

Standing Committees for

- Life, Earth and Environmental Sciences (LESC)
- Physical and Engineering Sciences (PESC)

ESF Exploratory Workshop on

**Biom mineralization:
From Biology To Materials**

SCIENTIFIC REPORT

Århus, Denmark, 11 - 14 May 2007

Convened by:
Henrik Birkedal

Executive Summary:

The ESF exploratory workshop *Biom mineralization: from biology to materials* involved 18 researchers from 9 countries. The topic of the workshop encompasses several fields of research reaching from biology to synthetic materials science while crossing medical aspects along the way. The workshop touched upon a broad range of the implicated fields – a key purpose of the workshop being to bring together a diverse group of researchers to create a common ground.

The talks covered the biology of sponges (Ilan) and the discovery of ferromagnetic inclusions in some sponges (Maysel). There has recently been a development and/or improvement of experimental tools for studying complicated materials such as biom mineralized tissues. At the workshop advances and applications of state-of-the art NMR techniques (Duer, Brunner), small angle X-ray scattering (Birkedal, Gupta), nanoindentation (Schöberl) and synchrotron scattering and imaging techniques (Epple) were presented. These techniques were clearly shown to provide novel insights into the complex materials that were the focus of the workshop. The organic matrix is of key importance for structure and properties of biom mineralized materials and the characterization of the biochemical make up of the organic matrix is of key importance for the field (Marin, Giraud Guille) as is also the influence of the organic matrix on mineral organization (Birkedal, Gupta, Epple, Duer, Brunner) and mechanical performance (Gupta, Schöberl). Mineralization in single cell organisms plays an important role in geology and a better understanding of e.g. the role of fossil organic matrix in chalk is likely to be of importance for improved oil recovery from oil fields in e.g. the North Sea (Stipp). The biom mineralized materials are a source of inspiration for the synthesis of novel materials e.g. by direct use of the organic matrix from sponge spicules (or in recombinant form) (Tremel). Further manipulating organic matrices, in particular that of crab cuticle is one promising avenue towards biomaterials (Reis). The manipulation of crystallization by organic mediators and/or control over kinetics can lead to new oriented assemblies of crystals dubbed mesocrystals (Cölfen), a concept which may also be of relevance in biological mineralization. Further insights into the role of the organic matrix can be gleaned from model studies by advanced cryo-TEM studies of organization at the air water interface (Pichon). Studies of silification in biology combined with physico-chemical insights into oligomer formation has allowed making novel silica and aluminum oxide materials (Perry). Calcium phosphate materials are important in bone and teeth medical research and routes to make such materials with diverse compositions, phases and crystal sizes have been developed (Boanini). The sugar based organic matrices of some biological systems can be used to make matrices for synthesis of materials very remote from biology such as high T_c superconductors that are normally made by high temperature syntheses (Hall). This very diverse set of topics made for a very exciting whole – the participants were very active in discussions that proceeded in a constructive atmosphere. The participants generally expressed satisfaction with the theme and format of the workshop and it is the belief of the convenor that new collaborative efforts will be generated from the workshop in the near future.

The organizer wishes to thank the ESF for its support and the ESF overseer, Prof. Beschkov, for his kind participation and for his interesting input on the ESF organization.

Scientific content of the event

The talks presented at the workshop covered areas from biology of sponges to bioinspired synthesis of potential superconductors. A main goal of the convenor was to bring together a broad group of researchers to establish a common ground in the very diverse field of biomineralization and bioinspired synthesis. Hence there were speakers focusing on biology, on experimental methodology and on synthesis. Below I summarize the scientific content of the event with these divisions. Some of the material discussed below is unpublished and this report should therefore be considered confidential.

Many biomineralized systems are hierarchical and structured on the 1-100 nm length scale. Small angle X-ray scattering (SAXS) probes this length scale and is very useful for the study of biomineralized system as the signal originates from electron density differences on this length scale. As the inorganic mineral phase most often is more electron dense than the surrounding matrix, SAXS provides insight into the shape, size and orientation of the mineral crystals. Scanning the sample through a restricted beam size provides spatial resolution for the study of inhomogeneous materials, such as biomineralized tissues, of the order of the beam size. This technique, scanning SAXS, has proven particularly useful in studies of bone. Henrik Birkedal (Denmark, the convenor) described laboratory experiments (see e.g. ¹) and the development of a new model for interpreting the SAXS signal from bone². Investigations of the femoral growth plate in pigs provided a clear picture of how the thickness of bone crystals evolves with bone age. The data also showed a surprisingly large degree of alignment of the bone crystals around the growth plate, an observation that does not immediately fit with current models of the structure of calcified cartilage³. H. Gupta (Germany) also showed how scanning SAXS provides insights into bone structure and described synchrotron experiments, where the higher flux allows using smaller beams that in turn gives better spatial resolution than for lab-based experiments. By stretching bone samples and measuring SAXS and apatite wide angle diffraction at the same times allow determining how stress is distributed over the individual components – fibers and crystals⁴. The SAXS/WAXS experiments described by Gupta of bone under mechanical stress showed that the hydroxyapatite take up only a part of the load. For dry samples, the fraction of the mechanical load carried by the crystals is larger than for wet samples. By further measurements, Gupta showed that plastic deformation is a thermally activated process⁵.

