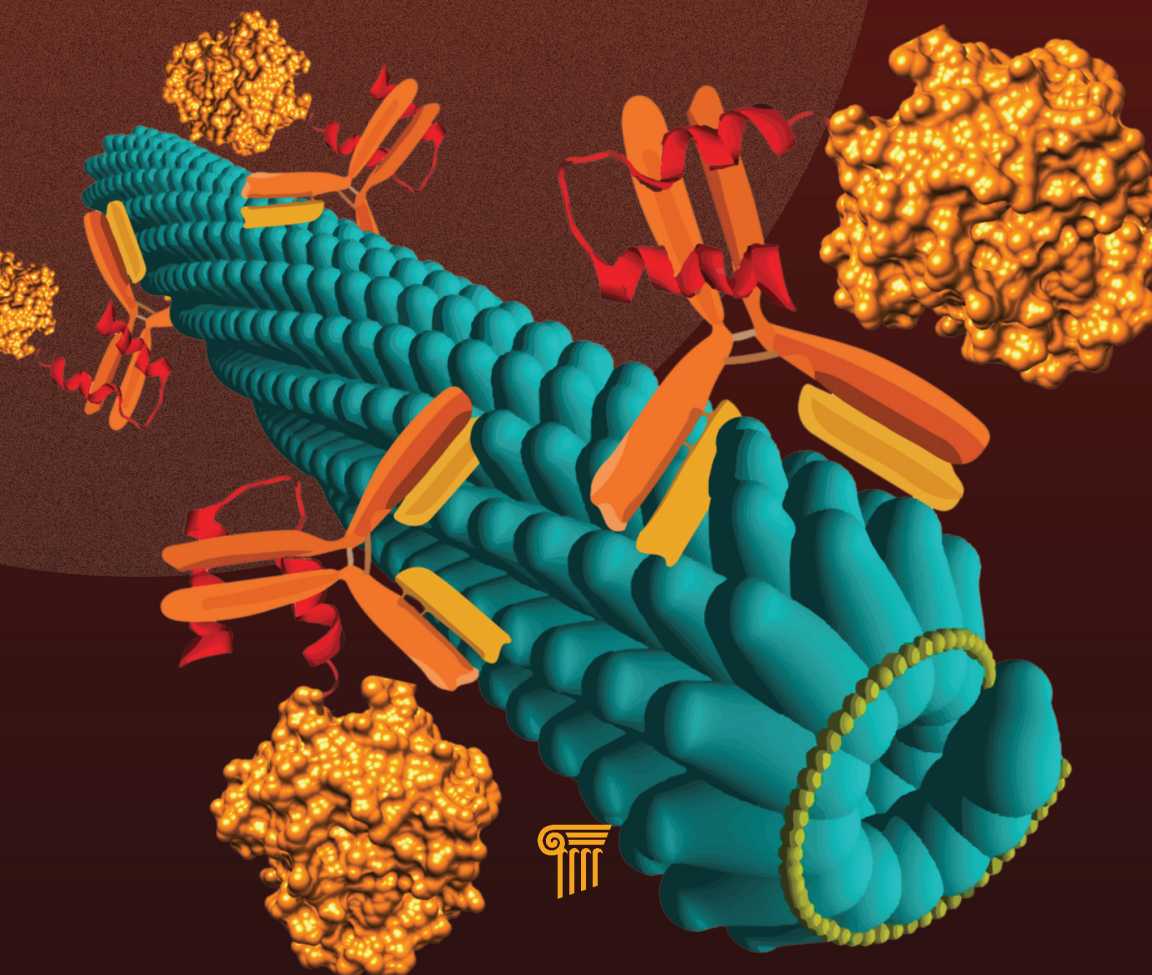
Enzyme Nanocarriers

edited by
Daniela Cardinale | Thierry Michon



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Preface

Enzyme immobilization has over 50 years of well-documented history, with discoveries spanning a large field of applications from sustainable chemistry to medicine through enzyme-targeted therapy. With its increasing understanding of living systems, the scientific community developed a new interest for biologically ordered structures having the potential to become "enzyme nanocarriers" (ENCs).

In nature, proteins interact by means of specific domains. They also combine with lipids and nucleic acids. From these biocomposites originate the three classes of carriers discussed in this book: viruses, polymersomes (inspired from lipidic membrane structures), and DNA origamis. A deep knowledge of the structure of these systems at the atomic resolution and of the physical rules allowing their assembly demonstrated that protein components are key factors for these assemblies. Because of the high power of genetic engineering in designing new protein properties, it has become possible to achieve a high positional control of enzymes on these complex composites. Today, biologists desiring to get into the promising field of bioinspired nanocatalysts have at their disposal a large choice of structures and the tools to modify them.

