Molecules: Nucleation, Aggregation and Crystallization

Beyond Medical and Other Implications

edited by
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Paolo Riccio

Karolinska Institutet
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Preface

This volume, entitled *Nucleation, Aggregation and Crystallization — Beyond Medical and Other Implications,* is a compilation of articles written based on the lectures presented by leading experts during their participation in the homonymous "International Course"[a] held at the Karolinska Institutet (2007) and in the scientific meetings of the MARIE Network of the European Science Foundation (2004–2006) on "Myelin structure and its Role in Autoimmunity."

The word "nucleation," derived from "nuclear family," is based on the concept of a progenitor, or the mother and father of any family. As a physico-chemical process, *nucleation* is followed by two poorly understood phenomenon: *aggregation and crystallization. Aggregation* underlies disorders like

[a] The following organizations supported/sponsored the course "Nucleation, Aggregation and Crystallization — Beyond Medical and Other Implications," (in alphabetical order):
BioVectis Sp.z.o.o www.biovectis.com, CHEMILIA AB www.chemilia.com,
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ESF MARIE Network, Molecular Dimensions Ltd. www.moleculardimensions.com,
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Wenner-Gren Foundation www.wennergren.org,
Åke Wiberg Foundation www.ake-wiberg.com.

Alzheimer's and "mad-cow" disease (aggregation of amyloid plaque), and cardiovascular diseases (deposition in coronary vessels of cholesterol and lipids). *Crystallization* refers to the appearance of crystals under physiological conditions (gout, silicoses, and liver or kidney stones).

Crystallization is a physicochemical process appearing in nature or artificially in test tubes, leading to formation of solid crystals from a uniform concentrate of slightly supersaturated solutions. In biological crystallization, crystals are usually needed for the determination of the three-dimensional structure of proteins and other biological macromolecules by X-ray crystallography. The structure is needed for a better understanding of protein function and in order to design effective drugs. If the structure of a protein is precisely solved, the designed drug is supposed to be more effective.

The selection of 20 chapters in this book covers important topics on the principles and methods of the crystallization procedures in one-, two- and three-dimensions, including those regarding organic fine chemicals and pharmaceutical compounds. Moreover, the book describes the use of oils and magnetic fields to grow better quality protein crystals. Other chapters deal with the relevance of bioinformatics and mass spectrometry in crystallomic, and discuss both the role of molecular mimicry and the structure of amyloid proteins and myelin proteins in diseases such as polyneuropaties, Alzheimer's disease and multiple sclerosis.

In recent years, the MARIE Network, funded by the European Science Foundation, re-introduced the concept that recognition of biological structures such as the myelin sheath, is fundamental for the understanding of specific diseases and the design of better therapies. Through MARIE, structural studies on myelin and multiple sclerosis are now enjoying a renaissance after a period of low interest. This can be ascribed to new theoretical or technological developments and to the progress in several fields, including bioinformatics, X-ray crystallography, and proteomics, but also to the MARIE idea to encourage the integration of different kinds of expertise in the fields of biophysics, biochemistry, molecular biology, neurology, neuroimmunology, and bioinformatics. This cooperation between experts in structural studies and specialists in

cell biology and neuroimmunology has enabled deeper probing into the underlying structure of the myelin sheath and to understand the processes by which various components involved in immune response, such as cytokines, antibodies, proteinases, and free oxygen radicals, may degrade the myelin over time, causing multiple sclerosis.

MARIE has helped to revive multiple sclerosis research at the structural level. Some aspects of this renaissance, in particular those regarding crystallization, form the fundamentals of this book, which provides a unique perspective of the physical and chemical sciences on one hand, and the biological and medical sciences on the other, and should be of considerable value to scientists, physicians, students.

One of our teachers, Nobel Laureate Dr. Gajdusek, passed away on December 12, 2008, few weeks after the 2nd proofs reading. The 3rd proofs were planned in January 2009. We have decided to treat his chapter, entitled, "From Kuru to Nucleation, Aggregation, Polymerization and Crystallization in Biology and Medicine" as his "scientific testament," and to leave a few very minor mistakes in the text. Chapter I of this book is probably the last scientific writing of Dr. Gajdusek.

I (JS), met Dr. Gajdusek for the first time in Chengdu, Sichuan, China, June 25–28, 2006, during the "First International Conference of Nanobiomedical Technology and Structural Biology." He gave a lecture "Nucleation, Medical Diseases and Beyond." It was a beautiful and informative lecture. He told us why he went to study kuru among the Fore people of New Guinea. New Guinea is the place he loved so much. His research on kuru was never supported financially by any organization, except his own funds. He never wrote any grant application for the whole of his life. From China (2006), at the age 82, he left for Tibet to continue his research. He was an old fashion scientist; he never used a computer for word processing, and he never had a secretary. He wrote this chapter by hand. I will remember him as a very energetic person, full of new ideas and new concepts, very friendly and supportive of teaching. We will miss him in other courses we will organize in the years to come.