Solid state NMR is a rapidly developing technique that probes the local environment of the target nucleus irrespectively of whether or not it is crystalline. Several biominerals contain nuclei well-suited for NMR: ³¹P in bone and ²⁹Si in the silica containing species such as plants, diatoms and sponges. By advanced cross-polarization sequences the excitation of one nucleus can be transferred to a neighbor of a different kind thus providing direct measures of proximity. This is one of the most direct ways of obtaining insights into the elusive mineral/matrix interactions. E. Brunner (Germany) discussed measurements on diatoms and on model systems of the silification process. Diatoms produce about 30-40% of the oceans primary organic production and are thus of enormous importance for the biogeological cycle of carbon. Several species can be held in culture and E. Brunner discussed measurements of several species grown in the laboratory in a manner that allows ²⁹Si labeling hence strongly increasing signal strength. In *C. granni*, ²⁹Si {¹H} HETCOR (correlation spectroscopy involving Si and H), Brunner showed that the obtained results depend on

¹ M. H. Büniger et al. *Bone* **2006**, 39, 530-541; Büniger et al. *Eur. Cell. Mater.* **2006**, 12, 81-91.

² Birkedal, Büniger & Skov Pedersen **2007**, in preparation.

³ Büniger et al. **2007**, in preparation.

⁴ Gupta et al. *Nanolett.* **2005**, 5, 2108; *Int. J. Fract.* **2006**, 139, 425; *PNAS* **2006**, 103, 17741.

⁵ Gupta et al. *J. Roy. Soc. Interface* **2007**, 4, 277.

sample preparation. By studying air dried samples, as opposed to the freeze dried samples studied previously in the literature, he could show that tightly bound water protons correlate with all Si sites including the fully saturated Q⁴ (Si(O-)₄) and the partially saturated Q³ (Si(OH)(O-)₃) and Q² Si sites. This shows that the silica of *C. granni* is in fact porous and can be penetrated by water. In studies of *T. pseudonana*, time series (by dosing the ²⁹Si label in rhythm with the growth cycle) showed that the cell wall is partially polymerized silica. Brunner speculated that it may in fact be in the form of silica nanoparticles. By further ¹³C and ¹⁵N labeling, Brunner investigated the organic matrix of *S. turris*. About 12 wt% of the cell walls is organic material and 13C solid state NMR showed that groups corresponding to peptides, proteins and sugars were present, possibly in the form of glycosylated silaffins. Some of the organic material is water accessible while some is not. The organic matrix is in part formed by silaffins such as silaffin-1A which is phosphorylated and forms aggregates as shown e.g. by ³¹P solution NMR line width analysis as a function of salt concentration⁶. The molecules are polyamines displaying a large degree of variability between species⁷. The self assembly by a combination of phosphate and polyamines into spheres could be extended to simply polyamines such as polyallylamine⁸.

Melinda Duer (UK) also used solid state NMR but to study bone. From the average crystal structure of geological hydroxyapatite, a geological model of the mineral phase of bone, one would expect a single sharp ³¹P peak in solid state NMR. This is not observed, rather a distribution is found corresponding to the presence of several P sites in the crystals. Advanced DFT calculations indicate that there are large rearrangements due to the ultrasmall crystal size in bone apatite. Double quantum spectroscopy, which allows mapping correlations between peaks, show that there is order to P distribution. This lends support to the surface restructuring model proposed by the DFT calculations. ¹H-³¹P correlations showed that there are HPO₄²⁻ substitutions (suggested to be surface states). In humans, old bones display a shoulder on the OH⁻ peak and a water peak as compared to young bone. This suggests that some changes in the mineral may take place with age, which could in turn be related to the poorer performance of old bone. By doing ³¹P-¹³C REDOR correlation spectroscopy, which is very hard to do on natural abundance samples such as bone and thus represent the absolute state of the art, Duer could shed light on the crystal/matrix interface. The results show that sugars are most strongly bound to the surface suggesting that proteoglycans may be the part of the matrix that directly binds to the crystals. This is an extremely important result in bone biology in the view of the convenor. Only further away (at longer correlation times) are ³¹P collagen correlations suggesting that collagen is only in the 'second coordination sphere' of the crystals.