Many smart applications of these composites have already been proposed. For instance, virus-like particles (VLPs) depleted from viral genetic material can be engineered and used as potent enzymatic nanoreactors or for targeting cytotoxic enzymes to cancer cells.

This book covers some of the most recent advances in this fast-evolving field. We hope that it will help the reader in the conception of new tools to create some ENCs of the future.

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Summer 2015

Introduction

Enzyme Nanocarriers: What Are They?

Enzyme immobilization on solid supports has been considered for a long time as an attractive solution to perform sophisticated organic synthesis, as required in the preparation of fine pharmaceutical chemicals. A good example is penicillin-G-acylase (PGA), which was immobilized on an epoxidic support. It improved its catalytic features, especially its efficiency in the synthesis of β-lactamic antibiotics and in other kinetically controlled reactions, such as the resolution of racemic compounds. Indeed, PGA is capable of catalyzing acylation reactions far more simply than the chemical methods commonly used in industrial processes. Enzymatic synthesis normally requires mild reaction conditions that are compatible with product stability. The resulting compounds are purer than those obtained by the classic processes of chemical synthesis. Moreover, by avoiding the need to use toxic reagents and solvents, the impact on the environment is greatly reduced. Besides this, the possibility of linking the enzyme with a solid support so as to be able to recover and reuse it effectively renders the study and development of enzymatic processes for the synthesis of penicillin and cephalosporin increasingly interesting for industry. Integration of redox enzymes with an electrode support and formation of an electrical contact between the biocatalysts and the electrode are the fundamental subjects of bioelectronics and optobioelectronics. But with the development of life sciences, chemists became increasingly interested in the performance of enzymes in their genuine environment. In living systems, the cellular organization of cooperating enzymes into supramolecular complexes is a metabolism key feature. A major advantage of such organization is the transfer of biosynthetic intermediates between catalytic sites without diffusion into the bulk phase of the cell. This so-called metabolic channeling combines an enhancement of the catalysis efficiency with a fine-tuning of metabolic pathways. The growing accumulation of knowledge in the field has inspired new technologies to design bioreactors coupling several enzymatic reactions. Consequently considerable effort is produced, aiming at

mimicking natural enzyme organization for optimizing the synthesis of valuable metabolites with industrial and medical importance. Today, enzyme immobilization on carriers is considered in several fields, including both fundamental studies covering modern aspects of enzyme kinetics (substrate confinement, limiting diffusion, single-enzyme kinetics, biocatalyzed cascade reactions) and more applied strategies like high-throughput screening of catalytic specificities, bioreactors, sensor ships for analysis, medical diagnosis, and therapy.

In the late nineties, the tremendous progresses of molecular biology fundamentals opened up the possibilities to feed a toolbox for building new bioinspired nanotechnologies. Among them was the goal to reposition biocatalysts in environments mimicking their genuine working place, the cell. The research presented in this book was selected among the most impressive achievements in the field of enzyme bioconjugation to bioinspired nanosupports. It opens up potential applications in nanocatalysis, lab-on-a-chip and biosensor devices, drug delivery vectors, nanometrology, and many more, letting the reader feel that imagination will be the only limitation. We wish this book to be a source of inspiration for the researcher seeking to build smart materials requiring a nanoscale positional control of functional proteins on various carriers. All the supports described herein pertain to soft materials (cells, virus, polymers, DNA). Most of the examples benefit from the amazing properties of proteins and DNA to self-assemble according to the "bottom-up law," a specific feature of all living systems. But these ENCs have the potential to be grafted on solid supports through "top-down technologies," spanning orders of magnitude from the nano- to the mesoscale and above.

Positional Control: A Key

The bottleneck in combining several different enzymes working cooperatively comes from the difficulty to control their relative positional assembly on the support. This control can be achieved by coupling the enzymes of interest with a compatible highly ordered protein scaffold. In the course of evolution, nature has optimized complex architectures conjoining perfect positioning in space with highly specialized functions. These smart materials can be regarded as composites made of a restricted number of building blocks (lipids,

