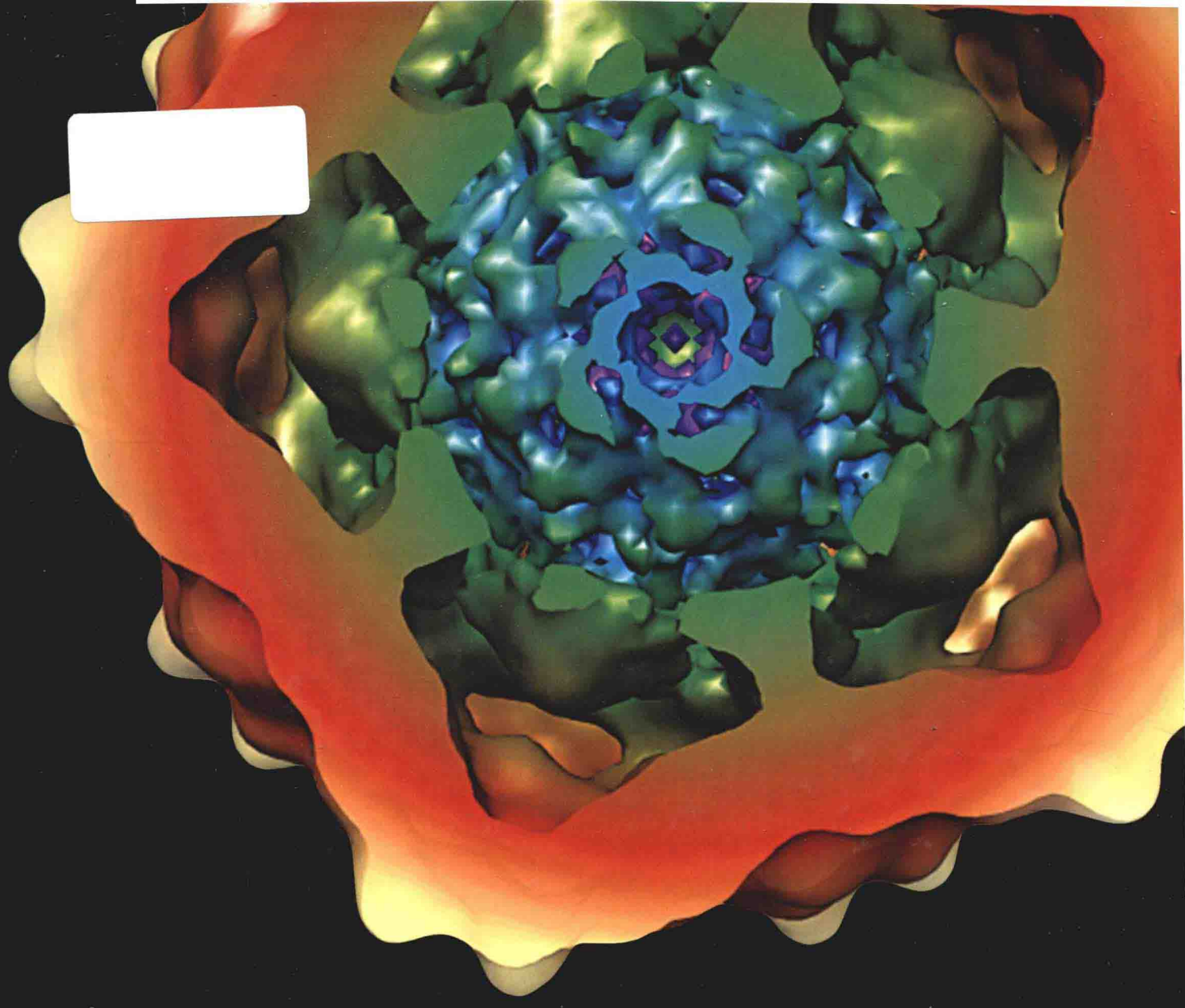

VIRAL NANOTECHNOLOGY

Edited by
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VIRAL NANOTECHNOLOGY

Preface

Nature works as small as it wishes.