Many biomineralized tissues play a mechanical role. To obtain a full picture of the relationship between mechanical properties and chemical composition and structure, local measures of the mechanical properties are desirable. T. Schöberl (Austria) discussed the use of nanoindentation (or instrumented indentation) to measure local mechanical properties. He first presented data on teeth where there is a good accord in the literature for measurement made on dry samples but a large variation on values obtained for wet samples. This raises the question of how one should hydrate the sample for nanoindentation in order to be as close to the biologically relevant state as possible. For studies of modulus as a function of distance from the dentin-enamel junction, there was a qualitative agreement in terms of distance dependence between wet and dry samples, but the individual measurements varied by a factor of two between wet and dry, the latter being the stiffest. The difference is larger for the hardness where dry samples were found to be three times harder than the values obtained by full immersion in buffer (but note that the true biological

⁶ Kröger et al. *Science* **2002**, 298, 584.

⁷ Sumper et al. *FEBS lett.* **2005**, 579, 3765; Sumper & Brunner *Adv. Func. Mater.* **2006**, 16, 17.

⁸ Brunner et al. *PCCP* **2004**, 6, 854; Lutz et al. *PCCP* **2005**, 7, 2812.

hydration state is probably in between – and unknown). Schöberl then showed data for the Cu-salt reinforced jaws of the marine worm *Glycera*⁹ and the non-mineralized jaws of the desert locust and the marine worm *Nereis*¹⁰. The latter two are interesting as they show how hardness and stiffness can be controlled by metal coordination chemistry even in non-mineralized systems. For the desert locust, Schöberl found a large difference in abrasive properties between dry and wet samples in the Zn-replete parts but no difference in the Zn-poor regions. This difference was smaller in *Nereis* indirectly hinting that the underlying structures may be different. The abrasion rate appears to generally be reduced in the wet state vs. dry but the size of the change varies from case to case. This indicates that the water content can be optimized for minimum abrasion and further strengthens the image of water as an important player in both structure and mechanical properties of hard biological tissues.

M. Epple discussed how synchrotron instrumentation can provide valuable insights into biomineralized tissues and discussed the use of synchrotron X-ray powder diffraction, single crystal diffraction, Extended X-ray absorption fine structure (EXAFS) and microtomography (μ -CT). He presented four examples: a freshwater snail, *Biomphalaria*, statoliths in the jellyfish *Periphylla periphylla*, woodlice and fish bones. In *Biomphalaria*, the snail house is CaCO₃ aragonite with traces of vaterite (<1 wt%). The observation of vaterite naturally poses the question of how the shell formation takes place: which polymorph comes first? When does the shell start to crystallize. SEM showed that shells form between 48 and 60 h after oviposition but that these are soft and not very mineralized. After 96 h the shell is ‘hard’ and after 120 h a ‘proper’ shell has developed. X-ray powder diffraction showed peaks corresponding exclusively to aragonite at 140 h (hatching) but none at 72 h. At 72 h EXAFS indicate that the CaCO₃ is amorphous but with local aragonite clusters. This suggests that the final polymorph structure is programmed into the amorphous precursor phase as was also pointed out by Perry in the discussion. Finally the shell evolution could be followed at about 1 μ m resolution by μ -CT. The jellyfish contains plaster of Paris, CaSO₄·1/2H₂O, single crystals of a size of 10-40 μ m. The structure could be determined by synchrotron single crystal X-ray diffraction confirming the structural assignment and showing the singly crystal nature of the particles. The observation of the hemihydrate plaster of Paris is puzzling because common wisdom says that this phase cannot be made in water! When the jellyfish die, the hemihydrate transforms into gypsum, which has a lower density and is hence less suited for gravity sensing. Investigation of six species shows that they all maintain the hemihydrate. In studying the terrestrial crustacean woodlice, Epple showed that the CaCO₃ is stored in fully X-ray amorphous sternal deposits, whereas there later in the life cycle also occurs Mg-calcite.

The biological organisms that present biomineralized tissues are extremely diverse and range from bacteria to humans. Micha Ilan (Israel) gave an overview of sponge biology with a particular emphasis on mineralized skeletal structure that plays a wide set of roles in these organisms: support of soft tissue, defense against predation, support muscles, feeding, boring, attachment to substrates, gravity sensing and possibly light sensing. The sponge *Tetilla* sp. displays phenotypic plasticity e.g. with the spicule (the mineralized part of the skeleton) weight fraction depending on the environment (smaller weight fraction in deep waters compared to shallow waters with larger currents)¹¹. Prof. Ilan then gave examples of each of the above mentioned uses of biomineralization in sponges. As an example, defense against predation involves chemical defenses (which are interesting as drug targets in their own right)¹² but in some species physical defences in the form of biomineralized spicules are also used to deter predators but only when spicules are

⁹ Lichtenegger, Schöberl, Bartl, Waite & Stucky *Science* **2002**, 298, 389.

