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Biomineralization

From Biology to Biotechnology and Medical Application

Second, Completely Revised and Extended Edition

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Biomineralization
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Second, Completely Revised and Extended Edition

WILEY-VCH Verlag GmbH & Co. KGaA

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Cover Illustration (Designed by Felix Bäuerlein):
Top left: Elongated prismatic magnetite crystals in membrane vesicles of a magnetic bacterium (D. Schüler, Chap. 4, p. 62).
Top right: Calcein-stained calcified skeletal structures in the caudal fin of zebrafish larvae (S. J. Du, Y. Haga, Chap. 17, p. 296).
Bottom left: From aragonite to calcite. The change of shape from "ear-stone" through star-like aragonite to pur calcite crystals in a down-regulation of the starmaker protein in the zebrafish (C. Söllner, T. Nicolson, Chap. 14, p. 236).
Bottom right: A micromechanical method to study stability of diatoms (C. Hamm, R. Merkel, Chap. 18, p. 322).

Library of Congress Card No.: applied for

A catalogue record for this book is available from the British Library.

Bibliographic information published by Die Deutsche Bibliothek
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at http://dnb.ddb.de

First Edition 2000
Second, Completely Revised and Extended Edition 2004

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Printed in the Federal Republic of Germany.
Printed on acid-free paper.

Typesetting Asco Typesetters, Hong Kong
Printing betz-druck gmbh, Darmstadt
Bookbinding Litges & Dopf Buchbinderei GmbH, Heppenheim

ISBN 3-527-31065-7

This book is dedicated to

my wife Cornelia

for her permanent encouragement and
editorial support also for this 2. edition
and to

my daughter Henrike and *my son Felix*

for their indefatigable cooperation in difficult computer work

Foreword

The term biomineralization summarizes the natural processes by which living organisms form materials from bioorganic molecules and inorganic solids. This is a fascinating topic, uniting the living and the (not always really) dead world surrounding us. In fact, all of us have a direct relation to biomineralization, as we are "biomineralisateurs" – producing each day crystals of a calcium phosphate (apatite), embedded in an organic matrix (mostly collagen), as part of the formation of bone material. These crystals are mere nanometers in size and are arranged in a well-defined hierarchical structure, so that any of us may be rightly called a bio-nano-engineer (a feat that might prove useful in grant applications).

The field of biomineralization not only connects the living and the mineral world, but also brings together scientists from very diverging fields, ranging from geology, mineralogy and crystallography via chemistry and biochemistry to biology and medicine, as well as, possibly, biotechnology. Whereas research in this field was dominated by a mostly descriptive approach throughout much of the last century, the last ten to twenty years have witnessed an increasingly profound scientific understanding of the formation mechanisms of biominerals. This progress has been fuelled by the application of modern molecular biology methods and the advent of novel solid-state analytical techniques, but, most significantly, by their mutual interaction. From the point of view of biology, the ability of an organism to form an inorganic solid material is a special feature that provides evolutionary advantages, and thus certainly deserves elaborate biochemical and molecular-biological studies. The growing interest among solid-state chemists and materials scientists lies in the processes by which the often complex and intricate hierarchical architectures of biominerals can be formed under conditions, which are incredibly mild, compared to the usual techniques of preparative solid-state chemistry. This is combined with a common general interest in the structures and processes occurring at the interfaces between organic matter (not necessarily of biological origin) and inorganic solids which are of utmost importance in many topical regimes of modern science (for example, in heterogeneous catalysis, organic-inorganic hybrid materials, biomaterials or in the attachment of cells to electronic devices). The idea of using synthesis methods taken from nature in order to generate materials with superior properties leads to bio-inspired preparation procedures (which take some key elements from biomineralization, for example the templating action of bioorganic polymers during precipitation of a solid) or bio-mimetic syntheses (which try to fully exploit the mechanisms active in biomineral formation and which may thus also provide an

insight into the natural processes themselves). Finally, if organisms could be convinced by genetic engineering to produce certain materials with selected properties, the biotechnological production of high-tech materials might become feasible.

The importance of biomineralization and its possible applications has recently been reflected in the set-up of dedicated research programs, such as the establishment of an Institute for Biologically Inspired Materials by NASA in the United States. In Germany, research on the "Principles of Biomineralization" has been focused on the priority research program of the Deutsche Forschungsgemeinschaft.