***Contemplation de la Nature* (1764)**

Charles Bonnet (1720–1793)

The field of viral nanotechnology is new. As many novel scientific enterprises in their early days, it is frequently confused for something else and under-recognized. To our surprise, when we started developing and discussing the idea of this book, many of our colleagues questioned the sheer existence of such a discipline, interpreting nanotechnology as an elaborate disguise of genetic and protein engineering. The common genealogy in all these disciplines is undeniable. Viral genetic engineering and protein engineering are clear methodological founders and forerunners of viral nanotechnology. However, the focus on application sets viral nanotechnology apart from genetic and protein engineering, both of which focus on constructing.

The transition from molecular technologies of constructing to novel technologies of application is a major event in the field of molecular research over the last two decades. Molecular engineering provides technologies for building molecular modules from genetic elements, while viral nanotechnology explores how these modules can be used. This is similar to the transition from the invention of bricks and other construction materials to advancing the architecture and construction of a variety of buildings and complex structures, including private homes, castles, laboratory facilities, city blocks, and entire cities.

The field of viral nanotechnology is vast and rapidly expanding. The manipulation of atoms and molecules for the fabrication of materials with *novel properties*, which cannot be otherwise obtained, offers opportunities for a multitude of unique applications affecting all human activities. The unique properties of viral proteins, such as their capability to be robustly assembled into well-defined multivalent architectures with a variety of shapes and sizes, which can be easily modified genetically or chemically, serve as a foundation for the major use of viral nanotechnology in the fields of biomedicine, photonics, catalysis, and energy.

The shift from *how to construct* to *how to use* is most crucial, leading to disruptive innovations in all the three areas of medicine—prevention, treatment, and diagnostics—and opens a way to *singularity* in medicine, assuring a total control over health and the fundamental changes in health care. Arnold Schoenberg once wrote in *Theory of Harmony*, "Our noblest impulse, the impulse to know and understand (erkennen), makes it our duty to search." We are confident that this collection of works of eminent researchers will induce such "noblest impulse" to discoveries in the field of viral nanotechnology, promoting the search for unprecedented improvements in human life.

This book, which you hold in your hands, presents a unique opportunity for direct communication with many practitioners of this exciting discipline. We hope that, despite skeptics, you will get this sense of imminence of major breakthroughs, which will convert dreams of controlled health and betterment of humankind into the firm reality of our times.

Yury Khudyakov
Paul Pumpens

Editors

Yury E. Khudyakov, PhD, is chief of the Molecular Epidemiology and Bioinformatics Laboratory, Laboratory Branch (LB), Division of Viral Hepatitis (DVH), Centers for Disease Control and Prevention (CDC), Atlanta, Georgia. He earned his MS in genetics from the Novosibirsk State University, Novosibirsk, Russia, and his PhD in molecular biology from the D.I. Ivanovsky Institute of Virology, Academy of Medical Sciences, Moscow, Russia. He started his research career in the laboratory of gene chemistry at the M.M. Schemyakin Institute of Bioorganic Chemistry, Academy of Sciences, Moscow, Russia. He was a research fellow in the laboratory of viral biochemistry and chief of the Genetic Engineering Section in the laboratory of chemistry of viral nucleic acids and proteins at the D.I. Ivanovsky Institute of Virology, Moscow, Russia. In 1991, he joined the Hepatitis Branch (HB), Division of Viral and Rickettsial Diseases (DVRD)/CDC, as a National Research Council Research Associate, National Academy of Sciences, United States. Since 1996, he has served as chief in the Developmental Diagnostic Unit, Molecular and Immunodiagnostic Section/ HB/DVRD/ NCID/CDC, and later as chief of computational molecular biology activity, and deputy chief of the Developmental Diagnostic Laboratory, LB/DVH/CDC.

Dr. Khudyakov's main research interests are molecular epidemiology of viral diseases, development of new diagnostics and vaccines, molecular biology and evolution of viruses, and bioinformatics.

Dr. Khudyakov has published more than 170 research papers and book chapters. He has edited the books *Artificial DNA*, CRC Press (2002) and *Medicinal Protein Engineering*, CRC Press (2009). He is an author of several issued and pending patents. He is a member of the editorial board for the *Journal of Clinical Virology* and the academic editor for *PLoS ONE*.

Paul Pumpens, PhD, graduated from the Chemical Department of the University of Latvia in 1970 and earned his PhD in molecular biology from the Latvian Academy of Sciences, Riga and DSc from the Institute of Molecular Biology of the USSR Academy of Sciences, Moscow, Russia.