proteins, polysaccharides, and nucleic acids). But ultimately, most of these combinations are finely tuned by precise surface overlaps between proteins. The diversity of the combinations of the 20 natural amino acids along the polypeptide sequence results in peptide folding into 3D domains, ensuring a very high selectivity of the assemblies. Virus particles are supramolecular edifices unsurpassed in nature. The simplest of these systems are made of noncovalent combinations of proteins and nucleic acids, which are precisely arranged in space. The information for this self-assembly is programmed in the virus genome through the amino acid sequence of the protein monomers (capsomers) forming the viral particle, or capsid. Although the natural function of capsids is the storage and transport of genetic material, their defined size has made them attractive building blocks from a materials science and nanotechnology point of view. Coupling enzymes to viruses' highly ordered protein backbones is an attractive way to achieve positional control. A very interesting feature of virus scaffolds resides in the diversity of their shapes and physical and chemical properties. Viruses are precious building blocks for nano- and micromaterial design. Their interior space is accessible to small molecules but often impermeable to large ones. Bacteriophages and to some extent plant (cowpea chlorotic mottle virus [CCMV], tobacco mosaic virus [TMV]) or mammalian (hepatitis B virus [HBV]) viruses can serve as good carriers for enzymes. The main advantages are their nanometer size range, their propensity to efficiently self-assemble into monodisperse nanoparticles of discrete shape and size, their stability and robustness, their biocompatibility and bioavailability, and the ease of production in large quantities. In Chapter 1, "Virus Diversity to Explore Various Kinds of Enzyme Nanocarriers," Makinen, Besong-Ndika, and Walter present the vast potentialities offered by virus diversity to design ENCs. Viruses appear to constitute the most abundant and robust biological entities on earth. For instance, the discovery of icosahedral viruses resistant to extreme acidic environments opens up the possibility of using viral architectures for applications that previously seemed unlikely. Ultimately, our creativity might well be the only limitation to the potential use of viral capsids.

Whatever the enzymatic device considered an important issue concerns the topology of enzyme grafting on the support. In an ideal situation, the enzyme must be suitably orientated to facilitate the substrate access to the active site. In Chapter 2, "Strategies for Virus–

Enzyme Coupling," Cardinale, Carette, and Michon review various strategies allowing a positional control of protein grafting inside and outside VLPs using both covalent and noncovalent solutions. The possibility to genetically engineer specific recognition motives on enzymes has opened up new routes to finely tune the orientation of the catalyst on or in the support.

In the cell, the main factor limiting the efficiency of reaction cascades is the Brownian diffusion of reactants. Living systems use essentially two strategies to overcome this limitation. The first strategy consists of the confinement in cellular compartments of the enzymes involved (lysosomes, mitochondria, etc.). The enzymes cannot escape, but these little chambers remain permeable to reactants. Once in the compartment, the substrate is exposed to a high local concentration of enzymes by the confinement effect. The efficiency of catalysis is significantly accelerated compared to reactions in an open medium. In the second strategy, enzymes involved in a given reaction sequence are distributed close enough so as to limit the diffusion. Most of the time, intermediate reactants are trapped in a matrix anchoring the enzymes and diffuse from one catalyst to the other. This matrix can be a phospholipid membrane bilayer, such as the inner membrane of the mitochondria, which contains the adenosine triphosphate (ATP) synthase complex surrounding the respiratory redox cascade. The synthesis of ATP requires a strict positional control of these enzymes related to each other at the nanoscale. In Chapter 3, "Viruses as Model Nanoreactors to Study Enzyme Kinetics," Rurup, Koay, and Cornelissen discuss the use of VLPs' reconstitution to produce a biomimetic confinement of enzymes according to the first strategy described above. A microbial lipase was selectively addressed to the inside surface of a VLP using a peptide-based noncovalent reversible "molecular Velcro" between the coat protein of the virus and the enzyme. In this confined space, the probability of collisions leading to efficient conversion of the substrate is increased. Following this strategy, it is possible to confine cascade reactions catalyzed by coupled enzymes in VLP systems. In addition the authors demonstrate how the limited dilution method allows the confinement of a single enzyme per VLP, providing a tool to study single-enzyme kinetics. Fluctuations in the catalysis rate or velocity distribution of a single enzyme over time (dynamic disorder) cannot be addressed by conventional kinetics. The real-time behavior of a population of isolated enzymes reveals

that enzyme conformational dynamics are linked to the catalytic event, an aspect previously only addressed in silico by molecular dynamics simulations. For such studies, the experimental design requires passive adsorption on glass supports or a chemical modification of the enzyme, two processes frequently accompanied by enzyme denaturation. As a solution, VLPs can mimic the small reaction volumes in a cell. The kinetic behavior in real time of a single molecule of horseradish peroxidase (HRP) trapped inside a VLP of CCMV was reported using fluorescence microscopy. The dilution of HRP was adjusted in the presence of a dissociated capsid protein (CP) to generate a mixture of empty VLPs and VLPs containing a single enzyme. When peroxidase was adsorbed to the VLP's outer surface, the product diffused freely and the fluorescence was less localized. In both cases, a significant fluctuation of fluorescence over time was observed.