Of course, another clear indicator of the topical nature of this field is this volume and the great success of its predecessor. Within only four years, it has become necessary to publish a sequel, and as opposed to movies, in science sequels usually represent true progress. Many chapters are new (in that they were not part of the first edition) or novel (in that they have been totally rewritten by the authors) and most of the others have been thoroughly reviewed, a stringent necessity in view of the current progress in the field. Edmund Baeuerlein, as a *professor emeritus* now (mostly) freed from his time-consuming research work at the bench and the burden of administration, has devoted a lot of time and much effort to make this volume not only a compendium of the latest research results but also a valuable introduction for newcomers to this field. He did this by careful selecting the contributions and authors and by rigorous editing.

My wish is that this book will be at least as successful as its predecessor. May the research results and ideas compiled here enlighten the reader.

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"Prinzipien der Biomineralisation"

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Preface

Modern research in biomineralization was first summarized in 1989 in the basic work ‘‘On Biomineralization’’ by two of the pioneers in the field, M. A. Lowenstam and S. Weiner. Parallel to this more biological review, its inorganic counterpart was published the same year ‘‘On Biomineralization: chemical and biochemical perspectives’’ by three pioneers of the chemical approach, S. Mann, J. Webb and R. P. R. William. These perspectives were highlighted in 2001 in ‘‘Biomineralization: Principles and Concepts in Bioinorganic Materials Chemistry’’ by its guiding chemical initiator Stephen Mann. It is obvious by these comprehensive volumes that biomineralization was dominated for about 20 years by excellent and extended structural and physiological research.

At the end of this period I had began to study magnetite crystal formation in bacteria. Parallel to progress in bacterial genetics the conviction was and is still growing on me that mechanisms in biomineralization will be predominantly elucidated by methods of molecular biology. The term ‘‘mechanism in biomineralization’’ is permanently discussed between chemists and biologists, whether the molecular process or the coupled process of transport, directed saturation and interaction of several organic compounds may be designated a mechanism.

A ‘‘Workshop on Biomineralization and Nanofabrication’’, organized by Richard B. Frankel in May 1996, inspired me to edit a multi-author volume on ‘‘Biomineralization. From Biology to Biotechnology and Medical Application’’ in November 2000. The aim of this edition was to compare structure formation of inorganic materials in those organisms that were expected to be analyzed most likely in the near future by genetics and molecular biology. At this time, prokaryotic and eukaryotic unicellular organisms, the magnetotactic bacteria and the mineralizing algae, coccolithophores and diatoms, were the prime candidates for these very biological approaches in biomineralization.

Almost complete genome sequences of 15 bacteria, including those of two magnetotactic bacteria, have been made available to the public domain surprisingly fast by the Joint Genome Institute (IGE) of the U.S. Department of Energy. These two genome sequences allowed studying magnetite nanocrystal formation at the genomic level. The human genome project was accomplished just before this work. In addition sequencing of the genome of the zebra fish, an important model organism for the human being, began in 2001 (and should be finished in 2005). Because of extended mutant analyses, I intended to introduce studies on mineral formation of

this simple organism in this new edition, and at the beginning of this year I fortunately succeeded in finding two such reports.

This progress by modern biological methods was paralleled by extraordinary developments in modern physical methods, the highlight of which is cryo-electron tomography, elaborated by W. Baumeister. Research in biomineralization not only comes together with material science from the very edges of its biological and physical parts, but also directly in the recent, epoch-making publication by M. O. Stone and co workers on ''Peptide Templates for Nanoparticle Synthesis derived from Polymerase Chain Reaction-Driven Phage Display''.

I am very grateful to Professor Dieter Oesterhelt for the opportunity to stay as a guest in his department, an opportunity which remarkably facilitates my task as editor and author.

I thank very much to Professor Peter Behrens, the Organizer of the DFG-Priority Program ''Principles of Biomineralization'', for inviting me to several workshops of this very interdisciplinary project.

The various information encouraged me to select new topics for this new edition, among these are models of human biomineralization and modern physical methods.

