

# Methods in ENZYMOLOGY

Volume 571

Rational Design  
of Enzyme-Nanomaterials

*Edited by*

Challa Vijaya Kumar





VOLUME FIVE HUNDRED AND SEVENTY ONE

# METHODS IN ENZYMOLGY

## Rational Design of Enzyme-Nanomaterials

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Enzyme-Nanomaterials

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## PREFACE

Enzymes are nature's catalysts and they are responsible for accelerating most chemical reactions in biology. The high selectivity or specificity of these catalysts, which operate under mild conditions of biological systems, makes them very attractive for use in the laboratory or industry. But this is quite challenging, because enzymes have evolved to function in the complex biological media at biological temperatures, and hence their stability/function under nonbiological conditions of a common chemical laboratory such as organic solvent media, extreme pHs, or high temperatures is often poor. Most enzymes are also quite expensive when compared to many chemical reagents, and hence special methods are required to render enzymes usable in the context of a chemical laboratory or for industry. This volume addresses these two important issues related to enzymes in much detail, and builds on the important contributions made to this topic from previous volumes of this series.<sup>1</sup>

Recent developments in nanochemistry are brought to bear on the above two problems of enzyme stability/function in the current volume. From a fundamental chemical point of view, enzyme stability is a thermodynamic issue, while kinetic stability is also important. Enzymes are thermodynamically stable at room temperature but the denaturation free energy ( $\Delta G$ ) decreases with increase in temperature. It reaches a numerical value of zero at the denaturation temperature, and at this temperature, the native and denatured states of the enzyme are in equilibrium with equal concentrations. Above this temperature, the denatured state is thermodynamically preferred over the native state. Similarly, the pH and solvent dielectric can also influence  $\Delta G$  and render enzymes unstable. To combat this important issue, significant progress has been made in improving enzyme stability by reducing the conformational entropy of enzymes by encasing them in nanomaterials with a variety of architectures described in this volume. One important point is that entropy of denaturation ( $\Delta S$ ) is positive and any reduction in  $\Delta S$  raises  $\Delta G$  and hence contributes to the enhanced stability of the enzyme. Many methods are developed to take advantage of this thermodynamic principle, directly or indirectly to lower the conformational entropy of the denatured state, and nanochemistry methods have become

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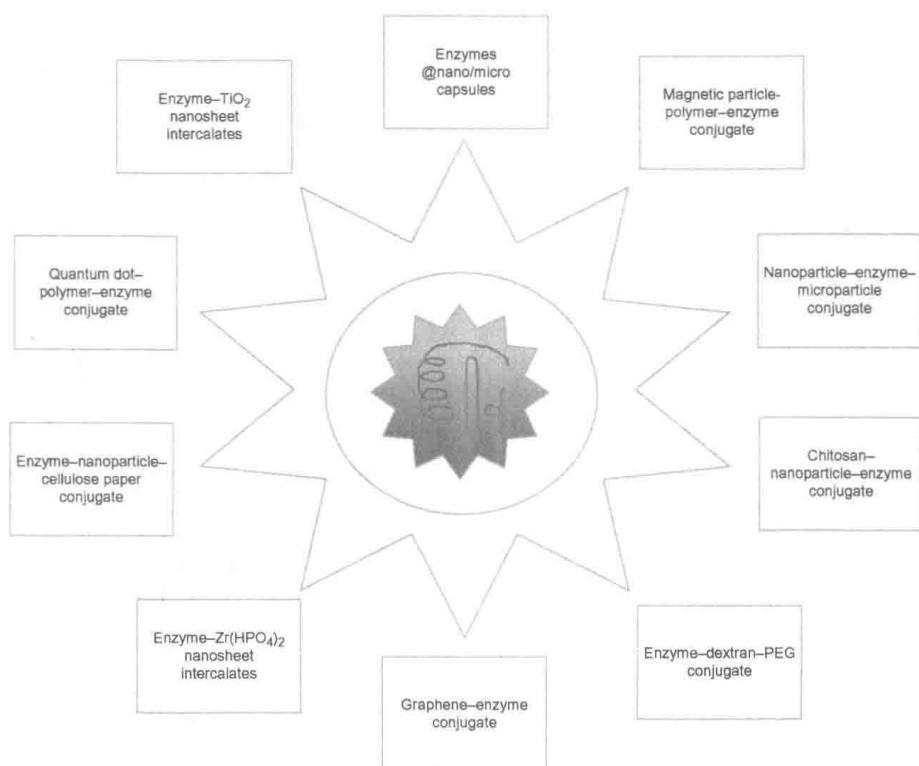
<sup>1</sup> Methods in Enzymology Volumes 137, 136, 135, 64, and 44.

handy for this approach. Our understanding of these issues, therefore, is rooted in fundamentals so that the tree of knowledge can grow and prosper. This is what the authors of the chapters in this volume have striven to do.

