LETTER

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LETTER

Assembling high activity phosphotriesterase composites using hybrid nanoparticle peptide-DNA scaffolded architectures

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Abstract

Nanoparticle (NP) display potentially offers a new way to both stabilize and, in many cases, enhance enzyme activity over that seen for native protein in solution. However, the large, globular and sometimes multimeric nature of many enzymes limits their ability to attach directly to the surface of NPs, especially when the latter are colloidally stabilized with bulky PEGylated ligands. Engineering extended protein linkers into the enzymes to achieve direct attachment through the PEG surface often detrimentally alters the enzymes catalytic ability. Here, we demonstrate an alternate, hybrid biomaterials-based approach to achieving directed enzyme assembly on PEGylated NPs. We self-assemble a unique architecture consisting of a central semiconductor quantum dot (QD) scaffold displaying controlled ratios of extended peptide-DNA linkers which penetrate through the PEG surface to directly couple enzymes to the QD surface. As a test case, we utilize phosphotriesterase (PTE), an enzyme of bio-defense interest due to its ability to hydrolyze organophosphate nerve agents. Moreover, this unique approach still allows PTE to maintain enhanced activity while also suggesting the ability of DNA to enhance enzyme activity in and of itself.

Introduction

Beyond their inherent catalytic roles in biochemistry, enzymes are now indispensable for many biomedical as well as industrial activities including production of therapeutics, specialty chemicals, and as bioremediation agents. Critical to all of these applications is the ability to deploy enzymes effectively so as to provide high throughput [1]. One widely pursued approach is that of synthetic biology and a key advantage is that the enzymes operate in their confined native cellular environment where they can function stably and with extraordinary efficiency [2]. Offsetting this benefit are the implications of working with living cells, the need to keep them alive, and the difficulties of continuously supplying reactants and purifying product. The alternative of employing natural enzymes in vitro, whether in solution or attached to surfaces, has a complementary set of tradeoffs with important acellular virtues but with decreases in stability and function typically seen, especially in industrial applications [3]. To improve on this situation, there is currently much interest in new nanoscale scaffolds for controlled enzyme display that also have the potential for better maintaining, or perhaps even enhancing, stability and function [4].

Recent research has shown that displaying enzymes on nanoparticles (NPs) can significantly increase their kinetic activity, although the underlying mechanism(s) are still not fully clarified [3–6]. Seeking insight into this question, we have performed extensive studies with assorted enzyme-NP systems under a variety of experimental conditions [7–12]. These efforts have provided some preliminary understanding about NP-
enhanced enzymatic activity, including showing that there is not one single mechanism that is responsible. For example, work with alkaline phosphatase on semiconductor quantum dots (QDs) found that its ‘random’ or heterogeneous attachment led to a decrease in activity whereas site-specific, oriented attachment enhanced the turnover [7]. Analogous research on phosphotriesterase (PTE) conjugated to QDs showed that the enhanced PTE activity resulted from the NP’s interfacial environment promoting product (nitrophenol) release, which was the rate-limiting step [8].

