using geometry to program multiple 3D states of an actuator, which could be interesting for a variety of applications such as soft robotics and cell manipulation.

Our group, together with the group of M. Eder, have also been involved in research and teaching within the context of the DFG excellence cluster Bild Wissen Gestaltung in the area of Active Matter. With the biologist T. Stach and cultural historian C. Vagt (Humboldt University) we have been investigating the structure of self-moving materials from a variety of different perspectives. We focus on understanding two biological examples, the house of the tunicate and the opening of seed protecting structures in plants, and equivalent structures in architecture. On one hand the interaction with scientists from very different disciplines can give new insights into our understanding of our biogical materials, but also we hope can also inspire new concepts in design and architecture.


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Mechanobiology

The ability of biological materials for structural adaptation and self-healing depends often on an adequate response to mechanical stimulation. Mechanobiology studies this link between a mechanical stimulus and the resulting structural change in biological systems. Bone is a relevant mechano-responsive material not only from a material science perspective, but also due to important medical implications. Age-related bone loss is a serious problem in our aging Western societies. Although structural adaptation of bone was already described more than 100 years ago by Julius Wolff, only now serious attempts are undertaken to obtain a more quantitative description between the local mechanical stimulation and the probability to resorb or deposit bone at this location. Wolff’s law states a higher probability for bone formation at sites of high mechanical loading, and preferred resorption at locations of low load. Aiming at a quantitative formulation of Wolff’s law, most fundamental questions that are arising concern the universality of such a formulation: Does Wolff’s law depend on the species and/or the skeletal site, does it change with age?

The structural adaptation of bone is enabled by specialized cells. Beside the bone resorbing and bone forming cells (osteoclasts and osteoblasts, respectively), osteocytes recently receive particular attention of researchers. These most abundant bone cells are embedded in the mineralized bone matrix. They use a network of thin channels – the canaliculi – to connect with each other via their long cell processes. Multiple functions have been attributed to this osteocyte network: (i) mechanosensation via the detection of the fluid flow through the canaliculi; (ii) contribution to mineral homeostasis by using the large surface area of the network; (iii) transport of nutrients and signaling molecules. The aim of the research group is to obtain a more quantitative description of how mechanical stimuli influence processes in bone. Using a combination of experimental and computational methods, the research focuses on the processes of bone remodeling and healing.

References:


Mechano-regulation of Bone Remodeling

With the aim of an experimental assessment of Wolff’s law, experiments on living mice were performed at the Julius Wolff Institute, Charité, (Bettina Willie, Sara Checa). Multiple micro-computed tomography images with a time lapse of about 5 days allowed the determination of the exact location where bone was remodeled [1]. An non-invasive in vivo loading device combined with finite element calculations provided the information about the local mechanical stimulation in the same mouse bone [2]. The combination of the information where bone was remodeled and how large the mechanical stimulation was at this site allows a quantitative assessment of the mechano-regulation (Fig. 1). The experiments were performed in mice of three different age groups (young, adult and elderly). The comparison of the mechano-regulation in adult and elderly animals demonstrate that in both age groups remodeling is mechanically regulated with the highest probability for bone formation at large mechanical stimulation (strain), while low mechanical stimulation results preferentially in bone resorption. However, the mechanical control of remodeling becomes more dysregulated with age. This is most obvious by the broader range of strains where both formation and resorption occurred in the elderly animals [3] (Fig. 1). Evaluation of remodeling events on the same long tube-like bone, but distinguishing events at the inner (endosteal) surface of the hollow tube compared to the outer (periosteal) surface showed a reduced mechano-responsiveness of the periosteal bone surface [4].

Structural Analysis of the Osteocyte Network

To make progress towards an understanding of the cellular implementation of the mechano-regulation in bone, we analyzed the structure of the osteocyte lacuno-canalicular network (OLCN). It is thought that fluid flow through this network caused by the loading of the bone is sensed by the cell processes of the osteocytes. The investigations focused on human osteons, the cylindrical building blocks of cortical bone formed during remodeling (Fig. 2). The network structure is imaged using rhodamine staining followed by confocal laser scanning microscopy. An image analysis provides information about the density and connectivity of the network, and its orientation with respect to the overall osteon structure [5]. The network was found to be impressively dense with one cubic center of healthy human bone comprising a network of 74 km in length. However, osteons show a substantial variability of the canalicular density with roughly 10% of the volume having a canalicular density twice the average value and large regions lacking an accessible network. Hints about the formation of the network are provided by our observation that the network density increases in direction of bone formation from the outer cement line towards the Haversian canal [6]. Future investigations have to show the diagnostic potential of our method, in particular with respect to a structural deterioration of the OLCN with age.
Bone Structural Adaptation and Healing