¹⁰ Lichtenegger, Schöberl, Ruokolainen, Cross, Heald, Birkedal, Waite & Stucky *PNAS* **2003**, 100, 9144.

¹¹ E. Meroz-Fine, S. Shefer & M. Ilan, *Marine Biology* **2005**, 147, 243-250.

¹² E. Burns, I. Iirach, S. Carmeli, J. R. Pawlik & M. Ilan, *Mar. Ecol. Prog. Ser.* **2003**, 252, 105-114

larger than 250 μm ¹³. Boaz Maysel described efforts to understand the observation of many of the specimens (19%) of the sponge *Niphates rowi* display ferromagnetism. They appear to contain magnetite as evidenced by the observation of the Verwey transition. SEM show Fe containing cubes and hexagonal crystals up to 1-2 μm in size adhering to the sponging skeletal fibers. These sponges have a preferred placement in vitro: horizontal facing east. This observation suggests that the magnetic particles may be involved in positioning the sponge with respect to the substrate. Further studies are needed to reveal the full identity of the iron minerals and their magnetisms. In the ensuing discussion F. Marin suggested that a genetics test for magnetotactic bacteria should be performed as these might be responsible for the presence of magnetite while E. Brunner suggested that Mössbauer spectroscopy might shed light on the type of mineral and its magnetic properties.

The organic matrix is clearly of key importance for the properties of the hybrid biomineralized materials. The characterization of this phase is, however, not trivial. F. Marin (Dijon, France) described some of his group's advances in extraction and characterization of proteins from the organic matrix of mussel shells. Understanding of the matrix proteins involved in shell formation is important for understanding the basic principles of biomineralization, the diagenesis of biogenic carbonates, the origin and evolution of biomineralization but also for applied research such as biomimetic and hybrid materials, searches for bioactive factors and for aqua- and pearl culture. Extraction of proteins from shells is done with either cold dilute acetic acid or EDTA resulting in both soluble and insoluble organic matrix fractions typically corresponding to 0.1-5 wt% of the total shell. From the fan mussel shell (*Pinna nobilis*), which contains prismatic calcite and aragonitic nacre, the group has extracted several proteins including the 636 amino acid, 67 kDa nacre protein mucoperlin that presents tandem repeats and repeats rich in Pro and Ser. Additionally they found putative O-glycosylations suggesting that the protein is mucin-like and group of proteins often found to be mineral inhibitors. Immuno gold labeling showed that the protein is predominantly found on the tablet sides¹⁴. In the prismatic calcite, very acidic proteins were found such as calprismin that is a 38 kDa very glycosylated protein containing 15% Asx, 16% Ala, 12% Thr, 11.6% Pro, 8.7% Glx and 8% Val and weakly binds Ca^{2+} . The 15 kDa Asp-rich (67%) caspartin is also obtained from the calcitic prisms. It binds Ca with low affinity but is a strong inhibitor of calcite growth. Immunogold staining shows that the protein is located within prisms (intracrystal) and just around prisms. Marin suggested that the protein may play a role in crystallographic orientation of the crystals. From the freshwater bivalve *Unio pictorum*, which is a 8-9 cm long mussel living in Burgundy rivers with a purely aragonitic nacre and a prismatic layer, contained glycosylated proteins in the acid soluble fraction of the organic matrix where the acidity is carried by the sugar part¹⁵. Marin also discussed work on crustaceans and focused on the terrestrial *Orchestia cavimana*. In its molting cycle the calcified skeleton is cyclically renewed in a process involving calcium storage granules in the form of concretions, sternal plates and statoliths. In *Orchestia* the granules contain amorphous CaCO_3 and the organic matrix contain a Ca-binding phosphorylated protein containing 108 amino acids, pI 4.4, 13% Asp, 16.7% Glu, 18% (P)Ser and 2 residues of (P)Tyr; the Thr residues are not phosphorylated. Ca binding involves the phosphoserines but not the (P)Tyr¹⁶. This type of knowledge of the organic matrices involved in biomineralization paves the way not only for a deeper understanding of biomineralization per se but also for the creation of artificial molecules for the synthesis of novel materials.

M. M. Giraud Guille (France) showed that the organic matrices in crab cuticle and human bone may be self assembled into liquid crystalline phases. Crab cuticle is made from

¹³ E. Burns & M. Ilan, *Mar. Ecol. Prog. Ser.* **2003**, 252, 115-123.