July 2004

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Abbreviations

AAS	atomic adsorption spectroscopy
ADP	adenosine diphosphate
ATCC	American Type Culture Collection
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
AFM	atomic force microscope
Bfr	bacterioferritin
BMP	bacterial magnetic particle
CA	carbonic anhydrase activity
CCM	carbon concentrating mechanism
CDF	cation diffusion facilitator
CEA	carcino-embryonal-antigen
CM	cytoplasmic membrane
CN	central nodule
CP	chloroplast
CV	coccolith vesicle
DEAE	diethylaminoethanol
DIC	dissolved inorganic carbon
DSi	dissolved silicon
DSM	Dt. Sammlung für Mikroorganismen
EDTA	ethylenediaminetetraacetic acid
ESI	energy spectroscopic imaging
EL	extracellular loops
ER	endoplasmatic reticulum
FAD	flavin adenine dinucleotide
FESEM	field emission scanning electron microscopy
FISH	fluorescence *in situ* hybridization
FMN	flavin mononucleotide
GA	N-acetylglucosamine
GFP	green fluorescent protein
GUT	grand unified theory
HRTEM	high resolution transmission electron microscopy
HPLC	high pressure liquid chromatography
ICS	intracellular carboxy segment
IgG	immunoglobulin G

IL	intracellular loops
INS	intracellular amino segment
kDa	kiloDalton
LPS	lipopolysaccharide
MA	N-acetyl muramic acid
MM	magnetosome membrane
MMP	many-celled magnetotactic procaryote
MRI	magnetic resonance imaging
MTB	magnetotactic bacteria
Myr	million years
NAD	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide, reduced
NADPH	nicotinamide adenine dinucleotide phosphate, reduced
NMR	nuclear magnetic resonance
OA	ornithineamidelipid
OATZ	oxic–anoxic transition zone
ORF	open reading frame
OM	outer membrane
P	peptidoglycan layer
PC	phosphatidylcholine
PCR	polymerase chain reaction
PE	phosphatidylethanolamine
PET	positron emission tomography
PG	phosphatidylglycerol
PM	plasma membrane
R 123	Rhodamine 123
rRNA	ribosomal ribonucleic acid
SATA	succinimidyl-S-acethylthioacetat
SAED	selected area electron diffraction
SCID	severe combined immunodeficiency
SD	single-magnetic-domain
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SDV	silica deposition vesicle
SEM	scanning electron microscopy
SER/THR	serine/threonine
SIT	silicic acid transporters
STEM	scanning transmission electron microscope
STV	silicon transport vesicle
TEM	transmission electron microscope
TEOS	tetraethyleneoxysilane
TMPD	tetramethyl-p-phenylenediamine
TPR	tetratricopeptide repeat
UTP	uridine triphosphate

1 Peptides, Pre-biotic Selection and Pre-biotic Vesicles

Edmund Bäuerlein

1.1 Peptides as Templates for Inorganic Nanoparticles: From Functional Groups to "Peptide Group Selectivity"

The "Combinatorial Phage Display Peptide Library" is the result of a gigantic random peptide synthesis initiative, based on molecular biology. In a special library [1], about one billion (10^9) peptides are present as combinations of 12 random amino acids. Each of these 12-amino-acid peptides is expressed as a fusion with the small, surface-displayed protein III of the bacteriophage M13. In addition, each phage molecule has five copies of protein III and, consequently, five identical peptides on its surface.

1.1.1 A Phage Display Peptide Library in "Regular Panning" for Mineral Binding and Synthesizing Peptides

This molecular biology approach became an important link to materials science, as it was not only used to select for peptides that bind specifically to inorganic materials – it was the brilliant idea of M. O. Stone that, vice versa, such peptides should also be capable to generate inorganic structures to which they had bound. The standard procedure [2a] describes a technique to select surface-specific peptides and to subsequently identify a subpopulation of silica-precipitating peptides from a larger pool of binders. This procedure, which includes multiple rounds of target binding, elution and amplification, was designated "biopanning". It is an additional advantage of this method that the low number of eluted peptide bacteriophages could be multiplied by infection of particular *Escherichia coli* host cells. It was then easy to sequence them because the DNA sequence of an individual peptide of the library is fused with that of the small surface-displayed protein III of the bacteriophage – a sequence which is well known and used as primer.