Implementing a simple version of Wolff’s law into a computer model, we studied the influence of mechanoregulated bone remodeling on the structural stability of foam-like trabecular bone. Our simulations demonstrated that the rather complex loading pattern in the dynamic foam-like structure does not support the commonly believed hypothesis that thinner trabeculae are mechanically protected from resorption. Remodeling rather led with age to structural deterioration by preferential loss of trabeculae in confined regions [7].

The jaw bone offers a particular interesting example of bone structural adaptation. Alveolar bone comprises the thickened ridge of the mandibular and maxillary bones that serves as primary support structure for teeth. The maintenance of the alveolar bone relies completely on a continuous mechanical stimulation due to mastication with mechanical disuse resulting in alveolar bone loss. The high mechanobiological sensitivity of this bone and high remodeling activity enables fast tooth movement, but has also detrimental effects on the progression of periodontal diseases. Together with the Japanese company LION Corporation, we studied the effects on the progression of periodontal diseases. Together with the Japanese company LION Corporation, we studied the effects on the progression of periodontal diseases.

beside adaptation, biological materials exhibit fascinating healing abilities [10]. During bone healing the differentiation of stem cells migrating to the fracture site is another process which is mechanically influenced. With computer simulations we tested hypotheses how mechanical stimulation is regulating bone formation, which can occur either directly by the action of osteoblasts or indirectly via a transient formation of cartilage. Comparing the simulation results with experiments on sheep bone showed that the healing process is so robust that active mechanoregulation only during crucial healing phases is sufficient for a successful healing outcome [11].

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Fig. 2: Top, circular structure of a human osteon with the central Haversian canal (red circle) housing a blood vessel. The red fluorescence signal shows further the lacunae, in which the cell bodies of the osteocytes find their place, and the fine canals (canaliculi) for the cell processes. The green second harmonic generated signal of collagen highlights the lamellar arrangement of the bone matrix. Scale bar 30 µm. Below, three-dimensional rendering of the canalicular network within an osteon as the result of the image analysis. Lacunae are shown in blue, canaliculi are colored from blue to red depending on their distance from the Haversian canal (green).

Fig. 3: The mineral nanostructure of alveolar bone and tooth of a diabetic KK mouse obtained by scanning SAXS/WAXS experiments. The 5 lines correspond to 5 horizontal scans from the buccal side (left) to the lingual side (right). The position of the tooth in the middle of the plots is highlighted by the gray shading. The black line corresponds to the uppermost scanning line closest to the tooth crown. Obtained parameters included the mineral particle thickness T (top) and the degree of mutual alignment ρ of the particles (bottom).

Understanding structure-function relations within biological materials highlights biological, physical and chemical principles, which can be beneficial for bio-inspired materials research. In our group, we use combinations of materials science approaches (i) to answer 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. 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), X-ray fluorescence (XRF), polarized light microscopy (PLM), confocal laser scanning microscopy (CLSM), electron microscopy (EM), 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).

### Fragility and Toughness of Bone

Bone material exhibits a complex multiscale arrangement of mineralized collagen fibrils. In the prevention of fractures, the most important mechanical property is toughness, which is the ability to absorb impact energy before complete failure. Toughness depends in a complex way on the internal architecture of the material on all scales from nanometers to millimeters. The related mechanisms include plastic deformation of glue-like organic layers between mineral platelets and fibrils, micro cracking, crack deflection and crack bridging. Therefore, bone fragility has several different mechanical causes. We described these mechanisms for bone material and put them in a clinical context [1].

### 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. We investigated bone during healing by means of µCT and different two-dimensional methods [2]. Backscattered electron images (BSE) were used to assess the tissue’s calcium content and served as a position map for other experimental data (NI and SAXS). Together with visualization experts from Zuse Institute Berlin we developed a software package enabling a combined visualization of information from these two-dimensional methods and three-dimensional µCT data. Fig. 1 shows the combination of (a) µCT, (b) BSE-images and (c) the combination of (a), (b) and corresponding X-ray scattering data.