¹⁴ Marin et al. *J. Biol. Chem.* **2000**; Marin et al. *Prot. Expr. Purif.* **2003**.

¹⁵ Marie et al. *FEBS J.* **2007**.

¹⁶ Testemère et al. *Biochem. J.* **2002**.

(calcified) chitin fibers surrounded by proteins¹⁷. Mineralization starts at the external, top side of the cuticle a few hours after molting. The work on cuticle structure prompts the question of how the organic template phase is assembled: is it by *cell* or *self* assembly? The observation of arched patterns in oblique sections by TEM by Bouligand (1972) prompted the development of a model of the existence of a cholesteric liquid crystalline phase¹⁸. Turning to bone, Giraud Guille showed how acid solubilized collagen can be made to self-assemble to various structures depending on concentration¹⁹ even giving liquid crystal fingerprint patterns in SAXS at concentration $\gg 100$ mg/ml without fibers. At a concentration of 90 mg/ml, intertriplehelices were shown to occur by SAXS. From acid solubilized collagen, liquid crystalline phases could be obtained²⁰ as well as microgels²¹. Turning to biomimetic hybrid materials, Giraud Guille mineralized dense liquid crystal collagen matrices (conc. 100 mg/ml) by addition of Ca^{2+} (110 mM), PO_4^{3-} (66 mM) and osteoblasts in a manner that indicates that the cells govern the mineralization process. She finally suggested that such materials may be suitable for implantation²² also by increasing concentration and pH that yields gel that can be seeded with cells for biomaterial use²³.

S. Stipp (Denmark) described her NanoGeoScience centers work on the role of biology in geosciences. Bacterial passive biomineralization of iron leads to insoluble Fe(III) compounds due to bacterial oxidation of Fe(II) resulting in ochre, goethite and/or hematite. An example is *Gallionella* whose exopolymer stalk acts as Fe_xO_y precipitation sites. This species is involved in Danish waterworks where the reducing groundwater is oxidized. Fine grained iron oxides are being investigated as filters for water treatment. Active biomineralization plays an important role in pine forest culture. Around the pine roots, a network of bacteria and fungi are present. The groups work showed that the fungal hyphae are responsible for dissolution and transfer of nutrients to roots while the bacteria don't seem to contribute. The single celled coccoliths make beautiful CaCO_3 shells. Chalk is ~95% coccoliths (calcite). Interestingly chalk retains many 'biological' properties in terms of retention of morphological characteristics and performance under water etching. This suggests that the structural components (organic matrix?) that are responsible for crystal shape regulation and organization are active even after the million of years that has passed since the deposition of the chalk layers. As oil in many places are included in chalk layers, a better view of how to manipulate solubility and microstructure of chalk may become an important tool in oil recuperation from e.g. North Sea oil fields showing how biogeo nanoscience and biomineralization research may provide important input into applicative megascience.

C. Perry (UK) presented work on bio- and synthetic silicas. The goal of the work is to develop the smallest organic groups that yield control over mineralization, in particular in oxide preparation, to yield financially compatible materials. Studies of biosilica formation shows that the silica precursor $\text{Si}(\text{OH})_4$ transforms into dimers then trimers and then higher oligomers. The charge of the oligomers is strongly pH dependent²⁴. The group also investigated the polymerization properties of other precursors such as Si-catechol complexes²⁵. If charge and uncharged regions are present on the same oligomer, spherical assemblies result and methylation of Si leads to faster assembly reactions. Hence to control the assembly reaction from oligomers into materials, one

¹⁷ Giraud Guille **1984**.

¹⁸ Giraud Guille *Curr. Op.* **1998**.

¹⁹ Giraud Guille *J. Mol. Biol.* **1992**.

²⁰ Bessau & Giraud Guille *J. Mol. Biol.* **1995**; Giraud Guille & Bessau *Conn. Tiss. Res.* **1998**; Mosser et al. *Matrix Biol.* **2005**.

²¹ Eglin et al. *Soft Matter* **2005**.

²² Bessau et al. *Biomaterials* **2000**.

²³ Helary et al. *Biomaterials* **2006**.

²⁴ Perry & Keeling-Tucker *Chem. Commun.* **1998**, 2587-2458; *J. Colloid and Interface Sci.* **2003**, 281, 652-664.

²⁵ *J. Mater. Chem.* **2004**, 14, 2231-2241; *Chem. Commun.* **2006**, 1521-1523.

needs alternately charged/uncharged amines with sufficient hydrophobicity to give droplets to enhance condensation rates²⁶. Changing to Al-oxides, Perry discussed the groups work towards nacre mimics propelled forward by use of model ions by ion exchange resins²⁷. Moving towards functional materials, Perry described the synthesis of hybrid materials from Al₁₃-oligomers with elastin components²⁸.