Alternative Routes to Make Biomimetic ENCs

A system mimicking the natural compartmentalization of enzymes in the cell organelles is described in Chapter 4, "Nanoscale Compartmentalization Techniques in Cascade Catalysis," by van Hest, Willemsen, and Rutjes. This system, defined as a porous polymersome reactor, is based on block copolymers of isocyanopeptides and styrene. Enzymes have been anchored at three different locations, namely, the lumen (glucose oxidase [GOx]), the bilayer (Candida Antarctica lipase B [CalB]), and the surface (HRP) of the polymersomes. Such an approach, applying the most sophisticated modern chemistry, allowed the authors to demonstrate that it is possible to control an enzymatic cascade of a reaction using a differential compartmentalization of the biocatalysts. This book is not intended to be exhaustive in the field of ENCs. Hence, we focused on a selection of approaches illustrating the most significant advances in the field. The reader will have noticed that however elegant and sophisticated they may be, these achievements mainly aim to be a proof of concept. However, they already participate in a box of molecular tools that technologists have begun to use. In terms of promising applications, we could not omit the use of ENCs in the medical field. Chapter 5, "Nanocarriers for Therapeutic Enzymes," treated by Howard, Hood, and Muzykantov, focuses on different concepts supporting these applications.

Assessing the effect of spatial organization on enzymatic activity in multienzyme systems is of primary interest. It will not only help to understand the molecular mechanisms involved but also will have a high impact for the design of nanotransducers and lab-on-a-chip applications. To this aim, nanoscale experimental platforms need to be designed. There are very few methods available to systematically evaluate how spatial factors (e.g., position, orientation) influence enzymatic activity in multienzyme systems. This limitation notably comes from the fact that their small size makes it extremely difficult to organize biomolecules onto surfaces in order to form fully active supramolecular complexes amenable to experimental studies. Recent advances in DNA–protein conjugates make the absolute control of enzymes positioning on well-defined 2D DNA origami possible. This provides a true genetically programmable bottom-up self-assembly of enzyme complexes on DNA templates. Using a chemical conjugation of enzymes and DNA staples specifically hybridizing with topologically defined sequences on the 2D DNA supports, the catalytic efficiency of the cascade can be finely tuned as a function of the distance between enzymes, so demonstrating the channeling effect discussed above. However, enzyme orientation on the support remains uncontrolled because of conjugation sites all around the protein surface. Far more sophisticated is the use of a whole biological organism as microplants to build networks of enzyme cascades on RNA templates. Such an approach belongs to the very active field of synthetic biology. Bacteria were genetically programmed to produce RNA self-assembling in large areas displaying anchoring sequences for small RNA aptamers allowing specific binding to the enzymes composing the cascade. It was demonstrated that the efficiency of the cascade is modulated by the assembly geometry [1]. In Chapter 6, "DNA Origamis as Protein Nanocarriers," Elezgaray, Aimé, and Arbona, after a general introduction on DNA origamis, present the potential of 2D and 3D DNA constructs as carriers. Although representing a remarkable experimental achievement, this approach has, up to now, presented notable drawbacks for "out of the cell" applications. This supramolecular organization of enzymes using a totally bottom-up approach is difficult to extend from nano- to mesoscopic-length scales. Moreover, in terms of applications, DNA-based structures are sensitive to temperature and many other physical and chemical parameters. However, very recent works demonstrate that such difficulties are progressively overcome. In particular, the organization

of origami units at the mesoscale and above could be accessible in the near future [2]. Chapters 3–6 deal with nano-object carriers, which can be used for the transport of enzymes. It was discussed above that, because of their highly ordered nature, virus-like structures can be precisely decorated with enzymes and can be used as ENCs. It appears that ENCs are easier to position on a support than single enzymes using top-down processes such as nanolithography or convective-capillary deposition. In Chapter 7, "Nanopatterning for Nanobiotechnologies: Emerging Methods Based on Soft Lithography and Directed Assembly," Cerf, Thibault, Trévisiol, and Vieu present the last developments of top-down technologies enabling a precise patterning of single nano-objects such as virus particles or DNA molecules on various supports. This illustrates how bottom-up and top-down approaches begin to meet for the preparation of smart materials. This approach aims at bridging the gaps between the mesoscale, the microscale, and higher.

The interest for virus-based technological applications increases in a variety of emerging fields from self-assembled nanoscale computers and machines to drug delivery vehicles, biochips, and self-healing regenerative tissues. In addition, alternative enzyme nanocarriers such as polymersomes and DNA are under investigation. This field of expertise will become increasingly important in the coming decade.

This edition of the book gets now to completion but science is still running. Recent developments in synthetic biology have led the concept of ENCs much farther. Bacterial cells can now be redesigned for the in situ assembly of enzymes networks on RNAs origamis. An actualized table content of the book should certainly include a very last chapter from a team such as the one of Delebecque et al. [1] whose work allows to envisage the engineered bacterium itself as a true sensor chip.

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Bordeaux, France
Summer 2015

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2. Teshome, B., Facsko, S., and Keller, A. (2014).Topography-controlled alignment of DNA origami nanotubes on nanopatterned surfaces. *Nanoscale*, 6(3), 1790–1796.

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