Here, we continue work with PTE (EC 3.1.8.1), which is an obligate dimer that catalyzes hydrolysis of organophosphate ester compounds having a phosphate center surrounded by three O-linked groups such as the nerve agents sarin and tabun along with the insecticide paraoxon [13]. Because of the practical importance of this chemistry, PTE is undergoing concerted development for a variety of industrial, agricultural, bioremediation, and biodefense applications [14]. In our earlier efforts with PTE on QDs, we used CdSe/ZnS core/shell QDs that were surface-functionalized with small, zwitterionic DHLA-CL4 ligands [8, 15, 16]. The PTE proteins were then directly coordinated to the QD surface by a metal-affinity interaction between a terminal hexahistidine or (His)_6 motif on the PTE and the QD’s ZnS surface [8, 15]. This coordination has high affinity (equilibrium binding constant K_eq ~1 × 10^9 M^-1), occurs nearly spontaneously when the two species are mixed together, and provides control over both the display ratio and the relative protein orientation [15]. Although DHLA-CL4 ligands are effective for many bioassays and other cellular utility [8, 11, 16–19], it is still highly desirable to functionalize the NPs with poly(ethylene glycol) or PEG-based ligands in order to enhance biocompatibility and fouling resistance for a variety of other applications [20–24]. Unfortunately, the
extended length and bulk of these ligands prevents most proteins including PTE from directly penetrating through and attaching to the QD surface [16, 22, 25]. One approach to addressing this is to couple an enzyme of interest directly to the PEG termini surrounding a NP. However, the inherent complexity of PEG chemistry and paucity of available reactive groups or chemical handles to functionalize them typically does not allow for site-specific or controlled display of the enzyme on the NP [22–25]. As an alternate way to address this, we engineered monomers of PTE coupled to a collagen-like domain that self-assembled into an extended-linear triple helix structure to allow QDs to display PTE even when functionalized with PEG [26]. Also attractive was the fibril-like nature of this trimeric construct that could potentially allow it to be spun into fibers for weaving into protective fabrics and cover materials. While the reengineering of the PTE did succeed in attaching it directly to the QD surface through the PEG layer, it also resulted in a significant and deleterious loss in catalytic activity (10× decrease in \(k_{\text{cat}}\)) [26]. The main goal of the present report is to address this deficiency by investigating the use of DNA as an extended linker that replaces the collagen-fibril approach and allows native PTE to be displayed around PEGylated QDs while still giving rise to the desired high-activity QD-PTE composite, see schematic in figure 1.

### Materials and methods

#### QDs and PTE

CdSe/ZnS core–shell QDs with emission maxima centered at \(\sim 525\) and \(\sim 625\) nm were synthesized and solubilized with either dihydrolipoic acid based zwitterionic compact ligand 4 (DHLA-CL4) or poly(ethylene glycol)-, or PEG, - appended DHLA where the PEG (MW \(\sim 750, \sim 15\) ethylene oxide repeats) terminates in a methoxy group (DHLA-PEG\(_{750}\)-OMe) as previously described [7–12, 16, 22, 26]. (His)\(_5\)- appended PTE was prepared as described [8, 26]. All PTE utilized here is expressed with the terminal (His)\(_6\).

#### DNA hybridization

DNA sequences listed in table 1 were purchased from Integrated DNA Technologies, Inc. (Iowa, USA). The 18 bp sequence was labeled with N-(5-(3-maleimidopropylamido)-1-carboxy-pentyl)iminodiacetic acid to derive the 18 bp DNA-NTA. The 38 bp DNA-(His)\(_5\) was produced from a 38 bp DNA oligo synthesized with a terminal amine that was subsequently covalently linked to the thiol on a Cys(His)\(_3\) peptide using the heterobifunctional linker GMBS (N-(γ-maleimidobutryloyloxy)succinimide ester). Modified sequences were purified by ultra performance liquid chromatograph and the modifications confirmed by mass-spectral analysis [27].

Stock solutions of DNA were prepared at 0.5 \(\mu\)M in 1 \(\times\) PBS (phosphate buffered saline: 137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4). 200 pmol of 18 bp DNA-NTA was activated with 20 \(\times\) Ni\(^{2+}\) (4 nmol) overnight followed by a second overnight incubation in the presence of 200 pmol PTE. The stock solution containing 100 pmol each of 38 and 56 bp DNA were hybridized in a thermal cycler by heating to 95 °C, holding for 5 min, and then decreasing the temperature by 1 °C every minute until reaching the final temperature of 4 °C. To construct the QD-DNA bioconjugates, first QDs and 38 bp–56 bp DNA were incubated for 30 min in the presence of buffer at the desired molar ratio. This ensures the DNA attaches to the QD surface via metal affinity coordination between the (His)\(_5\) of the 38 bp DNA and the Zn\(^{2+}\) rich QD surface. This was followed by the addition of an equimolar amount of 18 bp DNA-PTE to complete the structure. The final volume for all samples was 120 \(\mu\)l. The assemblies were then placed in a thermal cycler followed by heating to 37 °C then decreasing the temperature by 1 °C every minute until reaching the final temperature of 4 °C. It is of note that the melting temperature (\(T_m\)) of the 18 bp oligo is higher than the annealing temperature (37 °C) that is used. Because this portion of the sequence is single-stranded (ss) and contains minimal secondary structure due to its unique sequence and its relatively short length, a full melting protocol is not necessary to hybridize these oligos since there is no energy barrier to overcome to reach the lowest free energy state of hybridization. The samples were removed from the thermal cycler and an additional 880 \(\mu\)l of buffer was added to each sample such that the

