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Increasing the activity of immobilized enzymes with nanoparticle conjugation

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The efficiency and selectivity of enzymatic catalysis is useful to a plethora of industrial and manufacturing processes. Many of these processes require the immobilization of enzymes onto surfaces, which has traditionally reduced enzyme activity. However, recent research has shown that the integration of nanoparticles into enzyme carrier schemes has maintained or even enhanced immobilized enzyme performance. The nanoparticle size and surface chemistry as well as the orientation and density of immobilized enzymes all contribute to the enhanced performance of enzyme–nanoparticle conjugates. These improvements are noted in specific nanoparticles including those comprising carbon (e.g., graphene and carbon nanotubes), metal/metal oxides and polymeric nanomaterials, as well as semiconductor nanocrystals or quantum dots.

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Introduction

Enzymes are biomacromolecular proteins that accelerate biochemical reactions with high efficiency and precise specificity in nearly all biological processes [1]. Accordingly, enzymes have been incorporated into a wide variety of fields and industries including those associated with pharmaceutical and biofuel production, environmental monitoring, and disease diagnostics [2,3]. Researchers have improved the activity and stability of enzymes for such applications through various protein engineering techniques [4]. However, these techniques often include error-prone polymerase chain reaction (epPCR) and *in vitro* recombination, which are time-consuming, expensive, and tedious [5]. Furthermore, not all enzymes lend themselves to recombinant improvement due to the need to maintain the inherent structure that is associated with enzyme–substrate binding and catalysis [6,7]. Enzyme immobilization onto macro/micro surfaces is also typically required in order to enable their use in non-native applications such as in biosensors, environmental remediation materials, bioreactors, and other applied biotechnology fields [8,9]. Immobilizing enzymes onto planar surfaces can limit their performance due to multiple factors including the distortion of native protein configuration [10,11], steric hindrance, and slower diffusion rates of incident substrate toward the bulk surface [12,13]. To circumvent both the need to engineer enzymes and to eliminate the negative effects of enzyme immobilization on micro/macro surfaces, researchers have begun to utilize nanoparticles (NPs) as enzyme carriers.

NPs (i.e., flakes, tubes, wires, and spheres with length scales <100 nm) offer many unique and advantageous physicochemical capabilities, due in part to high surface area to volume ratios that enhance catalysis; surface chemistry well-suited for bioconjugation/biofunctionalization; and length scales that integrate well with and accordingly influence biological processes such as cell uptake/metabolism and gene expression [14]. These inherent properties make NPs advantageous for a wide variety of biologically-geared applications including biological/chemical sensing [15,16], clean energy generation [17], biodiesel production [18], drug delivery [19], and disease diagnostics [20]. Perhaps one of the more promising aspects of NPs is their apparent ability to enhance, in some cases, the activity and performance of immobilized enzymes [21••]. This article considers the underlying mechanisms behind enhanced performance of enzyme–NP conjugates and highlights the state-of-the-art nanomaterials that are being incorporated into such systems.

Enzyme–nanoparticle physicochemical mechanisms

Recent research has begun to elucidate the underlying physicochemical mechanisms behind the performance of enzymes immobilized onto NP enzyme carriers [17]. Although the interactions and catalytic underpinnings behind the enzyme–NP system are complex, specific characteristics of enzyme–NP conjugates have been

associated with enhanced enzyme performance [21••,22]. These characteristics include enzyme density, mass transport, NP morphology, NP surface chemistry, and enzyme orientation (Figure 1).