**Fig. 1: Bone healing visualized by a combination of 3D and 2D methods**

(a) µCT image of an osteotomized rat femur, (b) backscattered electron microscopy image of a longitudinal section from the same femur as shown in (a), (c) combination of images (a) and (b) together with results from SAXS measurements: Color-coded measurement points represent the mean mineral particle thickness (T). The degree of orientation (α) and the predominant particle orientation are denoted by the length and orientation of the bar.

### Mineralization in Healthy and Diseased Bone

The course of bone mineralization is a crucial determinant that affects the properties of healthy and diseased bone. The detailed mechanism by which calcium is deposited during mineralization and removed during absorption is largely unknown. We investigated samples from patients with osteogenesis imperfecta (OI), also known as brittle bone disease. This 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 VI and compared it with a control group [3]. Main characteristics of OI type VI were (i) the coexistence of a highly mineralized bone matrix with seams showing abnormally low mineral content and (ii) a heterogeneous population of mineral particles with unusual size, shape and arrangement, especially in the region with lower mineral content.
Hybrid Materials with Specifically Designed Interfaces to Improve Mechanical Properties

Hybrid materials consist-like bone-at the nanoscale of an inorganic phase embedded in an organic matrix. In order to imitate such interfaces in synthetic materials similar to those of nature and to achieve enhanced mechanical properties, we produced hybrid materials with partners from HU Berlin (Prof. Hans Börner). This model system is based on magnesium fluoride nanoparticles (MgF₂) embedded in a PEO matrix [4]. The interface between these two phases consists of conjugates with a peptide side adhering specifically to the inorganic surfaces of the nanoparticles, and the PEO side bonding well with the matrix. In tensile tests we could show that stiffness and toughness of the composites increased with the amount of conjugate. Pure PEO showed a modulus of elasticity of about 700 MPa, an addition of 15% MgF₂ particles increased the elastic modulus to about 820 MPa (Fig. 2). These values rose to a maximum of approx. 1400 MPa due to the functionalization of the particle surface with 3 mol% conjugate. The addition of MgF₂ particles reduced the toughness compared to pure PEO as expected. However, the toughness of the hybrid material increased through the peptide-polymer conjugates, but there the maximum is reached at about 1 mol% conjugate [5].

Fig. 2: Elastic modulus of a hybrid material, made from a PEO matrix, MgF₂ nanoparticles (15 wt%) and interface conjugates. The elastic modulus increases with the amount of conjugate from about 700 MPa (pure PEO) to 1450 MPa (3 mol% conjugate). (Prof. Hans Börner). This model system is based on magnesium fluoride nanoparticles (MgF₂) embedded in a PEO matrix.

Nanocrystalline 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 CaCO₃ phase into nano-crystalline calcite [6]. The nano-crystallinity of the formed calcite minimized structural anisotropy and resulted in greatly reduced birefringent effects (Figure 3a). We examined the corresponding structural changes by mapping local lattice parameters and size of the calcite crystallites within the individual microlenses [7]. The driving force for producing a crystal size of around 10 nanometers in calcite is the minimization of residual stresses and the associated elastic energy by plastic deformation involving grain boundary formation and twinning. Local strains originate from the transformation-induced macroscopic volume changes (Fig. 3b), which arise due to differences in the specific volume in ACC and calcite, mostly due to water loss and short-term atomic rearrangements. These MLA represent a striking example of a stress-engineered nanocrystalline material produced by almost no energy costs by phase transformation.

Interestingly, also nature utilizes CaCO₃-based materials to produce optical functional materials. In a review, we illustrate basic strategies to produce such optical functional materials by manipulating the material structure [8]. These strategies are driven by the aim to eliminate or reduce the birefringent properties of calcite.

Fig. 3: Nanocrystalline CaCO₃ microlens arrays. (a) scheme of the light path through one nanocrystalline microlens. (b) map of relative lattice distortions (in percent), Δc/a showing relative differences in lattice parameters [8].

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Nanostructuring of Inorganic / Polymeric / Biological Interfaces

Today increased interest of scientists is focused on dynamic, non-equilibrium processes and materials. It involves needs for effective energy conversion with the focus on oscillation of inorganic's properties, chemical networking, autoamplification reactions, mimicking living systems, using cell metabolic life inspiration and ions, protons, concentration gradients. These themes are investigated in the group through three different topics: 1) non-equilibrium methods for solid mesoprocessing, 2) coupling of light and pH to regulate soft matter dynamics; and 3) dynamic, self-adaptive and stimuli-responsive systems for nanoscale biomanufacturing. We collaborate with scientists in the MPIKG, other German Institutions and abroad. Our main collaboration partners are Prof. H. Mühwald, Emeritus Group (Interfaces) MPIKG; Dr. D. Andreeva, Institute of Basic Science in Ulsan; Prof. D. Sviridov, Belarusian State University, and Prof. G.M. Whitesides, Harvard University.