![Image](image-url)

### Table 1. DNA sequences.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence 5′–3′</th>
<th>(T_m) (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>38 bp DNA-(His)(_5)</td>
<td>ACT CCC TGT ATC CCG TGA CTT AAC TCG TGA ACT CGT GT— linker— Cys(His)(_5)-NH(_2)</td>
<td>74.8</td>
</tr>
<tr>
<td>18 bp DNA-NTA</td>
<td>NTA AAC TCG TGT AAC TAG CCG</td>
<td>59.7</td>
</tr>
<tr>
<td>Complementary 56 bp DNA</td>
<td>ACA CGA GTT CAC GAG TTA AGT CAC GGG ATA CAG GGA GTC GGC TAG TTA CAC GAG TT</td>
<td>78.1</td>
</tr>
</tbody>
</table>

Linker = GMBS (N-(γ-maleimidobutryloyloxy)succinimide ester); NTA = nitrilotriacetic acid. \(T_m\) = melting temperature.
QD concentration was 0.12 or 2 nM and the PTE concentration ranged from 0.12 to 8 nM. Free PTE and DNA-PTE at the same concentrations of QD-DNA-PTE also underwent the same hybridization protocol as controls, see figure 1.

Dynamic light scattering (DLS)
QDs and QD-DNA-PTE constructs were characterized by DLS analysis using a ZetaSizer NanoSeries equipped with a HeNe laser source ($\lambda = 633$ nm) (Malvern Instruments Ltd, Worcestershire, UK) and analyzed using Dispersion Technology Software (DTS, Malvern Instruments). Approximately 20–30 nM solutions of QDs were loaded into disposable cells and data were collected at 25 °C. All the samples were prepared in 0.2 × PBS buffer pH 7.4. For each sample, the autocorrelation function was the average of five runs of 10 s each and then repeated three to six times. CONTIN analysis was then used to determine number versus hydrodynamic size profiles for the dispersions studied using DTS as described elsewhere [21].

Kinetic assays
The assay was performed in a similar manner as described before [8, 9, 26]. To a 384 well plate, 25 $\mu$l of sample was added to each well followed by 25 $\mu$l of substrate. The absorbance was immediately measured at 405 nm using a Tecan Infinite M1000 dual monochromator multifunction plate reader and a kinetic program consisting of shaking the plate for 3 s before measuring the absorbance every 25 s. The absorbance values were converted to p-nitrophenol concentrations utilizing a calibration curve composed of successive dilutions of stock p-nitrophenol. The linear portions of the progress curves were used to calculate the initial rates for each substrate concentration. These were fitted to the Michaelis–Menten equation using Sigma Plot’s enzyme module.