W. Tremel (Germany) derived inspiration from the desmosponge *suberites domuncula* that has silicatein and galectin the central canal of the spicules²⁹. The extracted or recombinant silicatein can be used for synthesis of artificial materials by binding the proteins to a template through the use of e.g. NTA chemistry³⁰. By binding silicatein to Au surfaces one can make hydrolyzing surfaces that allow synthesis of ZrO₂, TiO₂, FeOOH, V₂O₅ etc³¹. By phosphorylation of silicatein self assembly and aggregation of the proteins into filaments is induced in a process facilitated by addition of silica. This has been emulated binding silicatein to polymers by NTA chemistry including a carboxylate/catechol block copolymer³². Tremel also discussed results showing that calcite crystals may be involved in chirality induction, one of the key open questions in origin of life research. Tremel et al. found that l- and d-alanine gave different CaCO₃ polymorphs (vaterite and calcite, respectively) presumably due to different affinities for kink sites on the surfaces. Making a Strecker synthesis of alanine on calcite lead to ee of 23% d- over l-alanine showing that calcite faces are capable of chirality induction³³.

E. Boanini (Italy) presented work on bioinspired calcium phosphates. Starting with apatites, she described how Sr²⁺ and Cl- substituted apatites can be made. For the Sr-system, the crystallographic axes were found to follow Vegard's law but the Sr-substitution leads to morphological changes with a more disordered morphology at intermediate substitution³⁴. Hybrid materials were made by addition of poly-Asp. At 56m mM poly-Asp, the X-ray diffraction peaks broadened anisotropically. The β -sheet poly-Asp appears to preferentially bind to the (001) hydroxyapatite face³⁵. Combing poly-Asp with octacalcium phosphate instead of hydroxyapatite lead to hollow spheres of OCP via the deposition of an amorphous calcium phosphate, deposition of a shell and subsequent dissolution and reprecipitation³⁶. Bisphosphonates are a class of antiresorptive drugs used in the treatment of several bone disorders. In vitro, Boanini showed that the bisphosphonate alendronate is incorporated into apatite and induces an isotropic reduction in crystal size³⁷. Octacalcium phosphate transforms into hydroxyapatite in water. Poly-Glu and poly-Asp slow down this transformation in a concentration dependent manner³⁸. Similar effects was observed in the transformation of α -TCP to octacalcium phosphate in phosphoric acid were polyacrylate slow downs the transformation that proceeds via a dissolution-reprecipitation mechanism³⁹. Finally, Boanini described how phase transformations from metastable calcium

²⁶ *PNAS* **2006**, *103*, 9428-9433.

²⁷ *J. Mater. Chem.* **2005**, *15*, 3415-3423; Patent WO2006103092; *Adv. Eng. Mater.* **2004**, *6*, 836-839.

²⁸ Deschaume, Shafran & Perry *Langmuir* **2006**.

²⁹ *J. Struct. Biol.* **2007** in press; *J. Biol. Chem.* **2006**; *Cell Tissue Res.* **2007**.

³⁰ *Chem. Commun.* **2004**.

³¹ *Chem. Commun.* **2005**.

³² *Adv. Mater.* **2007** in press.

³³ *Adv. Func. Mater.* **2005**, *15*, 683-688; *Angew. Chem. Int. Ed.* **2007**, in press.

³⁴ Bigi et al. *Inorg. Chim. Acta* **2007**, *368*, 1009-1016.

³⁵ *J. Mater. Chem.* **2004**, *14*, 274-279.

³⁶ *Angew. Chem. Int. Ed.* **2002**, *41*, 2163-2166.

³⁷ *Adv. Mater.* **2007**, in press.

³⁸ *J. Inorg. Biochem.* **2003**, *95*, 291-296

³⁹ *Biomaterials* **2002**, *23*, 1849-1854.

phosphates can yield coatings on e.g. etched Ti₆Al₄V that are of relevance for biomedical implant applications⁴⁰.