Dr. Pumpens started his research career as a research fellow at the Institute of Organic Synthesis, where he conducted research from 1973 to 1989. He served as head of the Laboratory of Protein Engineering at the Institute of Organic Synthesis (1989–1990), as head of the Department of Protein Engineering at the Institute of Molecular Biology of the Latvian Academy of Sciences (since 1993, the Biomedical Research and Study Centre) in Riga (1990–2002), and as scientific director of the Biomedical Research and Study Centre (2002–2014). He served as a professor of the Biological Department of the University of Latvia from 1999 until 2013.

Dr. Pumpens pioneered genetic engineering research in Latvia. He was one of the first in the world to perform successful cloning of the hepatitis B virus genome and the expression of hepatitis B virus genes in bacterial cells. His major scientific interests are in designing novel recombinant vaccines and diagnostic reagents and development of tools for gene therapy on the basis of virus-like particles.

Dr. Pumpens is an author of more than 300 scientific papers and issues or pending patents.

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Introduction

> Yet the spirit of an epoch is reflected not in the arts alone, but in every field of human endeavor, from theology to engineering.
>
> ***Music in Western Civilization*** **by Paul Henry Lang**

Nanotechnology is a rapidly progressing field of science that unites a broad range of diverse disciplines from biomedicine, including microbiology, virology, immunology, and vaccinology, to material science, including organic and inorganic chemistry, semiconductor physics, and microfabrication.

Although many definitions are in use, nanotechnology is commonly defined as a knowledge-based manipulation of matter sized from 1 to 100 nm with a special interest in self-assembling and self-regulating systems. Considering that many viral structural proteins match this definition, it is not surprising that nanotechnology embraced and explored various applications for viral capsids and envelopes. At the dawn of genetic engineering in the late 1970s of the twentieth century, macrostructures spontaneously assembled from recombinant viral structural proteins and resembling viral capsids and envelopes were described as *virus-like particles* (*VLPs*). Over recent years, these structures became also known as *virus-like nanoparticles* and *viral nanoparticles* (*VNPs*), reflecting a changing attitude of molecular researchers in the field from observation to technology. This transition resulted in the formulation of *viral nanotechnology* as an independent discipline within *bionanotechnology*, which involves all biological materials.

The subject of viral nanotechnology and its methodological foundation and applications are still being conceptualized. Existing concepts of the field will be certainly challenged and developed further. Nevertheless, the field is already vast and cannot be captured in a single paper or a single book. This volume contains a collection of chapters that just reflect the current state of viral nanotechnology, including basic methodologies (Section I) and selected applications clustered according to VLP structures (Section II). The history of the discipline is still to be written; however, it can be somewhat discerned from the order of applications presented in the book.

Hepatitis B virus (HBV) is one of the very first objects explored by recombinant gene expression technologies (Chapter 10). The initial attempts to express the HBV surface antigen (HBsAg) or envelope protein in *Escherichia coli*, the most popular bacterial host cell for expression of recombinant proteins, resulted, however, in a protein product that only faintly reproduced antigenic properties of the plasma-derived HBsAg. This setback was, however, rapidly overcome by expression of recombinant HBsAg in yeast and mammalian cells. Detection of HBV VLPs in extracts of yeast cells transformed with recombinant plasmids was a stunning demonstration of self-assembly of viral envelope proteins expressed in the heterologous expression system (Chapter 10). Discovery of native immunological properties, including capacity to elicit strong neutralizing immune responses upon immunization, not only prompted vaccine applications of the recombinant HBV VLP (Chapter 10) but re-enforced the notion, common at the time, of importance for the envelope macrostructure to be accurately reproduced in order to model antigenic epitopes. Although experiments with synthetic peptides and recombinant proteins showed later that antigenic epitopes, including conformation-dependent antigenic epitopes, can be efficiently modeled without the actual reproduction of the native envelope macrostructure, self-assembly of viral proteins continues to be an attractive fundamental property guiding a multitude of viral applications (Chapter 2), with VLPs remaining desirable targets for the development of vaccines and diagnostic reagents (Chapter 7).