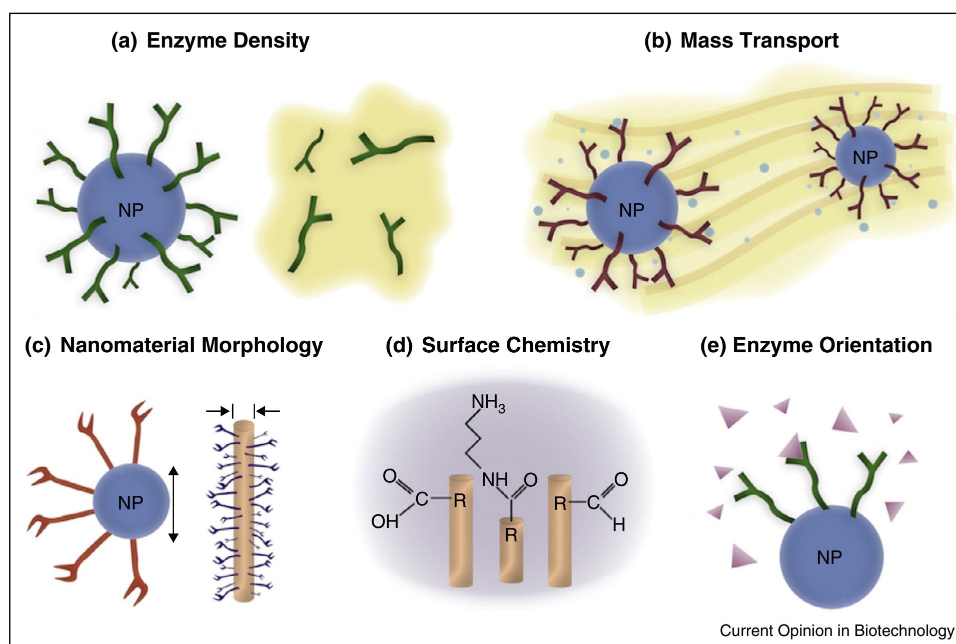
Immobilizing enzymes on NPs can increase their effectiveness for a variety of reasons. Enzyme configuration, orientation, and density can be controlled by changing the nanomaterial surface chemistry [22,23•,24,25•]. The ability to control both the enzyme configuration/density on the nanoparticle and the mobility of the enzyme–NP system has shown increased target-specific avidity [26]. The localized density of enzymes in a given volume can dramatically increase when multiple enzymes are attached to a single nanoparticle versus free enzyme [27•]. NP morphology can play a key role in impacting enzymatic enhancement as well. Since NPs/nanotubes maintain higher radii of curvature due to their smaller diameters, these materials allow for increased center-to-center distances between adjacent immobilized enzymes while limiting unfavorable protein-to-protein interactions [28]. Furthermore, enzyme orientation can be controlled by careful manipulation of the enzyme attachment chemistry, allowing for strategic orientation of the substrate binding pocket of the immobilized enzyme away from the NP surface and toward incident substrate [29•].

Additionally, the mobility of the NPs themselves enhances substrate-to-enzyme interactions via Brownian motion [30] while secondary interactions at the NP–enzyme interface, due in part to substrate–NP attraction through forces such as electrostatic attraction, can also increase the activity of NP immobilized enzymes [31]. In other words, enhanced activity can be attributed to the fact that with each collision between NP-immobilized enzymes and free-floating substrate, the weak association between the substrate and the NP interface results in multiple binding occurrences on one NP before the substrate moves elsewhere [31]. This proposed substrate–NP association or attraction would subsequently lead to a higher concentration of substrate near the periphery of the NP as opposed to the bulk environment which would further enhance the activity of enzymes immobilized on NPs than floating free in solution [32]. This motion is described in the literature as a process in which first reversible adsorption of the enzyme onto the NP surface takes place, followed by complete digestion of the substrate onto the NP, and finally desorption of the substrate for similar interactions with other NPs [21••]. These five enzyme–NP physicochemical mechanisms are succinctly outlined in Figure 1, illustrated in the representative enzyme–nanomaterial conjugates presented in the following sections, and summarized in Table 1.

Carbon nanomaterials

Graphene oxide (GO), the water-soluble derivative of graphene, offers a unique substrate for the functionalization and loading of molecules due to its double-sided geometry [33]. Functionalized GO has many potential applications in the biomedical field that include gene and

Figure 1



Five mechanisms that are critical to enhanced enzymatic activity with enzymes immobilized onto NPs: **(a)** higher enzyme density and higher localized avidity, **(b)** enhanced mass transport, **(c)** increased surface curvature, **(d)** favorable surface functional group interactions and **(e)** favorable enzyme orientation for enzyme–substrate interactions, *i.e.* optimized enzyme–substrate trajectory.

Table 1

Representative examples of enzyme enhancement when attached to select nanomaterials.