H. Cölfen (Berlin, Germany) introduced the concept of mesocrystals as a new concept in both biological mineralization and materials synthesis⁴¹. This idea is based on the observations that nanoparticles oriented with respect to each other can fuse as observed for anatase TiO₂ nanoparticles hydrothermally synthesized in 0.001 M HCl that merge through topotactic attachment at a given face⁴². Cölfen showed how this principle could be applied to make selfsimilar structures by 1D attachments starting with the formation of amorphous nanoparticles, aggregation on surface, crystallization and ending by rod formation through direct particle fusion. The nonclassical route to the resulting rod crystals was observed by video imaging and electrode measurements of the relative supersaturation. The concept can be extended to 2 and 3 dimensions such as 3D attachment of vaterite (991) by use of a cationically modified hydroxyethylcellulose and resulting in formation of a mesocrystal. Organic mesocrystals could also be obtained from d,l-alanine by reaction with PEG-PEI copolymers by cooling supersaturated solutions from 65°C to 20°C. With the amino acids, the supersaturation with respect to (meso)crystal formation can be controlled by pH. CaCO₃ can also be brought to form mesocrystals in the presence of PSS (polystyrenesulfonate) resulting in the various structures including rings depending on the concentrations of both Ca²⁺ and PSS. These materials have large surface areas up to few hundreds m²/g and their surface charges could be revealed by attachment of charged dyes. Using PEG-PSS also leads to CaCO₃ mesocrystals where one can move continuously from single crystals to partially ordered polycrystals showing that there is a continuous transition from classical crystallization over mesocrystals to disordered aggregates. Cölfen suggested that biomineralized structures such as the sea urchin spines that are apparently single crystals but with ‘impossible’ morphologies may be mesocrystals. C. Perry asked a the question of how far crystals can sense each other through the polymer – a question that remains open and is of central importance to much of biomineralization.

B. Pichon discussed the use of Langmuir self assembled monolayers (SAMs) for the control of CaCO₃ mineralization. The SAMs were made from long bisureas, where one side of the urea could be an amino acid. The bisurea functionality affords organization at the air/water interface through the formation of strongly hydrogen bonded structures⁴³. Pichon discussed studies of systems made from Gly terminated bisureas and Val terminated ones. The former gave rigid whereas the latter gave adaptable monolayers as evidenced by grazing incidence X-ray diffraction. The Gly-molecules showed the same packing on pure water as with Ca²⁺ in the subphase showing that the packing is governed by the bisurea hydrogen bondig. For the Val molecule, however, the subphase ions influence the organic phase. The early stages of the mineralization process were followed by in situ cryo-TEM. Initially, amorphous CaCO₃ was deposited that transformed into [10.0] calcite under the electron beam. Lower Ca-concentration (0.01 N) lead first to amorphous phase that was stable in the e-beam and below 20 nm in size; after 5 min vaterite (001) was observed and then after 10 min (100) calcite. This sequence of events is in accord with the Ostwald-Lussac ‘law’ of stages. The study illustrated how state of the art TEM can yield very detailed insights into even ‘wet’ reactions.

R. L. Reis (Minho, Portugal) presented the biomaterials research taking place in the 3Bs group (www.3Bs.uminho.pt) involving work on bone scaffolds, cartilage and skin replacements. The group’s work in bone is based on the standpoint that one cannot compete with bone itself as a material for implants, which leads to the conclusion that one has to recruit cells for

⁴⁰ *J. Biomed. Mater. Res. A* **2007**, in press; *Biomaterials* **2005**, 26, 4085-4089; *Biomaterials* **2004**, 25, 2539-2545.

⁴¹ Cölfen & Mann *Angew. Chem. Int. Ed.* **2003**, 42, 2350-2365.

⁴² Penn & Banfield *Geochim. Cosmochim. Acta* **1999**, 63, 1549-1557.

⁴³ Buijnsters et al. *Langmuir* **2001**, 17, 3623; Cavali et al. *Angew. Chemie* **2005**.

the formation of biomaterials and that scaffolds should be designed that best complete this task. Reis then gave a series of examples of the group's approach to this including the use of low cost starting materials based on starch and/or chitosan.

Hall (UK) showed how the principles and matrices of biomineralized systems can be used to make high T_c superconductors of complex shape. Such materials are normally only obtained in powder form by high temperature syntheses. The method being developed by Hall is based on the use of chitosan (from crab cuticle), dextran or alginate. These are all sugarbased materials. By using the organic matrix in solution together with a sol of precursors for e.g. $\text{YBa}_2\text{Cu}_3\text{O}_7$, thin flexible films can be cast of chemical homogeneous gels. Calcination at 920°C yields a superconducting material as evidenced by SQUID magnetometry⁴⁴. This work shows how the methodologies of bioinspired materials research can be extended to materials remote from biology and hence be of even wider use.

⁴⁴ Hall *Adv. Mater.* **2006**, *18*, 487-490.

Assessment of results

The workshop resulted in the following emerging ideas and avenues for future research:

1. Control over phase transformations and phase stabilization, in particular of amorphous phases, is an area that needs further attention both on the biological and synthetic materials side.
2. The interaction between organic matrix and mineral phase is still lacking.
3. Several advanced characterization tools are becoming available. Combining these is likely to lead to new paradigms for the field.
4. Our knowledge of bone biology and disease is still unsatisfactory and further research is needed for a better understanding of the building blocks of bone and their interaction is absolutely necessary for comprehending the properties of bone.