Methods for Solid Mesoprocessing
We have a specific interest in nonlinearity solids’ engineering and propose to exploit complex nonlinear dynamics (example shown in Fig. 1) to achieve superior technological functionalities, metastable materials, which may be difficult or even impossible to achieve with linear systems. High intensity ultrasonic (HIUS) treatment of solids is an ideal target platform for mesoprocessing with possibility to control process dynamics by parameters of HIUS treatment: solvent and additives, intensity and duration [1-3].

Other non-equilibrium methods can be envisaged: hot pulsed plasma discharge, laser focused exposure together with electrochemical processing.

We use metal surface nanostructuring to guide cell behaviour [1] with J. Dunlop (Biomaterials, MPIKG)) that is an attractive strategy to improve parts of medical implants, lab-on-a-chip, soft robotics, self-assembled microdevices, and bionic devices.

Coupling of Light and pH to Regulate Soft Matter
We suggest to investigate photocatalytically triggered local pH changes in semiconductor / polyelectrolyte (PE) Layer-by-Layer (LLbL) assembled interfaces, mimicking natural processes in a novel design strategy for inorganic / polymer interfaces. We have shown recently [4-6] that under irradiation of TiO₂, a series of photocatalytic reactions leads to a local change in pH, which modulates the pH sensitive LLbL assembly. Prime questions are: (i) how many photons are needed to locally change the pH on titania? (ii) what is the optimum LbL architecture to understand the basis of proton trapping and storage, the pH gradient under local irradiation? And (iii) how to achieve reversible actuation of different assemblies for advanced applications? [4]

The efficiency of the multilayers’ response (Fig. 2) was investigated with atomic force microscopy (AFM), in situ quartz crystal microbalance (QCM) and ion selective micro-electrode technique (SIET) for mapping the activity of protons over the surface under local irradiation.

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Eshelby Twist in the Axial Filament of the Sponge Monorhaphis Chuni

We studied the highly-ordered crystalline protein/silica axial filament in the anchor spicule of the marine sponge M. chuni, Fig. 1. Using microbeam synchrotron X-ray diffraction analysis, we discovered a specific lattice rotation (so-called Eshelby twist) propagating throughout the entire proteinaceous structure. This finding, together with the dislocation-induced deformation field visualized by transmission electron microscopy (TEM), indicated the presence of a screw dislocation situated along the axial filament [1]. The dislocation-mediated spiral crystal growth is thermodynamically favourable at low supersaturation levels due to the permanent presence of the dislocation-related kink on the growing surface of the crystal. Apparently, only this mode of growth can provide reasonable growth rates (at low temperatures of deep water) needed to form a very long (up to 3 meters) crystalline structure of the axial filament, consisting of perfectly arranged protein units and amorphous silica building blocks. It is fascinating that processes occurring in nature (the protein/silica hybrid crystal growth) and in the field of inorganic man-made materials (growth of nanowires and perfect bulk crystals, such as silicon) independently converged to the dislocation-mediated spiral growth mode, which is favourable from the viewpoint of free energy minimization.

Static and Dynamic Mechanical Characterization of a Single Interface in the Prismatic Structure in the Shell of Pincta Nobilis.

Quantification of the local physical properties of internal interfaces in biological structures is complex. Except for nanoindentation-based surface techniques, there was no viable in-situ methodology to directly measure static and dynamic mechanical response of an individual sub-micrometer-thick interface. We developed an indirect experimental-analytical framework designed for (but not limited to) quantifying the elastic and viscoelastic properties of a single organic interface in biocomposite materials and demonstrated it on the calcite-organic composite prismatic structure in the bivalve shell P. nobilis [2-3]. The methodology is based on force-modulation of a micro-cantilever that contains an individual organic interface and is cut-out from the biocomposite using the focused ion beam (FIB) milling technique, Fig. 2. During measurement, a modulating force is applied by a standard nanoindentation instrumentation at different locations along the micro-cantilever. By applying a theoretical treatment, the resulting time-dependent flexural deflection is then used to extract the elastic modulus and the damping coefficient of the interface alone.

Fig. 1: 3D model of the protein/silica axial filament in the anchor spicule of the sponge M. chuni.