Nanoparticles/nanomaterials	Functionalization	Enzyme	Enhancement (compared to the free enzyme)	Ref.
GO	Amine group	*Candida rugosa*	55% increase in relative hydrolytic activity	[23•]
CNTs	Amine group	*Candida rugosa*	10% increase in relative hydrolytic activity	[23•]
GO nanosheets	Amine-terminated polyethylene glycol	Serine proteases	Selectively enhance trypsin activity and improve the thermostability of trypsin	[33]
MWCNTs	Co^{2+} terminated nitrilotriacetate group	His-tagged NADH oxidase	166% increase in half-life, increase in thermal stability	[41]
CNTs	Co^{2+} terminated nitrilotriacetate group	His-tagged NADH oxidase	50% increase in half-life, increase in thermal stability	[41]
AuNPs	Multiple weak interactions with thiols, carboxylic acids and amines	Rhamnulose-1-phosphate aldolase	Four-fold enhancement of reaction rate	[22]
AgNPs	Polydopamine	Lipase	9% increase in biodiesel production yield	[48]
AuNPs	Adsorption	*Candida rugosa* lipase	The immobilization of AuNPs did not affect the value of K_{cat}, but the size of nanoparticles is inversely proportional to the catalytic efficiency of CRL	[50••]
PDA-coated Fe_3O_4 NPs	Catechol hydroxyl-groups present on the PDA layer	Trypsin	Increased binding capacity, enhanced mass transfer	[51]
ZnO nanocrystals (nanospheres, nanodisks, and nanomultipods)	The two aldehyde groups (–COH) of glutaraldehyde can bond separately to the amino groups of HRP and as-modified ZnO (crosslinking)	Horseradish peroxidase	Selection of a nanostructure morphology impacts enzyme performance	[27•]
$CaHPO_4$ nanocrystals (nanoflowers, nanoplates, and parallel hexahedrons)	Allosteric effect	α-Amylase	Reaction rate changes with NP shape; nanoflowers showed the highest catalytic rate (16.5×10^{-3} s^{-1}), then nanoplates (8.0×10^{-3} s^{-1}), and finally parallel hexahedrons (1.2×10^{-3} s^{-1}); all are improvements over free enzyme (4.4×10^{-4} s^{-1})	[52]
Polystyrene NPs	Carboxyl-functionalized groups	Zymogen Factor XII (FXII)	Increased functionality	[45]
PS-PNiPA microgel	Adsorption	β-D-Glucosidase	66% increase in K_M; three-fold increase in hydrolytic activity	[46]
CdSe–ZnS QDs	Polyhistidine group (His-6)	Endoglucanase	5 nm and 10 nm NPs had 4.9 and 5.6-fold increases in hydrolysis rate	[57]
CdSe–ZnS QDs emitting at 525 and 625 nm	Polyhistidine group (His-6)	Chimeric collagen-PTE trimer (PTE_3)	30–40% increase in enzymatic efficiency	[58]
CdSe–ZnS QDs	Single tertiary amine which terminates in two carboxyl groups	Alkaline phosphatase	V_{max} and K_{cat} increased 14–23% on the 525 nm QDs and slightly less than 10% on the 625 nm QDs	[59••]

drug delivery, cancer therapeutics, and biosensing [34,35]. Carbon nanotubes, rolled-up layers of graphene, exhibit similar properties to graphene but in a one-dimensional, tubular geometry rather than a two-dimensional, planar geometry [36]. Both of these forms of carbon nanomaterials have played a role in improving performance of immobilized enzymes.

The surface functionalization of carbon nanomaterials with distinct chemical functional groups can significantly affect enzyme performance [23•]. For example, the relative hydrolytic activity of *candida rugosa* lipase improved by 55% versus free enzyme (without NP immobilization) when immobilized on graphene oxide NPs that were functionalized with amine groups. Likewise, a 10% increase in hydrolytic activity was reported for the same enzyme on amine-functionalized carbon nanotubes versus free enzyme. In general, the catalytic efficiency, defined for these purposes as (V_{max}/K_M), increased up to 60% for all tested lipase enzymes (*candida rugosa* lipase,

pseudozyma (candida) antarctica lipase A, and *pseudozyma (candida) antarctica* lipase B) versus their free counterparts. However, the degree to which this improvement was realized depended upon the chemical moieties present on the surface of the nanomaterials and not all enzymes produced the same results; esterase enzymes immobilized on carbon nanomaterials yielded a 30% decrease in activity versus corresponding free enzyme. Furthermore, and of an interesting note, the thermal stability of the lipase enzymes seems to *decrease* when immobilized on carbon nanomaterials [23•]. This phenomenon correlates with some previous reports of enzyme–carbon nanomaterials conjugates leaving immobilized enzyme molecules in a more active but less stable form [37]. However, in our examination we found more examples of increased stability of enzymes immobilized on carbon nanomaterials than their free counterparts as demonstrated in the following examples.