The early experiences with HBsAg showed that the accuracy of functional reproduction of viral proteins is dependent on the used expression system (Chapter 10). However, selection of the appropriate expression system is not defined by functional reproduction alone but also by technological requirements that make the target nanostructures readily available for applications at low cost (Chapters 16 and 17). Technology of expression of recombinant biomaterials with desirable properties made giant strides since the time of the use of single-cellular bacterial and eukaryotic expression systems that dominated biotechnological applications years ago. Among many, plant expression systems have been recently developed into powerful biological factories for mass-scale production of nanoparticles from a large variety of viruses for vaccine applications (Chapter 16) and for manufacturing novel nanomaterials (Chapter 17).

Applications explored by viral nanotechnology, however, go beyond the selection of expression systems. Transition from functional reproduction of natural structures to rational design of particles with novel properties is a major trend epitomized by viral nanotechnology (Chapter 15). Harnessing of protein folding and assembly of proteins into higher-order structures is essential for application of viral proteins in nanotechnology. Recent advances in resolution of electron cryomicroscopy to the range of 4 Å for icosahedral and helical viral macrostructures and 20 Å for complex polymorphic virions (Chapter 3) and x-ray crystallographic analysis to the atomic resolution of 2 Å (Chapter 4) drastically improved our knowledge of viral architectures and prompted development of highly accurate computational approaches for prediction of protein 3D structures (Chapter 5). Availability of high-quality structural information is key to the rational design of VLP with predetermined properties. The introduction of different

functional entities at precisely defined sites of viral structures allows for the development of synthetic VLP (Chapter 29) and extends application of VLP beyond vaccines (Chapter 6).

Nothing in viral nanotechnology has a long history. All is recent, including applications such as nanomedicine (Chapter 8). However, even a short history has its champions. Although nanotechnology has explored structural proteins from almost all major viral families at one time or another (Section II), the contribution of the HBV core protein (HBc) to the field is most long-lasting. Three decades of research made this protein into a legendary model system of viral nanotechnology (Chapters 11 through 14). A robust assembly of HBc into a highly immunogenic structure, which is greatly amenable to introduction of exogenous epitopes, was discovered during the early days of genetic engineering and was immediately explored for the development of a novel generation of vaccines (Chapter 11). Using this model, several unconventional methodologies were introduced for the controlled formation of nanoparticulate structures with predefined properties (Chapter 12) and for boosting immune responses against DNA vaccines (Chapter 14). Unique nanotechnological properties of HBc led researchers to explore core proteins of the other members of Hepadnaviridae, of which HBV is a prototype species. Woodchuck hepatitis virus core (WHc) was used as a carrier for malaria antigenic epitopes. This antigen shares many vaccine-relevant properties of HBc except for HBV-specific immunoreactivity. Thus, the WHc-based malaria vaccine won't immunoreact with an antibody against HBc. Considering that ~15% of human population experienced HBV infection, cross-immunoreactivity with anti-HBc could've compromised efficacy of the HBc-based vaccines, especially in hepatitis B endemic regions of the world (Chapter 13).

To the general scientific community, vaccine development remains the most known application of VLPs (Chapter 9). Indeed, VLPs are natural vaccine targets. Surface geometry of repetitive 3D motives improves avidity binding of VLPs to B-cell receptors and formation of immune complexes, thus promoting strong immune responses that cannot be induced by a single protein (Chapter 7). Viral structural proteins form a variety of particles of different sizes and shapes (Chapter 1). Considering that the VLP macrostructure has a significant effect on immune responses (Chapter 7), VLPs formed from structural proteins of different viruses offer numerous opportunities for novel approaches to vaccines (Section II).

With certain exceptions, viruses are miniature genetic systems capable of encoding only a few different proteins. In order to support the infectious process, viral proteins must be polyfunctional. Structural proteins are responsible for packaging, protection, and delivery of viral genetic material, which is accomplished by binding to cellular receptors, internalization, and disassembly of virions. These proteins also play a key role in evading innate and adaptive immune responses. Polyfunctionality offers a wealth of opportunities and presents a multitude of challenges for nanotechnological applications of viral proteins. Manipulation of the protein structure allows for augmenting or boosting useful functions and suppressing or eliminating the undesirable functions of viral proteins (Section II). Different applications target different viral properties. For example, gene therapy makes use of the nucleic acid–transporting properties of VLPs rather than their capacity to elicit immune responses, while vaccine applications make use of the VLPs' high immunogenicity rather than their binding to cellular receptors and protection of nucleic acids.