Nanomaterials have several unique properties, such as high surface area for unit mass, rich surface chemistry arising from surface atoms which differ significantly from those of the interior atoms, and these materials also have versatile optical, thermal, and magnetic properties. When these interesting properties of nanomaterials are coupled with the versatile catalytic abilities of enzymes, one can generate novel biocatalytic nanomaterials. Many examples are documented in this volume. Very high loadings of the enzymes on the nanoparticle are often achieved due to their large surface-to-mass ratio. Due to the nanosize of the support materials, diffusion of the substrate to the active site of the bound enzyme is also facilitated.

One major difficulty in using nanomaterials for enzyme binding and recycling, however, is that the separation of the nanoparticle bound enzyme from the reaction media is often difficult due to the dispersion of the particles in the solvent. This important issue has been addressed by using several different methods in this volume, and one of them is the use of magnetic nanoparticles, and the other has been using innovative design architectures where the nanoparticles themselves are embedded in porous microparticles that could be readily separated from the reaction media and recycled (Fig. 1). Easy separation, reaction workup, and recycling of the biocatalyst could reduce the process cost and hence very important for industrial applications. This kind of hierarchical structure design from the nanoscale to macroscale with attention to the molecular details of the support matrix and its porous nature is critical for rapid progress in this area. This was the subject matter of several distinguished chapters in this volume.

The use of two-dimensional materials (nanosheets) for enzyme binding is another major breakthrough in the field. Because of their nm-thinness and large lateral size of several microns, nanosheets have very large surface-area-to-mass ratios and large ratio of length to thickness (aspect ratio) greater than their nanoparticle cousins. Therefore, large amounts of enzymes can be bound per gram of these nanosheets, and due to this unique aspect ratio, the bound enzymes are well exposed to the solvent for rapid reactions, overcoming the diffusional issues related to enzymes bound on nanoparticle surfaces. Furthermore, these nanosheets can be restacked with enzymes entrapped between the plates, and the nanogalleries formed by restacking provide protection against proteases, bacteria, and viruses, which can potentially degrade the biocatalyst. When properly designed,



**Figure 1** The variety of nanoarchitectures and methods used in the design of enzyme–nanomaterial hybrids for advanced enzymology applications.

enzyme/nanosheet biocatalysts also enhance thermal stabilities of bound enzymes due to a significant reduction in the conformational entropy of the bound enzyme. This latter hypothesis of confinement and reduced entropy of enzymes embedded between nanosheets (two-dimensional space) is now being tested by a number of different research groups around the world.

A number of two-dimensional materials are also being explored for enzyme binding, including the state-of-the-art materials such as graphene, which could be beneficial for enzyme binding due to its unique properties. For example, charge-conducting nanosheets might be able to promote redox activities of enzymes by wiring the charge-donor and charge-acceptor sites, via the layered material. This hypothesis remains to be tested, and facile access to two-dimensional materials reported in this volume might accelerate such exciting studies.

Cellulosic paper continues to attract attention for enzyme binding and sensing applications due to its versatile features as well as low cost. Paper

might be the medium of the future for enzyme binding where the nanofibers of cellulose could be exploited for functionalization as well as physical interlocking of enzymes in the nanofibers. Paper-based sensors, as reported here and elsewhere, are very exciting for the design of light weight, inexpensive, porous, high-capacity bioreactors or enzyme cartridges for small- or large-scale synthesis applications. One other area that could benefit from these types of developments could be synthetic organic chemistry where a multistep synthesis can be carried out by a number of different enzymes embedded in the layers of a paper matrix. Such futuristic goals are not far away, and numerous examples of paper-embedded enzymes are already known and it is only a matter of systematic studies that would one day produce reaction cartridges for multistep synthesis of high value or tedious chemical reactions.

Industrial exploitation of nature's biocatalysts is still rudimentary, when one compares the current status of the field with the potential number of enzymes that have not yet been examined for attachment to nanosurfaces. From nanoparticles to nanomicrocomposites to two-dimensional materials to fibrous networks (paper), there appears to be limitless opportunities to exploit and control the behavior of enzymes under nonbiological conditions. Enzymes can influence the production of molecules, materials, and devices, and they are at the service of the mankind on a global scale. Therefore, they have the potential to make progress in addressing social, economical, environmental, and political issues of the current-day society.

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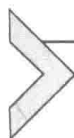
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# Preparation of Biocatalytic Microparticles by Interfacial Self-Assembly of Enzyme–Nanoparticle Conjugates Around a Cross-Linkable Core

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