Enzyme immobilization on amine-terminated polyethylene glycol (PEG) graphene oxide (GO) has further buttressed the correlation between graphene NP surface chemistry and immobilized enzyme performance. In experiments involving GO interacting with serine proteases, it was shown that PEGylated GO nanosheets affect the activity of trypsin in a substrate-dependent manner [33]. Casein was shown to digest much faster in the presence of trypsin functionalized GO nanosheets, whereas dephosphorylated whole casein (decasein) was digested at a fairly constant rate regardless of the presence of the nanosheets [33]. These results demonstrate that PEGylated GO nanosheets can enhance trypsin activity for casein digestion in certain scenarios. It was also noted that PEGylated GO *improved* the thermostability of trypsin by showing 60–70% retained activity at a temperature of 80 °C [33].

Researchers are also investigating the ability of chemically reduced graphene oxide (CRGO) to serve as a platform for enzyme immobilization, as it lacks the surface functional groups that, as previously discussed, can alter immobilized enzyme performance [38]. When GO is directly loaded with horseradish peroxidase (HRP) it has a maximum loading of 0.1 mg mg^{-1} [39], but loadings increase to 0.2, 0.7 and 1.3 mg mg^{-1} when applied to separate CRGO samples which were chemically reduced with L-ascorbic acid (L-AA) for 2, 4 and 12 h (CRGO-2, CRGO-4, and CRGO-12) [40]. Higher enzyme loading could be sustained on GO samples that were further reduced and it was also noted that the resultant hydrophobicity of the CRGO surface was proportional to the extent of its chemical reduction — a process which subsequently functionalized the GO surface with amine groups.

Carbon nanotubes (CNTs) have also shown increased stability of conjugated enzymes as opposed to free enzymes. CNTs conjugated with NADH oxidase (NOX) from *Bacillus cereus* achieved estimated half-lives of 800 hours, almost triple that of free NOX [41]. Furthermore, Figure 2 demonstrates how NOX immobilization on CNTs greatly increases enzyme stability at 50 °C and especially at 90 °C. Similarly, enzyme immobilization on multi-walled CNTs (MWCNTs) has also increased enzyme thermal stability [41]. Thus enzymes immobilized on carbon nanomaterials have shown significant improvement in both activity and stability.

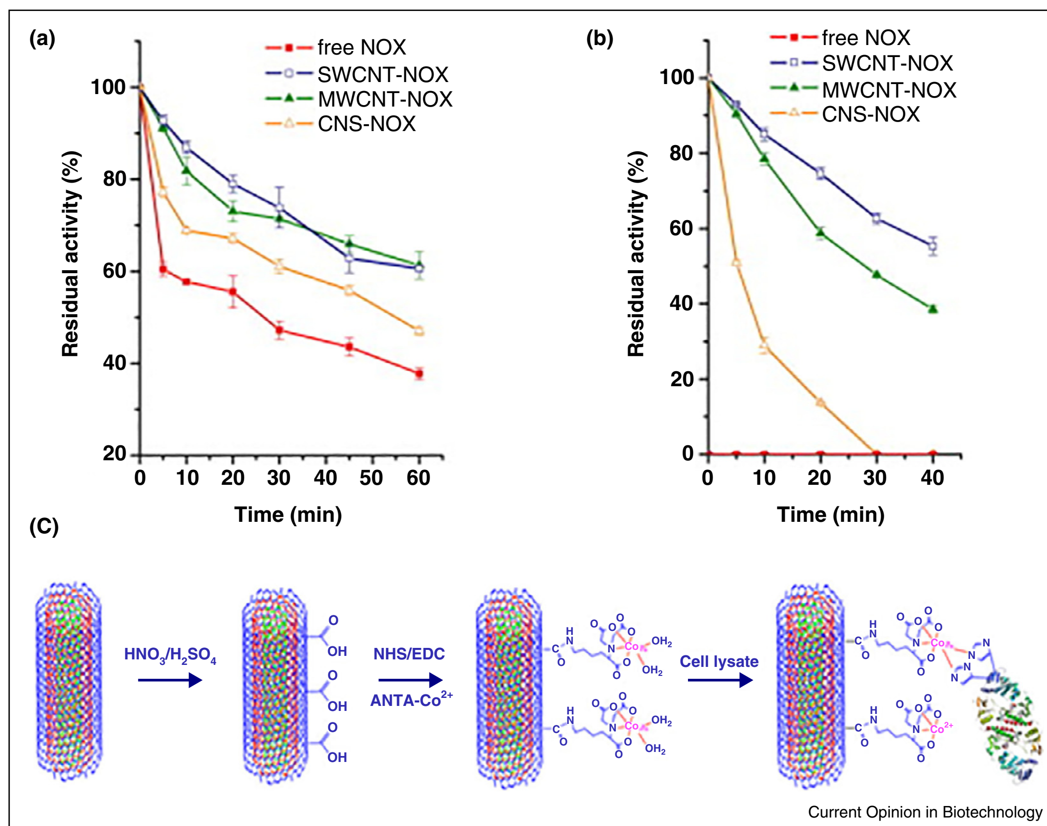
Metals/metal oxide and polymeric nanomaterials