Electrophoresis
To confirm hybridization of the 3 ssDNA fragments into a bifunctional 56 bp dsDNA spacer, a 3% (w/v) agarose gel supplemented with gel red stain was utilized. The gel was formed and run in 1 × TBE (89 mM Tris-borate and 2 mM EDTA, pH 8.3) buffer at 150 V. Each lane was loaded with 75 ng of DNA. After applying voltage for 60 min, the gel was imaged with a BioRad Molecular Imager ChemiDoc XRS+ system. When the 56 bp complementary sequence is hybridized with the 38 bp DNA-NTA a strong band appears around 50 bp. The band shifts with the addition of the 18 bp DNA-NTA, confirming assembly (figure 2). A 1% (w/v) agarose gel in 1 × TBE buffer was utilized to confirm assembly of DNA-PTE bioconjugates to 525 nm QDs surface functionalized with DHLA-CL4. Each lane contained 5 pmol of QDs. The QD-DNA-PTE were assembled at the indicated molar ratios as described above. The gel was run at ~90 V for 10 min and images were
Results and discussion

As a prototypical nanoparticulate material, QDs provide many useful properties when exploited as enzyme scaffolds. These include their small size with high surface-to-volume ratios which still allows the conjugate the ability to diffuse in solution as opposed to attachment on a solid surface [4, 28, 29]. Moreover, they have very low polydispersity making them quite uniform. Critically, they provide access to site-specific, homogeneous conjugation which allows for control over the number of attached molecules displayed on the QD along with their orientation [30].

Using DNA for the PTE attachment to QDs is also attractive for several reasons including its rigidity when in ds form and its ability to be modified site-specifically to display different chemical handles that facilitate both QD assembly and subsequent PTE attachment [34]. Intriguingly, Yan recently reported that DNA structures may themselves enhance the activity of an attached enzyme, though the mechanism (s) remains unknown [35].

Figure 3. Characterization of QD-DNA-PTE assembly. TEM images of (A) 525 nm and (B) 625 nm emitting CdSe/ZnS QDs with diameters of 4.2 ± 0.5 and 9.2 ± 0.8 nm, respectively. (C) Agarose gel imaged over time of 525 nm QD-CL4 assembled with increasing ratios of the DNA-PTE linker as compared to QD only controls. White arrows highlight the mobility shift between the QD control and the PTE-DNA assembled QDs. (D) DLS analysis showing the addition of each DNA fragment and PTE to the QD confirming successful assembly. 625 nm QDs functionalized with DHLA-PEG750-OMe were utilized as they are larger and thus easier to detect by DLS.

taken every five min with a BioRad Molecular Imager ChemiDoc XRS+ system to detect the QD photoluminescence with UV excitation.

Figure 3. Characterization of QD-DNA-PTE assembly. TEM images of (A) 525 nm and (B) 625 nm emitting CdSe/ZnS QDs with diameters of 4.2 ± 0.5 and 9.2 ± 0.8 nm, respectively. (C) Agarose gel imaged over time of 525 nm QD-CL4 assembled with increasing ratios of the DNA-PTE linker as compared to QD only controls. White arrows highlight the mobility shift between the QD and the PTE-DNA assembled QDs. (D) DLS analysis showing the addition of each DNA fragment and PTE to the QD confirming successful assembly. 625 nm QDs functionalized with DHLA-PEG750-OMe were utilized as they are larger and thus easier to detect by DLS.
(3-maleimidopropylamido)-1-carboxy-pentyl]iminodiacetic acid to display a NTA group. When these two oligos are hybridized to an unmodified 56 bp ssDNA complement, they form the full dsDNA linker whose extended length should be sufficient to cover the surface of the PEGylated QDs with the terminal (His)$_5$ coordinating to the QD surface and the NTA group available at the distal end of the structure for PTE to bind via metal affinity. Successful formation of the dsDNA hybrid linker itself was first confirmed with agarose gel electrophoresis as shown in figure 2. Furthermore, from prior work one would expect that this linker would coordinate with the QDs and maintain an extended length while still allowing some degree of movement [25, 36, 37], and this was indeed confirmed below.