We would like also to thank the publisher World Scientific Publishing, in Singapore, especially Ms Sook-Cheng Lim, for their support, encouragement

and to take on the "board" publication of our book. I would like to thank Prof. Gösta Winberg (KI, MIT) for introducing me to Dr. Gajdusek and for his support for the course; Prof. Zhang Shuguang (MIT) for inviting me to China, where I met for the fist time, Dr. Gajdusek.

This book is dedicated to all interested in the subject of nucleation, aggregation and crystallization of proteins, particularly to potential course leaders who may use this book during their teaching.

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Contents

Chapter 1

From Kuru to Nucleation, Aggregation, Polymerization and Crystallization in Biology and Medicine

*D. Carleton Gajdusek**,†

Nucleation is a word derived from nuclear family and refers to the concept of progenitor, the mother and the father of any family, which has been at the root of human life for many thousands of years. From it has emerged the concept of a breeding line of humans. Only in the last few centuries of civilization have physicists borrowed the word, and later biologists for Schwann's cell theory. Very recently it has passed to atomic theory, spectroscopy, radioactivity and to atomic bombs, fission and fusion. In physics any change in the free energy state of matter involves a nucleation or ordering of atoms into a new pattern as in any change to gas, liquid or solid or in the packing of atoms in carbon black, graphite, or diamond. Thus the word *nucleation* is not derived from atomic physics or cell biology. To be so deluded makes it difficult to understand the simple matter of pattern setting in any change of state.

Keywords: Crystallization; molecular casting; twinning of minerals; nucleation; amyloidoses; amyloid enhancing factor; nucleant; infectious nucleant; carbon; diamond; montmorillonite; epitactic.

Molecular Casting

Infectious amyloid nucleants

We have repeated confirmation of resistance of a portion of infectivity of scrapie to temperatures as high as 600°C (Brown *et al.*, 2000). The

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†1923–2008.

enormous resistance to dry heat of a small fraction (about one part in 10^6) of infectious activity may represent a molecular casting, fingerprints of the nucleants.

Infectious nucleant or prion activity is the result of very close three-dimensional matching. Any particle which can sufficiently mimic the ability of the molecule to be nucleated to crystallize, to fibrilize, or to form a two-dimensional molecular sheet can trigger the process. Matching must surely be at atomic distances, close enough to evoke van der Waals forces and Coulombic forces, even H-bonding.

Can we preserve biological specificity of antibodies, antigens, pheronemes, receptors, transmitters, ion channels and enzymes in organic molecular casts or atomic moulds?

The answer is yes, already for the first six items, and if we allow for synthetic hydrocarbon polymers for molecular casting, it may be so for enzymes.

Dermatoglyphic preserving of biological specificity

One of my adopted New Guinea sons has pointed out to me that a fingerprint using battery ink (MnO_2) is an example of such preservation with no atom of carbon and of biological specificity, more individual than the DNA sequence of identical twins.

Fossils show accurate speciation in paleobotany and paleozoology

Another of my adopted New Guinea sons has pointed out that fossil footprints allow for accurate classification and yet are not a source of DNA for speciation by polymer chain reactions. Nucleoli can be counted in inorganic fossils in cells extinct for millions of years.

Osmium shading in electron microscopy reveals details of molecular structure at nanometric distances

There is no contribution from carbon atoms to the image of an osmium or platinum shaded freeze-fracture electron microscopy photograph of individual molecules.

Mineral nucleants for crystal growth in outer space

McPherson and Shlichta (1988) sent a series of proteins into outer space to avoid the convection of fluids by the gravity of the Earth. They nucleated them with a selection of ground minerals. A subset of minerals usually initiated crystal formation in one or another of the proteins, a different set for each protein and differing forms of epitactic crystal growth for a given protein with each mineral.

What makes a diamond hold together?

Two polished ancient Chinese copper mirrors applied face-to-face stick together and require considerable force to separate them. A stack of newly opened clean microscopic cover slips slide one upon the other, yet it requires considerable force to separate them.

A diamond, the hardest of minerals, can scratch steel, yet it is still only made of carbon, the same as carbon black, coal or graphite.

Sulfur may be pure yet malleable, ductile, fragile or clay-like and of many colors depending how the S atoms are packed. Such is the nature of the van der Waals' forces that are brought into play at atomic distances.

Twinning of minerals

There are at least 200 twinning possibilities for quartz (SiO_2). Most possible forms have been found in the over 30 000 years of searching for them. When one new twin "form," "strain" or "species" is found, it is usually named for the region where it has been found. It is common to find

other examples of this particular "strain" of twinned quartz in mining shafts for many hundreds of kilometers around the first finding, yet nowhere else on Earth. Much the same is true for diamonds, emeralds and rubies, and for other examples of twinning in mineralogy.

Industrial viruses and "ice nine"

Kurt Vonnegut, Jr wrote of "ice nine" in his book *Cat's Cradle* (1963): a fictional approach to the problem of nucleation based on a sound understanding of the phenomenon. His brother, William, was a major meteorologist fully familiar with non-DNA or non-RNA containing viruses and the World War II ethylene diamine tartrate problem of the industrial viruses, which nucleated the slow appearance of bubbles of large crystals of the compound made for optical purposes. Kurt Vonnegut, Jr. got the idea right.