Inorganic materials also offer many unique properties that make them excellent candidates for enzyme immobilization platforms. Applications of metal NPs as enzyme carriers are quite diverse, and include urea sensing [42], glucose sensing [43] and biodiesel production [44], and polymeric NPs have applications including controlling blood coagulation [45] and drug delivery [46]. Here we review enzyme–nanoparticle conjugates with either metal/metal oxide NPs or polymeric NPs as the enzyme carrier.

The enzyme rhamnulose-1-phosphate aldolase from *Escherichia coli* (RhuA) was immobilized onto gold nanoparticles (AuNPs) through multiple weak interactions with thiols, carboxylic acids, and amines [22,47]. The initial reaction rate between dihydroxyacetone phosphate (DHAP) and (S)-Cbz-alaninal reached 4.5 mM h^{-1}, which amounts to over a four-fold enhancement of reaction rate as compared to free floating enzymes that experienced a reaction rate of 1.1 mM h^{-1} in the same conditions with the same enzyme concentration. Silver NPs have also acted as enzyme carriers for the enzyme lipase which was immobilized via a self-polymerized polydopamine called polydopamine. This complex was reported to produce biodiesel at a production yield of up to 95%, while free lipase displayed a yield of only 86% [48]. This enhancement was reported to be triggered by increased enzyme density and increased exposure of immobilized lipase to substrates due to enzyme immobilization on NPs.

Immobilization of lipase on metal NPs can also lead to enhanced mass transport of incident substrate. Without surface modification, the formation of linker-free lipase-AuNPs complex had a smaller Michaelis constant (K_M) and the same maximum velocity (V_{max}) compared to that of the free enzyme, and the K_M values were 9.10 μM and 23.91 μM, respectively. This proves that lipase had a higher affinity or selectivity, represented by a smaller K_M value, toward the substrate in the enzyme–AuNP complex, as the addition of NPs is an efficacious means of tuning the lipase-substrate association [49]. Furthermore, based on a diffusion-collision theory and Stokes-Einstein equation, the enzymatic activity of lipase-AuNPs conjugates is size dependent: smaller particle size resulted in a

Figure 2



Stability of free and immobilized NADH oxidase (NOX) 50 mM, pH 7.0 at **(a)** 50 °C and **(b)** 90 °C [41]. SWCNT stands for single-walled carbon nanotubes, MWCNT stands for multi-walled carbon nanotubes and CNS stands for carbon nanotubes. The immobilization of NADH oxidase onto a functionalized CNT is illustrated in **(c)**.
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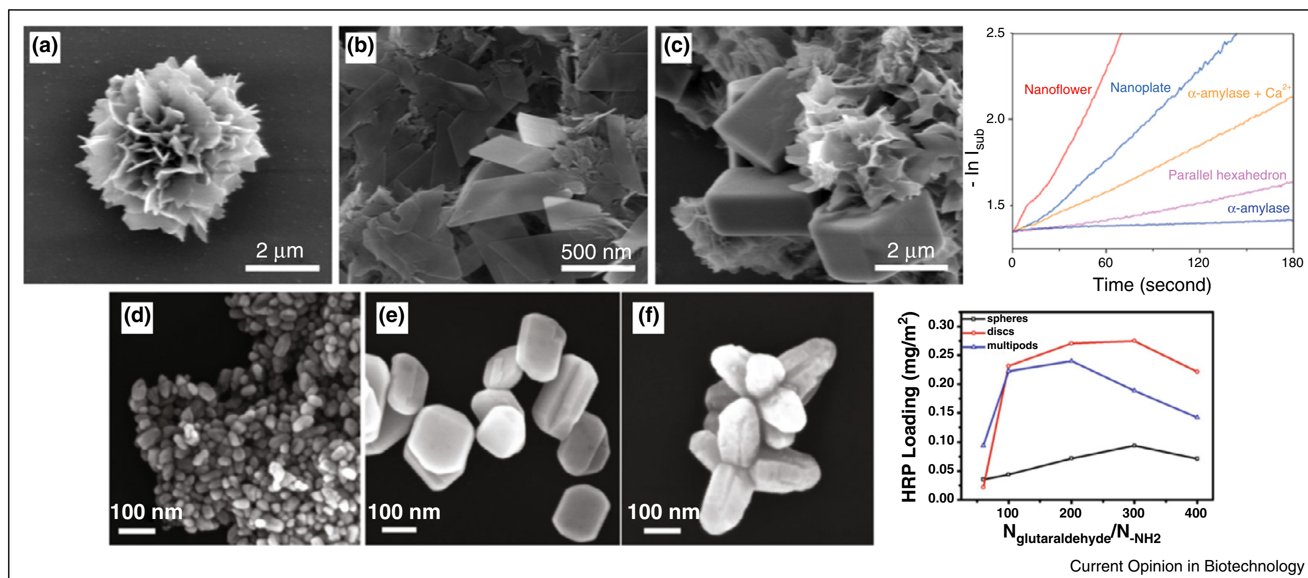
higher catalytic efficiency of lipase by increasing its kinetic affinity toward the substrate [30,50••].