In the mind of the organizer, it is very likely that collaboration between participants in the workshop will develop in the near to medium term.

Final Program

All invitees will speak during the workshop. Presentations will be 35 minutes followed by 10 minutes discussion.

Friday 11 May 2007

Evening *Arrival*

Saturday 12 May 2007

08.30-09.00 **Presentation of the European Science Foundation (ESF)**

Venko Beschkov (ESF Standing Committee for Physical and Engineering Sciences)

09.00-12.30 **Lectures from participants (including coffee):**

09.00-09.45 *Bio mineralization and biomineralization, what is it good for (sponges)?*
Micha Ilan

09.45-10.30 *Nature Made Hybrid Materials: Building Principles, Self-Assembly, Biomimetism*
Marie M. Giraud Guille

10.30-11.00 *Coffee*

11.00-11.45 *Inhibition of Crystal Growth and Biomineralisation*
Susan Stipp

11.45-12.30 *Mechanical Properties of Biotools investigated by an indentation instrument*
Thomas Schöberl

12.30-15.00 *Lunch and networking*

15.00-18.00 **Lectures from participants:**

15.15-16.00 *Structural Investigations of Bones and Byssi*
Henrik Birkedal

16.00-16.45 *The involvement of cooperative deformation, temperature activated plasticity and chiral springs in bone micromechanics*
Himadri S. Gupta

16.45-17.15 *Coffee*

17.15-18.00 *High-resolution synchrotron radiation techniques in biomineralisation research*
Matthias Epple

18.30 *Dinner (Department of Chemistry)*

Sunday 13 May 2007

09.00-12.30 **Lectures from participants (including coffee):**

09.00-09.45 *Ferromagnetic minerals in the Red Sea sponge *Niphates rowi**
Boaz Mayzel

09.45-10.30 *Solid-state NMR: a tool for probing the organic-mineral interface in biominerals*
Melinda Duer

10.30-11.00 *Coffee*

- 11.00-11.45 *Biom mineralization in Diatoms: An NMR Spectroscopic Study*
Eike Brunner
- 11.45-12.30 *Molluscan shell proteins : origin, evolution and applications*
Frédéric Marin
- 12.30-13.45 *Lunch*
- 13.45-16.00 **Lectures from participants:**
- 13.45-14.30 *Fine-tuning of surfactant monolayer self-organization for biomimetic mineralization of CaCO₃*
Benoît P. Pichon
- 14.30-15.15 *Inorganic chemistry at the biological interface*
With special reference to biosilicification in higher plants and model studies of silica and alumina-'organic' composites
Carole Perry
- 15.15-16.00 *Self-Assembly and Mineralization of Artificial Spicules of Marine Sponges*
Wolfgang Tremel
- 16.00-18.00 **Networking session** and coffee
- Evening *Dinner downtown*

Monday 14 May 2007

- 09.00-12.30 **Lectures from participants:**
- 09.00-09.45 *Non classical crystallization: Self assembly in Bio- and biomimetic mineralization*
Helmut Cölfen
- 09.45-10.30 *Biomimetic Strategies for the Tissue Engineering of Mineralized Tissues using Natural Origin Materials and different Stem Cell Sources*
Rui L. Reis
- 10.30-11.00 Coffee
- 11.00-11.45 *Bio-inspired Calcium Phosphates as Innovative Materials.*
Elisa Boanini
- 11.45-12.30 *Biopolymer Mediated Growth of Morphologically Complex Superconductors*
Simon R. Hall
- 12.30-14.30 *Lunch & departure*

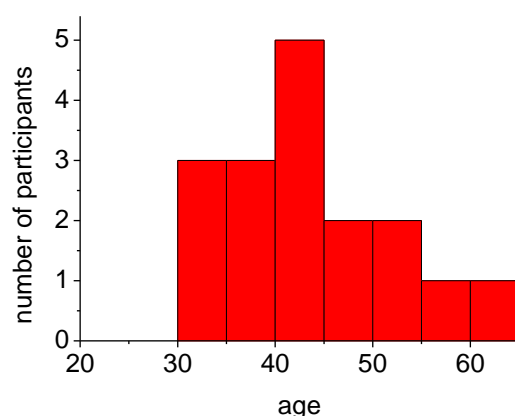
Statistical information on participants

Gender repartition: male: 13 female: 5

Countries of origin:

Denmark	2
Germany	5
Israel	2
Great Britain	3
Austria	1
France	3
Italy	1
Portugal	1
Total	18

Age structure:



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