Figures 3(A) and (B) show representative high-resolution transmission electron micrographs (TEMs) of the QDs utilized, while figure 3(C) shows agarose gel images of 525 DHLA-CL4 QDs assembled with increasing ratios of the PTE-loaded DNA linker. PEGylated QDs were not utilized for the gels as they have almost no charge and hence no mobility during electrophoretic separation. As seen here, a higher molecular weight, slower-mobility species emerges and grows in intensity as the DNA:QD ratio increases, while the intensity of the free QD band decreases. Additional confirmation of the final assembly comes from DLS analysis where the hydrodynamic diameter ($H_D$) of 625 nm emitting PEGylated QDs was found to increase from ~15.7 nm to 24.4 nm with addition of the 38 bp DNA-(His)$_5$ (see figure 3(D)) [21]. Hybridizing the 56 bp complement further increased the $H_D$ to 43 nm, but addition of the 18 bp NTA did not increase the size further, as expected since this would not extend beyond the 56 bp complement. Lastly, addition of PTE increased the conjugate $H_D$ to ~60 nm. We estimate that the DNA linker itself contributes about 14 nm, derived as (43 – 15.7 nm)/2, a value close to that estimated from just the predicted length of the dsDNA (18.5 nm) when accounting for some freedom of movement [30].

With confidence in our ability to display PTE on PEGylated QDs using extended DNA linkers in a controlled, ratiometric manner, we next investigated the PTE kinetic activity in this configuration. To assess the enzymatic activity of these conjugates, we compare their performance with other PTE configurations (see figure 1), namely: (i) PTE alone; (ii) PTE attached to a DNA linker but with no QD; and (iii) PTE displayed on CdSe/ZnS core/shell QDs functionalized with a DHLA-CL4 passivation ligand as in [8]. PTE activity is assayed using paraoxon (diethyl 4-nitrophenyl phosphate) as the substrate and monitoring formation of the p-nitrophenol product by its absorbance at 405 nm [8, 12, 26]. Initial rate data are shown in figure 4 and these data were collected under standard excess substrate conditions so that standard Briggs–Haldane assumptions are justified. The ratio of PTE:QD ratio was restricted to be either 2 or 4 because higher ratios lead to much faster turnover that rapidly consume the substrate and invalidates the steady-state approximation. At these low ratios, the sample assembly is heterogeneous following Poissonian statistics, and this needs to be taken into account in interpreting the data [15, 25]. The data were analyzed using the Michaelis–Menten model and the corresponding kinetic descriptors estimated, see table 2 [7–13, 26]. On this basis, several trends are readily apparent. Displaying PTE on the DNA alone enhances its catalytic rate ($k_{cat}$) by a factor of three over PTE by itself, while displaying it on the QD without the DNA linker and with the short DHLA-CL4 ligand enhances it by more than six-fold, with the largest increase occurring for the larger 625 QDs. The effectiveness of the DNA linker is demonstrated by the QD-DNA-PTE conjugate data that shows similarly enhanced activity despite the presence of the PEG ligands. Enzyme affinity as measured by $K_M$ decreases when PTE is attached to either QD, DNA, or the QD-DNA and this trend is also consistent with our previous reports [7–13, 26]. This, in turn, acts to reduce the corresponding enzyme efficiency ($k_{cat}/K_M$). Free PTE activity mirrors that previously reported [8]. More importantly, the specific activity (nmols of substrate consumed per min per mg PTE) of the QD-DNA-PTE constructs is almost 10-fold that of the free enzyme, figure 4(C). PTE activity in the presence of PEGylated QDs without DNA ligand, i.e. unassembled, was similar to that of PTE alone (data not shown).