Similarly, another research group showed that when immobilized onto polydopamine coated magnetic microspheres (Fe_3O_4@PDA) with diameter of approximately 220 nm, trypsin experiences a larger binding capacity and enhanced mass transfer [51]. When Fe_3O_4@PDA was mixed with a protein solution for 30 min at 37 °C, all the trypsin molecules were present on the surface of the magnetic Fe_3O_4@PDA, where they were easily accessible to the target proteins, increasing binding capacity. Interestingly, when the protein concentration was lowered to 5 ng μg^{-1}, detection was still successful, and is attributed to high concentrations of substrate near the immobilized trypsin on the Fe_3O_4@PDA surface [51].

The degree of immobilized enzyme activity enhancement can also depend upon the nanomaterial's specific morphology. For example, ZnO nanocrystals in the form of nanospheres, nanodisks, and nanomultipods (Figure 3, bottom row) were immobilized with horseradish peroxidase (HRP) via cross-linking with glutaraldehyde, as two aldehyde groups can form bonds with the amino groups of HRP [27•]. Results showed that nanodisks sustained the highest enzyme loading at 0.275 mg m^{-2}, followed by nanomultipods at 0.240 mg m^{-2}, and finally nanospheres at 0.094 mg m^{-2}. However corresponding catalytic efficiencies (K_{cat}/K_M) of 1.09 mM s^{-1}, 1, 1.28 mM s^{-1}, 0.78 mM s^{-1} for HRP immobilized on nanodisks, nanomultipods, and nanospheres respectively, demonstrate that NP morphology can play a more important role on immobilized enzyme activity than enzyme loading concentration. This research study concluded that nanomultipods had the highest catalytic efficiency because they directly improve enzyme loading and catalytic efficiency due to the increased space between pods for the glutaraldehyde chemistry-driven self-polymerization to occur. On the contrary, the space among nanopods is limited, consequently restricting glutaraldehyde from self-polymerization and decreasing the quantity of immobilized enzymes. Additionally, multi-pods can anchor amino groups, which subsequently results in higher efficiency of HRP immobilization [27•]. These results

Figure 3



SEM images of **(a)** nanoflowers, **(b)** nanoplates, and **(c)** parallel hexahedrons. The corresponding plot shows $-\ln I_{sub}$ versus time for five catalytic systems. The reaction rate constants (k) determined from this plot are $16.5 \times 10^{-3}\ s^{-1}$ for nanoflowers, $8.0 \times 10^{-3}\ s^{-1}$ for nanoplates, $4.5 \times 10^{-3}\ s^{-1}$ for free α-amylase with Ca^{2+}, $1.2 \times 10^{-3}\ s^{-1}$ for parallel hexahedrons, and $1.2 \times 10^{-3}\ s^{-1}$ for free α-amylase [52]. The figure also shows SEM images of **(d)** nanospheres, **(e)** nanodisks, and (f) nanomultipods of ZnO nanocrystals [27•]. The corresponding plot shows the possible enzyme loadings on different morphologies of ZnO nanocrystals.
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reinforce the idea that selection of nanostructure morphology does impact enzyme density/activity and hence increases avidity between enzymes and substrates.