Fig. 2: A micro-cantilever milled from the prismatic structure in P. nobilis containing a single interface.

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INTERACTIONS OF WATER WITH BIOLOGICAL MATERIALS

Biological materials are constituted by molecular/supramolecular building blocks which are assembled at several hierarchical levels. They are often complex materials, which evolved to exploit intereactions with water and ions in a desired way. In my group, we address, from the molecular level upward, the effect of water and electrolytes on the molecular components of natural materials and on material’s mechanical properties. The aim is to describe the thermodynamic of the interactions and understand what molecular mechanisms are responsible for the observed responses, with particular focus on passive actuation of tissues. Because of the various hierarchical levels of organization, we developed (in collaboration with many groups of the department) in-situ, multi-technique approaches, from which we can obtain information from the molecular to the macroscopic level.

Wood and Actuation in Plant Tissues

Although water interactions with plant tissues is of extreme importance for technological and physiological reasons, to date, they have been described only phenomenologically, without taking into account tissues’ composition and structure. In the last two years, in collaboration with prof. Thomas Zemb (ICSM – Marcoule - France) we developed a new model, using a force balance approach which, starting from compositional and structural data, describes how, for fibre reinforced polymeric composites, the chemical energy associated to water interactions is used to overcome the work of swelling and can be converted into mechanical work. This approach allows to establish the full thermodynamics of the actuation for non-living plant tissues [1]. Also, we recently established a way to account for electrostatic effects (impregnation from electrolytic solutions) which allows to describe the ions specific effects in such tissues [2]. This knowledge has fundamental consequences in wood technology: it enables the possibility to design new treatments able to turn these processes on or off so that water uptake can be either promoted or avoided at specific humidity ranges. This force balance approach could be used also to describe the actuation of synthetic biomimetic systems [3].

Collagen Based Tissues

Another molecule strongly interacting with water is collagen, which is the most abundant protein in mammals’ tissues. In collaboration with A. Masic (MIT - Boston), we could describe how, by changing the collagen hydration state the molecule undergoes structural changes that result in the generation of tensile stresses up to 80 MPa. In mineralized tissues, this contraction of collagen puts mineral particles under compression leading to strains of around 1%, which implies localized compressive loads in mineral up to 800 MPa. Interestingly, as collagen partially dehydrates when mineralized, this mechanism could be in place to protect the mineral phase from tensile loads in collagen based mineralized tissues [4]. In the last year, we have started to investigate the effect of the temperature on the structure and stability of collagen in hydrated conditions.

Effects of Water on Mechanics

Water content has a major impact on materials’ mechanical properties and structure. We found that water acts as a plasticizer in the case of sucker ring teeth and this can be exploited to shape materials at very mild conditions (T below 100 C and high humidities). Once dried after shaping, the materials recover their original mechanical properties [5]. Similarly, the same plasticizing effect in the intermiphasic layer of Pinna nobilis makes water an essential player in determining explicit intrinsic and extrinsic toughening mechanisms of the mollusks’ shell [6]. If confined in small pores, water affects also the mechanics of harder inorganic materials. In collaboration with prof. P. Huber (TUH) we studied adsorption-induced deformation of mesoporous silicon and we developed a general model to relate the pore-load modulus to the porosity and to the elastic properties of materials [7].

3D Imaging of Organisms and Tissues

Lately, the activity of the group has been focussing on the imaging and 3D reconstruction with nm resolution of organs and tissues in quasi-native hydrated conditions by means of FIB/SEM in cryogenic conditions. Using this technique, we could discover in the coccolithophorid alga Emilia- nia huxley a new Ca-rich cellular compartment likely involved in the biominalerization pathway [8]. Also, we could determine the spatial relationships between the magnetosomes and the cellular membrane in magnetotactic bacteria [9].
Amorphous Calcium Carbonate (ACC): Crystallization and Phase Behaviour
First the influence of particle size on ACC crystallization was investigated, resulting in an opposite behavior on crystallization in solution and upon heating [1]. In the presence of water, crystallization is predominantly caused by a dissolution-reprecipitation behavior leading to the less stable polymorph with smaller nanoparticles, whereas upon heating the smaller spheres are more stable.