A comprehensive study was begun with peptide-mediated synthesis of a target silica. It was called biogenic, because the synthetic peptide R5, which contains 19 amino acid residues, was used [3]. It is the non-modified analog of the repeating sequence R5 in native silaffin-1A (natSil-1A) [4], a major organic component of the silica cell wall of the diatom *Cylindortheca fusiformis* which was recently described

*Si3-3	A	P	P	G	H	H	H	W	H	I	H	H	
*Si3-4	M	S	A	S	S	Y	A	S	F	S	W	S	
Si3-8		K	P	S	H	H	H	H	H	T	G	A	N
*Si4-1	M	S	P	H	P	H	P	R	H	H	H	T	
*Si4-3	M	S	P	H	H	M	H	H	S	H	G	H	
Si4-7		L	P	H	H	H	H	L	H	T	K	L	P
Si4-8		A	P	H	H	H	H	P	H	H	L	S	R
*Si4-10		R	G	R	R	R	R	L	S	C	R	L	L
▶Ge4-1		T	V	A	S	N	S	G	L	R	P	A	S

Figure 1.1 Multiple sequence alignment of silica-binding peptides obtained after the third and fourth rounds of panning. The various phage display peptides were plated on LB plates containing x-Gal and isopropyl-1-thio-β-D-galactopyranoside (IPTG). DNA was isolated from at least 10 independent blue plaques and sequenced [2]. Si3-4 was the fourth clone selected from the third round of panning. Amino acids with functional side chains that are able to interact with silica surface are shaded. (According to [2a], courtesy of M. O. Stone.)

by Kroeger et al. [4]. A network of silica spheres with a diameter of 400–600 nm was obtained when R5 peptide was incubated in freshly prepared orthosilic acid for 2–5 min at pH 7.5 and room temperature. The biosilica particles were subsequently washed several times to remove residual R5 peptide before use.

The phage display peptide library was now incubated with these particles. After three or four rounds of panning the sequence of the peptide, the peptide phage which remained bound to silica particles after stringent washing was determined. The multiple sequence alignments of such silica-specific peptides are shown in Fig. 1.1 [2a]. Binding of the phage peptides to the surface of silica particles was substantiated with a phage immunoassay and is presented in Fig. 1.2 [2a] using relative units. Si4-1 and Si4-10 apparently interacted more strongly with the silica particles, compared to the six other selected peptide phages.

The first, unprecedented step into materials production was now to examine the phage peptides, which were selected by their binding capacity on silica particles, for the formation of silica particles. They were incubated in a freshly prepared solution of orthosilic acid as described above for peptide R5. The amount of silica generated by phage peptides was quantified by the molybdate assay [5], as shown in Fig. 1.3. The highest activity was repeatedly observed with Si4-1. The phage peptides Si3-3, Si4-10, Ge4-1 and M13 showed only minor or no silica-precipitating activity.

This first experiment of phage peptide-mediated material formation resulted in the highest amounts of silica when three types of amino acid, i.e. hydroxyl- and imidazole-containing as well as of high cationic charge, were present in the peptides. Histidine and serine were found previously by Morse to be essential for catalysis by silicatein, a protein with enzymatic activity similar to human protease Cathepsin L. Silicateins were able to hydrolyze orthosilicic acid ester as tetraethoxysilane at neutral pH by simultaneous polycondensation to silica. Silicones were produced when methyl or phenyl triethoxysilanes were used as substrates [6].

With respect to the following experiments and results, it should be emphasized that here a subpopulation of phage peptides was obtained by standard panning

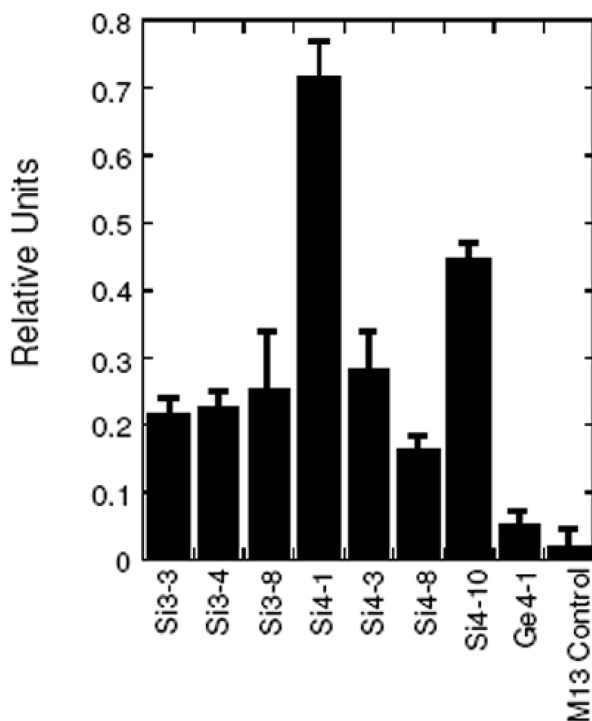

Figure 1.2 Binding of phage display peptides to silica by phage immunoassay. The binding of biotin-conjugated anti-Fd, an antibody raised against the pIII coat protein of M13 phage, was detected with the use of streptavidin–horseradish peroxidase. (According to [2a], courtesy of M. O. Stone.)

