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REVIEW

Understanding enzymatic acceleration at nanoparticle interfaces: Approaches and challenges

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Summary The ability of enzymes to catalyze reactions and engage in complex syntheses has long made them attractive for use in a multitude of industrial, biotechnological, and research applications. Although this utility grows with each passing year, the exploitation of enzymes in *ex vivo* formats is still hampered by relatively low rates of turnover, particularly when attached to planar surfaces. However, a growing number of reports suggest that assembling enzymes or their substrates onto nanoparticle (NP) surfaces can accelerate or otherwise improve catalysis when compared to freely diffusing enzyme in bulk solution. Here, we present an in-depth review and discussion of what is currently known about this phenomenon with an emphasis on inorganic NPs. The assembly of enzyme—NP and substrate—NP bioconjugates is first described, emphasizing their heterogeneity and the expected impact on enzymatic activity within the framework of collision theory, biomolecular interactions, and the classic Michaelis—Menten model. We next discuss representative examples from the literature where accelerated enzyme activity has been reported in conjunction with NP materials, the mechanisms that have been proposed to account for the accelerated activity, and the challenges that remain to fully understand and optimize this potentially valuable phenomenon. Finally, approaches to quantitative modeling of NP-associated enzyme activity are discussed, including a kinetic analysis that we suggest can provide insight into the underlying mechanisms that may drive the observed rate enhancements. We conclude with a perspective on the future evolution and broader impact of this growing area of nanoresearch.
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Introduction

The biorecognition and catalytic processes derived from antibodies, enzymes, nucleic acids, peptides, and the like have traditionally been exploited in solution or on surfaces for biotechnological applications. However, the last decade has seen a revolution in the approach to using these reagents with their incorporation into hybrid nanoparticle (NP) complexes. Such hybrid materials combine the advantages of their biological component (*i.e.*, biorecognition, binding, catalysis, inhibition, *etc.*) with the unique physicochemical properties of the NPs (*i.e.*, luminescence, magnetism, photoconversion, *etc.*) [1–6]. To exploit these opportunities, tremendous research efforts have focused on the generation of new and varied hybrid materials. Incorporating enzymes into nanomaterials is especially promising as it is predicted that their catalytic properties, in combination with NP architectures, can be exploited to create designer nanofactories or active composites suitable for energy conversion, bioelectronics, biosensing, drug delivery, and theranostics to name but a few applications [1,3–5,7,8]. A key feature of such enzyme–NP composites, and the converse substrate–NP conjugates, is increasingly frequent reports of enzymatic rates that are enhanced when compared to those reported for the same biomolecular components in the absence of the NP scaffold; see Table 1 for a representative listing [9].

Enzymes undergo dynamic conformational changes that allow them to rapidly sample their substrate(s) to find favorable interactions for binding and then catalyzing a chemical reaction [10,11]. The standard analogy for this induced fit model is that of a ''lock-and-key'' or, more appropriately, a ''hand-in-glove.'' Interestingly, the conformational flexibility that is crucial to enzymatic action can sometimes cause enzymes to be structurally or functionally unstable over the long-term. While immobilizing enzymes on surfaces can improve their long-term stability by constraining their movement, it almost invariably results in a concomitant reduction in activity [12,13]. The slower catalytic rates and efficiencies in the immobilized state are generally ascribed to steric hindrance, changes in tertiary structure, and slower diffusion. As we will describe with examples from the literature, the immobilization of enzymes on NPs can break this connection, often providing improved stability *and* enhanced reaction rates—a clear departure from the typical behavior of enzymes at bulk interfaces. If NP–enzyme and NP–substrate conjugates are to realize their full potential, it is crucial to have a complete understanding of their physicochemical properties and their catalytic activity. To date, this important area of research has received only very limited attention, especially when compared with the intense efforts devoted to the synthesis and application of composite bionanomaterials.

As individual entities, the properties of biomolecules and NPs are generally well understood. For example, the structure and function of proteins, peptides, and nucleic acids have been under intensive study for more than a century. As a result, biophysical models have been developed that provide quantitative understanding of their activities. Particularly prominent examples include bimolecular first-order interactions to describe antibody-antigen binding [14] and the Michaelis–Menten (MM) model for enzyme kinetics [15–17]. These models have been extended to describe antibody interactions on planar surfaces [18] and enzymatic catalysis in sample- and diffusion-limited environments [19]. Such extensions have empowered systematic improvements through identification of the important physical variables. Similarly, extensive research over the last two decades has resulted in a growing understanding of the physicochemical characteristics of most NP materials [20–24], and chemical and physical models are becoming available for designing and tuning their structure and function. NP-bioconjugates, on the other hand, are essentially novel composite materials that have the properties of both a NP and a bioreagent, but which may not behave as a simple sum of their parts. The standard models that are applied to biological reagents in bulk solution are often not directly adaptable to new NP systems because the constraints and assumptions used to derive those models are not necessarily consistent with the physicochemical characteristics of the NP system. For example, the assumptions of the MM model for enzyme kinetics are not easily satisfied by NP systems, even though the MM model has still been applied to such systems.

This review seeks to provide an overview of studies that have reported enhancements of enzymatic activity, stability, or other functional properties for enzyme–NP and substrate–NP composite materials relative to their bulk analogs. The advantages and drawbacks of applying traditional models of enzyme activity to the analysis of NP-bioconjugate systems are discussed both generally and in the context of specific examples. One particularly important issue is the mismatch between NP systems, which are frequently heterogeneous, and traditional models of enzyme activity, which are designed for homogeneous enzyme–substrate systems engaged in what, to a first approximation, would be ''steady-state'' conditions. With this in mind, the preparation and heterogeneous properties of such NP-bioconjugates are first described. Empirical examples of enhanced enzymatic activity with NP-bioconjugates are then presented, along with examples where enhancement is attributed to conformational changes or confinement of the enzyme, colocalization, or the special properties of NPs. Challenges in identifying the root causes of enhancement and its precise magnitude are also addressed. Finally, a NP-specific approach for developing enzyme kinetic models is outlined within a generalized MM framework. The intention is to convince readers of the need to think deeply about analyzing and interpreting the enzymatic activities associated with enzyme–NP or substrate–NP conjugates, and to provide a framework for reformulating models of enzymatic activity within these NP systems as this field slowly develops.

Nanoparticle bioconjugates

The integration of inorganic NPs with biomolecules has been motivated by the idea of bringing the unique, intrinsic properties of NPs into biological environments and experiments. The large surface-to-volume ratios offered by NPs are perhaps their most significant physical property. The volume of a NP is sufficiently small for dispersal in solution as a colloid, yet its surface area is sufficiently large to provide an interface for functionalization with biomolecules (*i.e.*, as a nanoscaffold or nanoplatform). NPs also offer a host