The dependence of ACC particle size on concentration and temperature was used to determine the phase behavior of ACC [2]. This led to the formulation of an unstable ACC region with a maximum at higher temperatures, which showed a good fit with spinodal decomposition theory. Using this data we also investigated the effect of commonly found additives like poly(aspartic) acid (pAsp), magnesium (Mg) and phosphate (PO₄) on the phase behavior of ACC. Both pAsp and phosphate have the ability to decrease particle size dramatically, whereas Mg has no effect at all. All changes in the phase diagram, however, were only observed with PO₄.

Amorphous Calcium Phosphate and Condensed Phosphates
In contrast to ACC, amorphous calcium phosphate (ACP) has a much more variable composition that depends on the pH of the reaction solution. Next to that it can transform into different calcium phosphate crystals with changing composition, which makes studying its properties as well as its crystallization a much more tedious task [3]. Additionally, especially in biology, also condensed phosphates like pyrophosphate and polyphosphate can be present, which are very poorly studied materials, and difficult to differentiate from amorphous calcium phosphate [4,5].

A common way of detecting condensed phosphates in Biology is by use of DAPI-staining. In a joint project with Sidney Omelion (Ottawa University), we discovered that the yellow staining is due to an autofluorescence effect upon concentration of DAPI [3]. A consequence of this result is that every negatively charged surface that is able to attract DAPI will show this fluorescence, inclusive polyphosphate but also amorphous calcium phosphate, whose negative charge is at the base of bone mineral structure [6].

Structural Studies on Amorphous Calcium Carbonate
To investigate the structure of amorphous calcium carbonate we focused on x-ray and neutron pdf-analysis. Neutron-pdf analysis allows us to look at the structural features of water, for which many speculations have been done.

In contrast to some studies we do not find any evidence for the presence of proto-structural features in any of the prepared ACC samples. The structure of ACC seems to be dominated by h-bonding between Ca and water, though above a certain water threshold we do see evidence for water clusters inside the ACC which are not h-bonded. More surprisingly, the local coordination of Ca with carbonates and water seems to resemble the arrangement of water molecules inside a saturated CaCl₂ solution.

References:
Physics of Biomolecular Interfaces

Biological tissues and cells are composed of diverse functional units such as organelles, protein complexes, and carbohydrate assemblies. They are often organized as functional interfacial molecular layers, the prototypical examples being biological membranes. In the congested biological environment membrane functions are sensitive to the composition of the aqueous milieu and to their mutual interactions.

But soft interfaces constituted by molecular layers play important roles also in context with bio- and wet-technological processes, for instance, when resulting from adsorption processes.

In our Emmy-Noether research group, supported by the German Research Foundation (DFG), we study the interaction of biological and technologically relevant soft interfaces with solutes (such as proteins) in their aqueous environment and also their mutual interaction in the aqueous milieu, with a specific focus on interactions involving biological membranes (see Fig. 1). 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 composition.

Fig. 1: Schematic illustration of a biological membrane interacting with a protein (left) and with the surface of another membrane (right).

X-Ray & Neutron Scattering Techniques and Complementary Computer Simulations

To address these questions, molecular-scale structural insight into the involved layers is required [1]. In order to obtain such information we prepare model systems of well-defined (bio-)molecular composition at solid/air, solid/water, liquid-water, and air/water interfaces [2-7] and study them with various structure-sensitive techniques based on x-ray and neutron scattering [2-6, 8]. In addition we employ complementary methods, such as ellipsometry, calorimetry, tensiometry, rheology, and spectroscopy. Finally, computer simulations carried out in collaborations provide a means to interpret experimental results on a mechanistic level [9-13]. To this end we have developed a simulation method that accurately accounts for the chemical potential of water between interacting surfaces. The simulation results have recently led to a better understanding of the long-debated “hydration repulsion” between phospholipid membranes [9-11] and of the tight cohesion between glycolipid-rich membranes, which naturally occur in the form of multilamellar stacks [13].

Protein Adsorption to Material Surfaces

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 soft hydrophilic polymer brushes like 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. Our results obtained after incubation with proteins highlight the importance of the brush parameters and the implications of PEG’s reported but often neglected antigenicity [2].

Fig. 2 (top) shows a set of reflectivity curves from a PEG brush in aqueous solution before (left) and after (right) incubation with solutions of anti-PEG IgG antibodies that can also be 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 H2O and D2O 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 top). The reconstructed protein density profiles (Fig. 2 middle) showed that the adsorption of antibodies occurred onto the brush itself (Fig. 2 bottom), an adsorption mode termed “ternary adsorption” in the theoretical literature [2]. In this configuration the antibodies display their Fc segment to the aqueous phase suggesting that foreign body reaction is promoted.