(later in Fig. 1.4 designated as "regular panning") which included amplification by infection of *E. coli* host cells with the selected phage peptides. This limited amplification [compared to the polymerase chain reaction (PCR), presented later] appears to preferentially result in peptides, which in terms of structure and possibly dynamics may be taken to be similar to active centers of enzymes [6], and, therefore, to act by definite residues in binding as well as in material production.

Such a similarity was supported by the first results of peptide-mediated synthesis and patterning of silver nanoparticles [2b]. These experiments were a kind of unexpected back reaction of Belcher's pioneering experiments with the "Combinatorial Phage Display Peptide Library". She used the library to identify peptides recognizing a range of semiconductor surfaces with high specificity, depending on the crystallographic orientation and composition of structurally similar materials (GaAs on silicon) [7]. Stone succeeded here in performing the back reaction – not the production of GaAs, but that of silver nanocrystals by a peptide of the phage display peptide library, which had been selected by regular panning (Fig. 1.4) [2b]. Only three different sequences, i.e. AG3, AG4 and AG5, of 30 independent assays were found

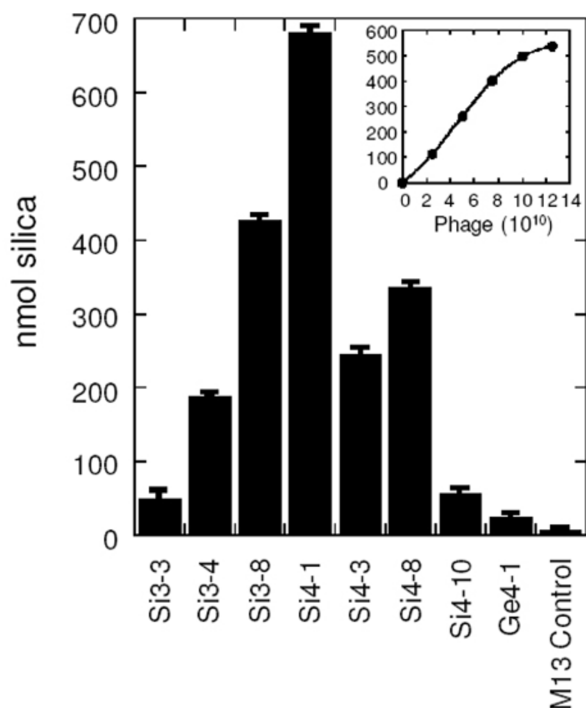

Figure 1.3 Silica condensation of the selected phage display peptides (clones). Equal amounts of phage particles (10^{11}) were incubated for 5 min in Tris-buffered saline (pH 7.5) with hydrolyzed tetramethyl orthosilicate. The silica precipitated was collected, washed and the amount of silica was measured with the spectrometric molybdate assay. The silica concentration obtained with R5 peptide (100 μg) was 1.05 μmol. The amount of silica precipitated is proportional to the amount of Si4-1 phage particles added (inset). (According to [2a], courtesy of M. O. Stone.)

in peptides which selectively bound to silver particles of a nano-sized activated powder (Table 1.1).

These silver-binding 12-amino-acid peptides contain preferentially prolines and hydroxyl amino acids:

AG3:	4 prolines	2 tyrosines	2 serines	
AG4:	2 prolines	2 tyrosines	3 serines	
AG5:	4 prolines	–	1 serine	2 threonine

For synthesis of silver nanoparticles, each of the three peptide phages was first incubated in a solution of silver nitrate for 24–48 h at room temperature. The reddish color of the solution and, after centrifugation, of the precipitate was observed using peptide phages AG3 and AG4, but not AG5. A characteristic surface plasmon resonance band about 440 nm was obtained in the ultraviolet-visible spectrum of the reddish solution, reflecting the size and shape distribution of the silver nanoparticles [2b].