Conclusions

Overall, we find increases in activity when attaching the PTE enzyme to the DNA along with the ability of the full conjugate structure to maintain this enhancement even when DNA is acting as an extended linker between the PTE and the central PEGylated QD. In addition to the previous contributing mechanisms behind NP–enzyme enhancement postulated earlier, there is also a possibility of substrate sequestration at the NP interface; this was previously considered with the enhancements noted for a diffusion–limited enzyme and is still being investigated [11]. One of the more intriguing results we find is that attaching PTE to the DNA linker also results in significant enhancement of $k_{cat}$. As mentioned, the Yan group has also reported on similar phenomena with dehydrogenase enzymes in DNA constructs [35, 38]. It is currently not clear if this is as widespread a phenomenon as that suspected for attaching enzymes to NPs [3]. Regardless, it is quite probable that the contributors to enzyme enhancement in both cases include the localized interfacial environment surrounding either the NP or the DNA. For NPs, this is believed to arise in the complex, hard-to-characterize interfacial region where the surface ligands...
Figure 4. PTE bioconjugate activity. Initial rates of p-nitrophenol product formation from paraoxon hydrolysis for PTE free in solution (PTE, yellow), PTE attached directly to the 525 nm QD surface (525 QD-CLA-PTE, green), PTE attached to the 56 bp dsDNA (DNA-PTE, red), or as displayed on the QD–DNA structure (QD-DNA-PTE, black). Data shown for ratios of either (A) 2 or (B) 4 PTE assembled per QD or their equivalents in the other configurations. (C) Specific activity (in units of nmols of substrate consumed min$^{-1}$ mg$^{-1}$ PTE) of QD-DNA–PTE conjugates versus that of the equivalent amount of free PTE. Averaged value for the 3 ratios of QD–DNA–PTE is 48 920 ± 8558 and 4760 ± 1472 for the free PTE. Data averaged from 4 replicates with error bars representing the standard deviations. No hydrolysis of paraoxon was observed from QD controls lacking the PTE enzyme.

Table 2. Estimated kinetic parameters.

<table>
<thead>
<tr>
<th>Ratio of PTE/QD = 2</th>
<th>$V_{\text{max}}$ (μM s$^{-1}$)</th>
<th>$K_M$ (mM)</th>
<th>$k_{\text{cat}}$ (s$^{-1}$)</th>
<th>$k_{\text{cat}}/K_M$ (mM$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>525 QD-DNA-PTE</td>
<td>0.20 ± 0.01</td>
<td>0.30 ± 0.04</td>
<td>101 ± 7</td>
<td>34.2 ± 5.0</td>
</tr>
<tr>
<td>625 QD-DNA-PTE</td>
<td>0.19 ± 0.01</td>
<td>0.23 ± 0.04</td>
<td>97 ± 7</td>
<td>42.9 ± 7.8</td>
</tr>
<tr>
<td>525 QD-PTE</td>
<td>0.10 ± 0.01</td>
<td>0.12 ± 0.02</td>
<td>51 ± 3</td>
<td>41.3 ± 6.5</td>
</tr>
<tr>
<td>625 QD-PTE</td>
<td>0.22 ± 0.02</td>
<td>0.19 ± 0.04</td>
<td>109 ± 9</td>
<td>56.3 ± 12.0</td>
</tr>
<tr>
<td>DNA-PTE</td>
<td>0.19 ± 0.01</td>
<td>0.25 ± 0.03</td>
<td>94 ± 6</td>
<td>37.3 ± 5.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ratio of PTE/QD = 4</th>
<th>$V_{\text{max}}$ (μM s$^{-1}$)</th>
<th>$K_M$ (mM)</th>
<th>$k_{\text{cat}}$ (s$^{-1}$)</th>
<th>$k_{\text{cat}}/K_M$ (mM$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>525 QD-DNA-PTE</td>
<td>0.85 ± 0.13</td>
<td>1.12 ± 0.23</td>
<td>212 ± 33</td>
<td>18.9 ± 4.9</td>
</tr>
<tr>
<td>625 QD-DNA-PTE</td>
<td>0.58 ± 0.06</td>
<td>0.70 ± 0.02</td>
<td>145 ± 16</td>
<td>21.9 ± 4.4</td>
</tr>
<tr>
<td>525 QD-PTE</td>
<td>0.34 ± 0.02</td>
<td>0.33 ± 0.03</td>
<td>86 ± 4</td>
<td>25.9 ± 2.8</td>
</tr>
<tr>
<td>625 QD-PTE</td>
<td>0.82 ± 0.11</td>
<td>0.73 ± 0.15</td>
<td>206 ± 28</td>
<td>28.2 ± 7.0</td>
</tr>
<tr>
<td>DNA-PTE</td>
<td>0.45 ± 0.03</td>
<td>0.29 ± 0.03</td>
<td>112 ± 7</td>
<td>38.6 ± 2.1</td>
</tr>
<tr>
<td>Free PTE</td>
<td>0.15 ± 0.03</td>
<td>0.10 ± 0.04</td>
<td>31 ± 3</td>
<td>310.6 ± 3.3</td>
</tr>
</tbody>
</table>