Depth Localization of Biologically Important Chemical Elements in Molecular Layers

In contrast to specular x-ray and neutron reflectometry, which reveal “global” matter density profiles perpendicular to soft interfaces and which today are widely used techniques, standing-wave x-ray fluorescence (SWXF) allows for the determination of density profiles specifically of chemical elements. The method is based on the standing wave (SW) created above a multilayered solid surface by interference of the
During a scan of the angle of incidence $\theta$ across the Bragg peak at $\Theta$, the maxima of the SW move along the surface normal and induce x-ray fluorescence with element-characteristic energies (see Fig. 3 A for a scheme of the experimental setup). The method thus allows reconstructing elemental depth profiles from the angle-dependent characteristic fluorescence.

While SWXF studies have so far dealt with relatively heavy elements, typically metal ions, as artificial labels for the molecular layers under investigation [8], we have made the technique applicable also to the comparatively light elements $S$ and $P$, which are found in the most abundant classes of biomolecules, for example in the headgroups of phospholipids (Fig. 3 B). Our measurements yielded element-specific insight into the architecture of various lipid monolayer architectures and into the conformations of proteins adsorbed to surfaces under various conditions [5]. Fig. 3 C exemplarily shows the angle-dependent P fluorescence (symbols) from a solid-supported phospholipid monolayer (panel D), together with the modeled intensity (solid line) corresponding to the best-matching average height $z_0$ of the P atoms above the solid surface ($z_0 \sim 4 \text{ Å}$, see Fig. 3 E). More recently we have used the same approach for interacting model membrane surfaces, composed of lipids and lipopolymers, of which the interaction was adjusted via controlled dehydration [6].

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Incident x-ray wave with the wave reflected from the periodic multilayers close to the Bragg condition.

Fig. 2: (top) Neutron reflectivity curves from a PEG brush in H$_2$O and D$_2$O as well as in H$_2$O/D$_2$O 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. (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. (bottom) Cartoon illustrating the interpretation of the density profiles.
BIOLOGICAL AND BIOINSPIRED MATERIALS

Adsorption of the Protein β-Lactoglobulin at Water/Air and Water/Hexane Interfaces – Effect of Solution pH and Ionic Strength

In many modern technologies, proteins play an important role. In particular, for the production of various foodstuffs, proteins are essential ingredients. For the tailored application of proteins and optimum solution conditions, fundamental knowledge is required. Although proteins do not decrease significantly the surface or interfacial tensions in foams or emulsions but rather the added surfactants, their presence is essential for the stabilization of these liquid colloidal systems. Due to the nature of foams and emulsions, knowledge not only on the equilibrium but also the dynamic interfacial properties of protein adsorption layers is of importance.

The adsorption of proteins at liquid interfaces is a process which does not only depend on the protein’s bulk concentration. Due to their nature, protein molecules adsorb at an interface and upon contact with the hydrophobic phase they can change their conformation. These conformational changes can affect also the dynamics of the adsorption process, including the response of the interfaces to mechanical perturbations. The so-called induction time, for example, depends not only on protein bulk concentration but also on the pH and ionic strength of the solution [1].

The present work was dedicated to the surface pressure isotherms for BLG solutions at three different pH values (3, 5, and 7), different ionic strengths and at two interfaces: aqueous solution to air (W/A) and to tetradecane (W/TD), respectively. Based on the data of interfacial pressure isotherms, the dynamic surface pressure dependencies $\Pi(t)$ are analysed using a new theoretical approach recently derived in [2]. The obtained adsorption parameters are discussed in terms of the pH effect and the particular impact of air [3] or tetradecane as the adjacent oil phase [4], respectively. In addition to the adsorption dynamics mechanism, also the relaxation mechanism due to compression/expansion perturbations was studied in [3, 5].

The interfacial tension isotherms of BLG adsorbed at the W/A and W/TD interfaces at the solution pH of 3, 5 and 7 can be well described by a thermodynamic model [1, 4]. All model parameters obtained by fitting the experimental data to a thermodynamic model are more or less identical for the three pH values, except the surface activity parameter $b$, which increases with the pH.