Moreover, polyfunctionality of viral proteins presents opportunities for the development of different approaches to the same applications. For example, not all VLP applications to vaccines are alike. VLP can be used directly as a vaccine (Chapters 10 and 19 through 21) or as a carrier of epitopes (Chapters 9, 11 through 13, 20, and 21). In the first case, the immunogenic VLP properties and capacity to elicit the VLP-specific immune response are used to induce protective immunity in vaccinated hosts. In the second case, though, the challenge is to use immunogenic properties of VLP to direct immune response against the inserted epitopes rather than against the carrier itself. Although structural proteins from all viral families can be used as epitope carriers (Section II), phage VLPs are most suitable to carry exogenous epitopes. These VLPs are highly immunogenic and incapable of interacting with antibody against human pathogens (Chapter 15). Antibody binding to the carrier protein reduces efficacy of vaccination against the inserted epitopes, especially in human populations with high prevalence of the carrier-specific antibody (Chapter 13).

VLP-based vaccines are not just dreams of a distant future; they are reality of the present. HBV (Chapter 10) and human papilloma virus (HPV) (Chapters 19 and 20) VLPs are efficient vaccines of today that have already saved many human lives from debilitating and deadly diseases. Both viruses cause malignant transformation of infected cells. HBV vaccine is heralded as the very first anticancer vaccine that prevents the development of liver malignancy. HPV VLPs offer a very potent treatment against HPV-induced tumors. A variety of VLPs from different viral families were generated for cancer therapy (Chapter 27) and evaluated for treatment of various forms of cancer, including melanoma, hepatocellular carcinoma, and malignant tumors of different organs (Chapter 28).

All viruses have a capsid that packages nucleic acids. Many viruses have an additional lipoprotein shell or envelope that covers the capsid. Both capsid and envelope proteins may form VLPs upon production in heterologous expression systems. The envelope VLPs are mainly applied to vaccine development (Chapter 10). However, applications of the capsid VLPs are much broader and not restricted to vaccines against infections with nonenveloped viruses or immunotherapy (Chapters 23, 27, and 28). Their capability to form complexes with exogenous nucleic acids is used for the specific delivery of DNA vaccines and for gene therapy by gene transfer and silencing (Chapter 20).

The range of VLP applications continuously increases, with each passing year bringing new ideas for the biomedical use of protein nanostructures. For example, application of the polyoma- and papilloma-derived VLPs for gene delivery was

extended to the use of the same VLPs as nanocontainers for exogenous proteins and peptides with pharmacological properties. Nanocontainers protect the enclosed cargo from proteases and immune responses, preventing its degradation. Such protection helps to sustain a pharmacological concentration of proteins and peptides, which significantly improves their therapeutic effects (Chapter 20). Development of nanoreactors from bacterial microcompartments, or closed capsid-like structures, which encapsulate enzymes and enzymatic pathways, is another extraordinary advancement in nanotechnology that revolutionizes the use of enzymatic reactions, making them considerably more efficient than can be attained by a simple mixing of enzymes (Chapter 22). Although viral nanotechnology is destined to revamp many fields of human endeavor, from electronics to medicine, none of them will be as affected as health care and public health (Chapter 8). In addition to prevention and therapy (Section II), viral nanotechnology will transform diagnostics by devising, for example, VLP-enabled imaging probes for optical and magnetic resonance imaging and for positron-emission tomography (Chapter 23).

Increase in demand for various applications motivates exploration of more complex viral nanostructures. If we look at the developments in the field over the last several years, we will see that viral nanotechnology is progressing from using mainly single-protein VLPs to nanostructures composed of more than one or several proteins (Chapters 18 and 26) and from icosahedral to asymmetrical VLPs (Chapters 24 and 25). These trends do not only reflect significant advancements of molecular technologies, enabling production of such complex protein systems, but point to a rapid conceptual evolution of nanotechnology from needs of yesterday to anticipations of tomorrow.

Nanotechnology is built on actionable ideas. Transition from mere exploring to active improving the world is a major development in scientific thinking of the time, with nanotechnology having been born from this development. The thrust forward experienced by the field of viral nanotechnology in recent years is overwhelming and inspires expectations that people of this generation will see improvement in health and life on a scale unprecedented in the history of humankind.

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