Amyloid enhancing factors are scrapie infectious amyloid nucleants

For some 35 years, I have been aware of the work of amylidologists in their attempts to accelerate the appearance on AA amyloid deposits in animals primed with inoculation of $AgNO_3$ or heterologous casein. Their discovery of amyloid enhancing factors, which were active in high dilutions and difficult to purify, remind me of our problems with the infectious agents of scrapie or kuru. I suggested that amyloid enhancing factors were scrapie-like agents (Gajdusek, 1977, 1988, 1991, 1994a, 1994b; Niewold *et al.*, 1987).

Any β-pleated polymeric assembly as a two-dimensional sheet or as a fibril may act as a heteronucleant for different amyloidogenic proteins

Amyloid deposits in man or animals are always found to be contaminated with other proteins similarly polymerized into fibrils — even copolymerized. These are all the proteoglycans and glycosoaminoglycans as well as

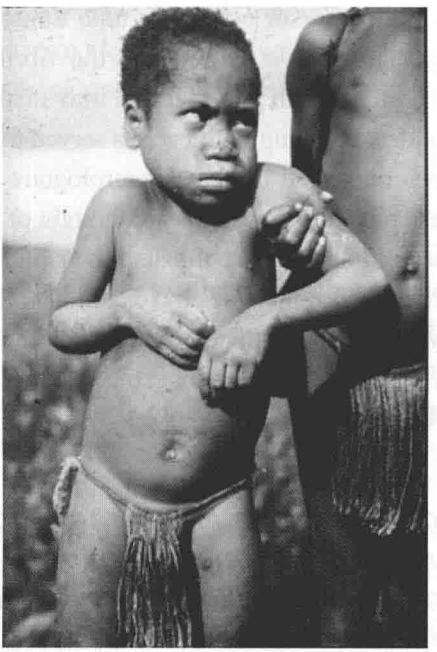

Fig. 1. A prepubertal Gimi boy of about ten with kuru who requires aid to remain standing, He shows the spastic strabismus which most children develop in kuru. He died a few months later in 1957 (DCG-57 NG-1150).

plasma P-protein, chymotrypsin, ubiquitin, light chains of gamma-globins, and other amyloidogenic proteins.

Tropocollagen is nucleated to fibrilize not only by submicroscopic fibrils of tropocollagen but also by dimers and polymers of glycosoaminoglycans or proteoglycans, and not by heparin which is a single-bonded dimer (Obrink, 1973).

Synthesis of prion-like infectious nucleants

Katarzyna Johan (Lundmark) and Per Westermark (1998) succeeded in getting *in vivo* heterologous nucleation of β-fibrillary protein polymerization into amyloid fibrils with synthetic amyloid enhancing factors. Such heterologous nucleants are synthetic peptides from the highly fibrilogenic

section of the amyloid precursor protein or both transthyretin and insulin associated amyloid. They serve to nucleate the fibrilization of the AA amyloid precursor protein when polymerized into small fibrils, but not as unpolymerized peptides. Labeling with I^{131} has served to locate AA amyloid fibrils that have been nucleated by these heterologous amyloid-enhancing factors. Thus, if these replicating systems are thought of as being alive, they have already synthesized "life" and published their findings.

Per Westermark and his colleagues have demonstrated the induction of AA amyloidosis by nucleation with heteronucleants such as silk and spider webs and by oral *paté de fois* in transgenic mice (Johan *et al.*, 1998; Solomon *et al.*, 2007).

Biological macromolecules all interact strongly with SiO_2, the most common solid mineral on the surface of Earth. Montmorillonite clay deposits cause delayed neurodegenerative diseases

Iler (1977) and Weiss (1981) have shown how silicon and oxygen in the form of SiO_2 can interact and bond to biological macromolecules or polymers in long series of strong attractions, whether they are carbohydrates, proteins, nucleic acids or fats. Silicon and oxygen are the two most common elements on the surface of Earth. The role of silicon is fully discussed in the Nobel Foundation's *The Biochemistry of Silicon and Related Problems*, in which Iler's article appeared. Thus binding to solids is the most likely origin of life, not a primordial oceanic liquid.

The high incidence foci of two very different diseases, Guamanian amyotrophic lateral sclerosis *(lytico)* and Parkinsonism dementia *(bodig)*, of the Chamoro people on Guam, also occurred in the few remote inland villages of Honshu Island in Japan and among the Auyu and Jakai people around Bade and Kepi in southern West New Guinea (Gajdusek, Salazar, 1987). It has virtually disappeared from all of these places with the introduction of civilization. These three foci were restricted to remote communities in which a depletion of calcium produced a chronic severe deficiency of calcium in the diet, such that calcium sparing resulted in soft tissue deposition of calcium-aluminum-silicon,