Another study on NP morphology corroborates the aforementioned results. When α-amylase was immobilized onto $CaHPO_4$ nanocrystals, the catalytic reaction rate of nanoflowers and nanoplates displayed stronger activity than free α-amylase (Figure 3, top row). Nanoflowers and nanoplates have a higher surface area-to-volume ratio than parallel hexahedrons, another structure analyzed in the research, and as a result of less significant mass transfer limitations, a larger portion of the immobilized α-amylase rests on the surface and has a higher likelihood of interacting with the substrate. This study also showed that reaction rate (k) changes with shape; nanoflowers yielded the highest catalytic rate ($16.5 \times 10^{-3}\ s^{-1}$), then nanoplates ($8.0 \times 10^{-3}\ s^{-1}$), and finally parallel hexahedrons ($1.2 \times 10^{-3}\ s^{-1}$) [52].

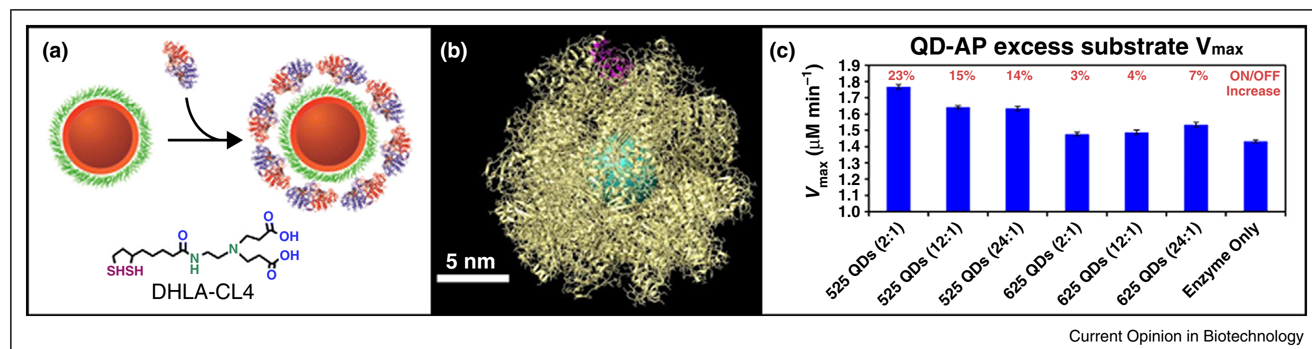
Nanomaterial morphology, specifically NP curvature and surface area, have been shown to directly affect enzymatic enhancement. Four different diameters — thus four different surface areas and curvatures — of carboxyl-modified polystyrene NPs (COOH-PS NPs) were loaded with an 80 kDa glycoprotein, zymogen Factor XII (FXII), which is activated by kallikrein (KaI). The activity of the absorbed proteins was studied, and results show that the larger NPs (220 nm) had a strong positive effect on the activity of the enzyme–NP complex [45]. The study also identified an inverse correlation between NP surface area and protein activity. A kinetics analysis on FXIIa using the Michaelis-Menten model concluded that proteins immobilized on NP surfaces are more functional (active) than free proteins in solution [45].

In another application involving polymeric NPs, β-D-glucosidase from almonds was immobilized via adsorption onto NPs comprising a polystyrene (PS) core onto which a cross-linked poly(N-isopropylacrylamide) (PNiPA) network was attached [46]. Interestingly, the NP structure, referred to as a PS-PNiPA microgel, is weakly negatively charged, resulting in repulsion between the NP and enzyme and requiring the formation of hydrogen bonds to link the enzyme to the NP. Calculations of kinetic parameters show that at 40 °C, K_M increased by 66% when immobilized onto PS-PNiPA as compared to β-D-glucosidase in free solution. In addition, hydrolytic activity increased by a factor of 3.2–3.5 on the PS-PNiPA microgel [46]. Though polymeric and metallic NPs may have fundamentally different material properties, they have both been successfully used as immobilization platforms to enhance enzymatic activity.

Luminescent semiconductor nanocrystals

Luminescent semiconductor nanocrystals, or quantum dots (QDs), have been utilized in fundamental research

Figure 4



(a) Schematic showing alkaline phosphatase (AP) binding to a 625 nm quantum dot (QD) and the dihydrolipoic acid-based compact ligand (DHLA-CL4) that is used to functionalize the QD surface. **(b)** Rendering of a 525 nm QD with AP attached. The enzymatic binding pocket is shown in purple to highlight outward-facing placement. **(c)** A comparison of the rate of enzyme production (V_{max}) with excess substrate for varying AP-QD ratios and QD sizes of 525 nm, 625 nm, and the enzyme in free solution. Ratios indicate the number of AP per QD.
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and technical applications due in part to their unique size-dependent physical and electronic properties, including resistance to photobleaching, size tunable photoluminescence, and large effective Stokes shifts [53–55]. Common applications involving QDs include cellular imaging, theranostics, *in vivo* biosensing, and smart molecular sensing probes [56].

Research groups have analyzed the effects of NP morphology on enzyme activity by immobilizing the enzyme endoglucanase onto CdSe–ZnS core–shell QDs with diameters of 5 and 10 nm, respectively, through metal-affinity coordination and an engineered C-terminus histidine tag [29•,57]. The histidine tag bioconjugation scheme permits moderate control over *both* the number of biomolecules per QD and their subsequent orientation on the QD surface. The hydrolysis of phosphoric-acid-swollen cellulose (PASC) was examined to analyze the catalytic activity of QD–enzyme conjugates [57]. Compared to the free enzyme and regardless of particle size, the hydrolysis rate was elevated two-fold when PASC was attached to the QDs via coordination with a flanking polyhistidine tag on the cellulose. When the particle size was taken into consideration and different sizes of QDs were studied separately, it was found that the initial hydrolysis rate by QD–enzyme conjugates was proportional to the nanometer dimension: 5 nm QD–enzymes had an initial hydrolysis rate of 461.1 um h^{-1}, while that of 10 nm QD–enzyme was higher (533.3 um h^{-1}). Corresponding to this explanation, 5 nm and 10 nm diameter QDs had 4.9 and 5.6-fold respective increases in hydrolysis rate compared to that of the free enzyme (94.4 um h^{-1}) [57]. This report supports the idea that nanomaterial morphology, size, and orientation can significantly improve enzymatic activity [56].

Another study related to QDs assembled a *de novo* chimeric collagen-PTE trimer (PTE_3) in controlled ratios to QDs. PTE_3 was assembled to CdSe–ZnS core–shell QDs, some of which emitted at 525 nm and some at 625 nm. When compared to enzyme in free solution, both QDs showed significantly enhanced enzymatic activity rates; enzymatic efficiency improved 30–40% overall [58]. Although the focus here has specifically been on enzyme activity when immobilized to NPs, it is also important to note that when substrate is attached to NPs such as QDs, marked improvements in enzymatic performance have also been reported [31].

One detailed study on enhanced performance of alkaline phosphatase (AP) immobilized on CdSe–ZnS core–shell QDs showed enhancement in both V_{max} and K_{cat} with successful orientational control of AP placement as compared to AP in free solution (Figure 4) [59••]. Though the increase in these performance metrics varied in magnitude from 3% to 23%, both 525 nm and 625 nm nanoparticles showed improvements at all enzyme-to-QD ratios tested. Interestingly, the enhancement on 525 nm QDs ranged from 14% to 23%, while the 625 nm QDs only saw improvements measuring slightly less than 10%, as can be seen in Figure 4. Researchers conducting this study postulate that the higher surface curvature of the smaller QDs better promotes native enzyme configuration and lower enzyme-to-enzyme neighbor interactions than the larger QDs. The greatest enhancement in enzyme performance was noted when fewer enzymes were immobilized on the QDs and therefore further corroborates the mentioned postulate [59••].

Conclusion

Enzymes are active accelerators of many biochemical processes and are widely used to catalyze reactions in

numerous applications. Nanoparticles have been shown to enhance the effectiveness of immobilized enzymes [9], which has fueled research interest in enzyme–NP systems (see Table 1). Although not all enzyme–NP conjugates show increased enzymatic activity, many of those that do also appear to be dependent on having discrete, controlled bioconjugation of the enzymes to NPs [60,61]. Furthermore, five major physicochemical mechanisms contribute to enhanced enzymatic activity on NP–enzyme conjugates: higher enzyme density; enhanced mass transport of incident substrate due to both the attraction of substrate to the NP surface and to the movement of the NP–enzyme bioconjugate; NP curvature/morphology; NP surface chemistry leading to more active enzymes; and favorable enzyme orientation for increased enzyme–substrate interactions. These mechanisms serve to provide a better understanding of the enhancement of activity of enzymes immobilized on NPs and can be applied to optimize the design of new NP–enzyme conjugate systems. As shown with the various examples presented here, enhancement is still not fully predictable, and additional research must be done to provide a framework to understand exactly how enzymatic activity is enhanced in NP systems.

Acknowledgements

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- • of special interest
- •• of outstanding interest

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