DHLA-CL4 ligand used for QD–PTE, DHLA-PEG750-OMe used for QD–DNA–PTE.
interact with the aqueous environment to provide colloidal stability. Putative alterations to the bulk environment here may include ionic/charge gradients, boundary layers and phase separation, along with changes to density, $pK_a$'s, viscosity and solvation [39, 40]. The limited area where these changes and their localized effects are found have made them almost impossible to directly characterize with any currently available analytical methodologies [39, 40]. For nucleic acid structures, the high localized density of charges in conjunction with their polymeric nature can be anticipated to give rise to something equally complex [41]. Indeed, using the model system of glucose oxidase coupled with horseradish peroxidase, Zhang and colleagues have recently suggested that DNA scaffolding contributes a more optimal pH environment towards the activity of anchored enzymes [42].

Beyond just a hybrid biomaterials approach to linking enzymes directly to the shell of PEGylated QDs, the current work also suggests use of DNA, either alone or in conjunction with NPs for creating designer catalytic nanostructures while still maintaining enhanced enzyme activity. We note that there is a growing interest in developing such DNA-based structures as generalized scaffolds in order to control enzyme placement relative to each other [29, 32, 42, 43]. Determining if the same mechanisms are responsible for enzyme enhancement when attached to DNA or other NPs will certainly help elucidate more about this unique enzyme-materials interfacial environment and contribute to assembling more advanced multifunctional bionanomaterials. These may, for example, include central NPs with designer DNA linkers extending outward and displaying multiple copies of enzymes or sequential enzyme cascades. NP materials are also not just limited to QDs but can include almost any example, include central NPs with designer DNA linkers extending outward and displaying multiple copies of enzymes or sequential enzyme cascades. NP materials are also not just limited to QDs but can include almost any material as long as the necessary functional groups are available for facilitating the assembly. The latter could certainly help promote transition to final applications as well without specifically requiring QD materials which have some associated issues of potential toxicity [44]. Although much remains to be elucidated about how these phenomena arise, this area clearly represents an exciting and growing opportunity at the biological-nanomaterials interface [4].

Acknowledgments

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References

[31] Medintz I L 2006 Universal tools for biomolecular attachment to surfaces Nat. Mater. 5 842
[36] Boeneman K et al 2010 Quantum dot DNA bioconjugates: attachment chemistry strongly influences the resulting composite architecture ACS Nano 4 7253–66
[38] Zhao Z et al 2016 Nano-caged enzymes with enhanced catalytic activity and increased stability against protease digestion Nat. Commun. 7 10619
[39] Pfeiffer P et al 2014 Interaction of colloidal nanoparticles with their local environment: the (ionic) nanoenvironment around nanoparticles is different from bulk and determines the physico-chemical properties of the nanoparticles J. R. Soc., Interface 11 20130931
[40] Zobel M, Neder R B and Kimber S A J 2015 Universal solvent restructuring induced by colloidal nanoparticles Science 347 292–4
[42] Zhong Y, Tsitkov S and Hess H 2016 Proximity does not contribute to activity enhancement in the glucose oxidase-horseradish peroxidase cascade Nat. Commun. 7 13982