When protein molecules adsorb at the interface, they are subjected to conformational changes. Protein molecules first adsorb at the interface in a folded conformation. At low bulk concentrations, they have sufficient space at the interface to unfold. Unfolded protein molecules occupy a much larger interfacial area. Moreover, at water/oil interfaces the hydrophobic parts of the protein molecules have the tendency to penetrate into the oil phase which is supported by the conformational change. The consequence of this changed conformation is taken into account in terms of a change in the adsorption parameter $b$ in the corresponding adsorption model:

$$\frac{\Pi \omega}{RT} = \ln(1-\theta) + \theta \left(1 - \frac{\omega}{\omega_0}\right) + a\theta^2$$

where $R$ is the gas law constant, $T$ is the temperature, $\omega$ is the average molar area of adsorbed protein molecules, $a$ is the protein intermolecular interaction parameter, $\omega_0$ is the molar area of a water molecule, $c$ is the subsurface concentration of the protein, and $\Gamma$ and $\omega_0$ are the "partial" adsorption and molar area of protein in the state with the smallest molar area (native, folded).

The adsorption behaviour generally shows increasing interfacial pressure and dilational elasticity with increasing protein concentration. A comparison of the experimental data and model calculations of the adsorption behaviour of BLG at the W/TD interface with those at the W/A surface is presented in Figs. 1 and 2. The interfacial pressure of BLG adsorbed layers at the W/TD interface starts to increase at concentrations many orders of magnitude lower than that for the W/A surface. In addition, the isotherms at the W/A surface are much steeper as compared to the corresponding ones at the W/TD interface. While the adsorption isotherms at the W/A surface reach the critical point of secondary layer formation at rather low protein concentrations, at the W/TD interface the critical points are reached at much higher protein bulk concentrations. In addition, the corresponding interfacial pressure values $\Pi^*$ at these critical concentrations (kinks in the isotherms) are larger at the W/TD interface than at the W/A surface by almost a factor of three. Hence, one could conclude that the adsorbed amounts of protein at the W/TD interface are much larger than at the W/A surface.

Fig. 1 Experimental interfacial pressure isotherms $\Pi(c)$ of BLG at the W/TD (solid lines) interface and at the W/A (dashed lines) surface; blue lines - pH 7, black lines - pH 5, red lines - pH 3.
in activity coefficients, referred to as the TIC model, fails to controlled adsorption model with time-independent adsorption between theory and experiment. The classical diffusion con-sensitive PAT. The red line in the dynamical interfacial tension data measured by drop profile cules and the TD molecules as the oil phase.

However, the corresponding adsorption values \( \Gamma \) shown in Fig. 3 are only slightly higher and the molar areas shown in Fig. 4 slightly lower than those calculated for BLG at the W/A surface. Therefore, we must conclude that it is not the total adsorbed amount that leads to the high interfacial pressure values observed at the W/TD interface, but it is caused by the interfacial structure resulting from a strong interaction between the hydrophobic parts of the adsorbed protein molecules and the TD molecules as the oil phase.

Also a new diffusion controlled model to describe the protein adsorption kinetics was proposed to improve the agreement between theory and experiment. The classical diffusion controlled adsorption model with time-independent adsorption activity coefficients, referred to as the TIC model, fails to adequately describe our experimental results. As one can see in Fig. 5, the dashed lines calculated for different diffusion coefficients do not adequately describe the experimental data.

In contrast, the new model with a time-dependent (or surface-coverage dependent) adsorption activity coefficient \( b(G) \), named as the TDC model, can be successfully applied to the dynamic interfacial tension data measured by drop profile analysis tensiometry PAT. The red line in Fig. 5 represents an example for the excellent quality of data interpretation.

The results make clear that the measured dynamic interfacial tensions cannot be properly described by a pure diffusion controlled model. In contrast, the proposed combined model of diffusional transport and an additional time process for conformational changes can describe the experimental data properly. The model assumes that the conformational changes of adsorbed protein molecules can be reflected by changes in the adsorption activity coefficient \( \phi \).

The rate (kinetic) constant k obtained by a best fitting of the experimental data using the proposed TDC model depends on the BLG bulk concentration and on the solution pH. The resulting kinetic rate constant for BLG solutions is the smallest at pH 5 (negligible net charge and compact molecular structure), which physically means that the protein molecules change their conformation at the interface to the smallest extent at these conditions. In contrast, it has the largest values at pH 3 (highest net charge and increased affinity to the adjacent bulk subphases) which means the conformational changes of the proteins are most considerable.

New experiments have been performed at a third interface, the solution surface to air saturated with alkane vapor. The presence of oil molecules adsorbed from the vapor phase, provide a special atmosphere that influences the protein adsorption process tremendously. These effects will be further investigated in up-coming experiments.


References: