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Hydrolytic enzymes conjugated to quantum dots mostly retain whole catalytic activity

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ABSTRACT

Background: Tagging a luminescent quantum dot (QD) with a biological like enzyme (Enz) creates value-added entities like quantum dot–enzyme bioconjugates (QDEnzBio) that find utility as sensors to detect glucose or beacons to track enzymes in vivo. For such applications, it is imperative that the enzyme remains catalytically active while the quantum dot is luminescent in the bioconjugate. A critical feature that dictates this is the quantum dot–enzyme linkage chemistry. Previously such linkages have put constraints on polypeptide chain dynamics or hindered substrate diffusion to active site, seriously undermining enzyme catalytic activity. In this work we address this issue using avidin–biotin linkage chemistry together with a flexible spacer to conjugate enzyme to quantum dot.
Methods: The catalytic activity of three biotinylated hydrolytic enzymes, namely, hen egg white lysozyme (HEWL), alkaline phosphatase (ALP) and acetylcholinesterase (AChE) was investigated post-conjugation to streptavidin linked quantum dot for multiple substrate concentrations and varying degrees of biotinylation.
Results: We demonstrate that all enzymes retain full catalytic activity in the quantum dot–enzyme bioconjugates in comparison to biotinylated enzyme alone. However, unlike alkaline phosphatase and acetylcholinesterase, the catalytic activity of hen egg white lysozyme was observed to be increasingly susceptible to ionic strength of medium with rising level of biotinylation. This susceptibility was attributed to arise from depletion of positive charge from lysine amino groups after biotinylation.
Conclusions: We reasoned that avidin–biotin linkage in the presence of a flexible seven atom spacer between biotin and enzyme poses no constraints to enzyme structure/dynamics enabling retention of full enzyme activity.
General significance: Overall our results demonstrate for the first time that streptavidin–biotin chemistry can yield quantum dot enzyme bioconjugates that retain full catalytic activity as native enzyme.

1. Introduction

Quantum dots are tiny (~10 nm) semiconductor nanocrystals that are excellent luminescent labels for biomolecules owing to their bright luminescence in visible-NIR region, size-tunable narrow emission band, superior photostability and broad absorption spectrum for simultaneous excitation of multiple probes in comparison with traditional organic dyes and fluorescent proteins [1]. QDs today find applications in improving photovoltaic devices [2,3], assembling sophisticated biophotonic logic devices [4,5], creating novel thermodynamic machines [6,7] apart from being used for sensing glutathione selectively [8], sensing NO_2 [9], and sensing clenbuterol and melamine [10], as light-emitting devices [11,12] and as active drug tracers *in vivo* [13,14]. Of late, they have also found extensive applications in biology as cellular probes for immunolabeling, multimodal *in vivo* and live animal imaging [15,16], cellular tracking and related applications [17–19].

QD bioconjugates have served as fluorescent labels for both *in vivo* cellular imaging and *in vitro* assay detection [20]. Combining the brightness of QD luminescence with remarkable ligand/substrate recognition specificity of an antibody/enzyme/aptamer has given rise to numerous biomedical applications like sensing glucose [21–23], urea [24], polyphenols [25], and H_2O_2 [26]. Similar conjugates have been useful in detecting toxins like ricin [27], target DNA [28], and cocaine [29] or in monitoring enzyme mediated phosphorylation [30] and release of doxorubicin into cancer cells [31]. Their utility in tracking activity of AChE *in vivo* under diseased conditions [32] or monitoring protein unfolding of human serum albumin [33] has been demonstrated. Such hybrid devices are predicted to find applications in areas ranging from energy harvesting and nanoscale electronics to biomedical diagnostics [34].

Over the past three decades, numerous reports of enzyme conjugated to nanoparticles/nanorods/QDs have appeared (see Table 1). However, in vast majority of such reports, the catalytic activity of enzyme in the conjugate has suffered significant loss rendering them futile. In spite of the diverse number of conjugation strategies employed, there clearly appears no universal method that can guarantee retention of whole enzyme activity in the QDEnzBio conjugate. It is likely that changes in native enzyme conformation, backbone/side-chain dynamics or substrate access to the active site in QDEnzBio might have contributed to

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Table 1
Enzyme–nanoparticle conjugates synthesized in the past and their consequence on enzyme activity. Entries are listed in the order they appeared in literature with most recent work reported first.

	Enzyme	Nanoparticle	Conjugation strategy	Inert spacer	Effect on enzyme activity		Reference
1	Tyrosinase (from mushroom)	Glutathione–CdTe QDs	Encapsulated with Poly-(diallyldimethylammonium chloride) in a hybrid film	No	−85%	Decreases	[43]
2	Sialyltransferase (PmST1, from *Pasteurella multocida*)	Magnetic nanoparticles	Cysteine functionalized magnetic NP reacting with protein α-thioester through native chemical ligation	Yes (5 atoms)	−20%	Decreases	[44]
				Yes (23 atoms)	+165%	Increases	
				Yes (45 atoms)	+225%	Increases	
3	Cytidine monophosphate sialic acid synthetase	Magnetic nanoparticles	Cysteine functionalized magnetic NP reacting with protein α-thioester through native chemical ligation	Yes (5 atoms)	−20%	Decreases	
				Yes (23 atoms)	−19%	Decreases	
				Yes (45 atoms)	−14%	Decreases	
4	Sialyltransferase from *Neisseria gonorrhoeae* (NgST) (membrane bound protein)	Magnetic nanoparticles	NgST-Biotin with Streptavidin-magnetic NP	Yes (5 atoms)	−66%	Decreases	
				Yes (23 atoms)	−92%	Decreases	
				Yes (45 atoms)	−82%	Decreases	
5	Type II restriction endonuclease (EcoRI)	CdS QDs	Amide bond using NHS[a] and EDC[b] chemistry	No	Qualitative estimate	No change	[45]
6	Butyrylcholinesterase	3-Mercaptopropionic acid coated CdSe/ZnS QDs	Electrostatic interaction	No	−50%	Decreases	[32]
7	α-Chymotrypsin	Ni nanoparticles	Chemical reduction in aqueous solution	No	−58%	Decreases	[46]
8	Glucose oxidase	CdTe QDs	QD-COOH + GOx-NH_2 with EDC and NHS	No	13 fold lower K_m	Increases	[21]
9	CMP-sialic acid synthetase	Magnetic nanoparticles	Intein expression system and native chemical ligation	Random No	−67%	Decreases	[47]
				Site-specific No	−23%	Decreases	
10	Trypsin	Gold nanorods	Click chemistry (acetylene–trypsin to azide on Au nanorods)	Yes (12 atoms)	−43%	Decreases	[48]
			Amide bond (EDC chemistry)	No	−87%	Decreases more	
			Electrostatic adsorption	No	−81%	Decreases more	
11	α-Chymotrypsin	CdS nanoparticles	Chemical reduction in aqueous solution using TCEP[c]	No	−50%	Decreases	[49]
12	Lipase from *Thermomyces lanuginosus*	Gold nanoparticles	Click chemistry (acetylene–lipase to azide-functionalized Au nanoparticles)	Yes (47 atoms)	0%	No change	[50]
13	Cytochrome $P450_{BS\beta}$	CdS QDs	Electrostatic interaction	No	−83%	Decreases	[51]
14	RNase S	Gold nanoparticles	S-peptide (Cys) with Au NP followed by self-assembly with S-Protein	Yes (3 amino acid peptide spacer)	−99%	Decreases	[52]
15	Pepsin	Colloidal gold	Interaction of Cys–SH or Lys–NH_2 with colloidal gold	No	−19%	Decreases	[53]
16	Aspartic protease from *Aspergillus saitoi*	Colloidal gold	Interaction of Cys–SH or Lys–NH_2 with colloidal gold	No	−10%	Decreases	[54]
17	Horseradish peroxidase	Colloidal gold	Adsorbed to colloidal gold sols that were later electrodeposited onto Pt gauze/glassy carbon	No	Between −7% and −16%	Decreases	[55]
18	Xanthine oxidase	Colloidal gold	Ibid	No	Between −13% and −59%	Decreases	
19	Carbonic anhydrase	Colloidal gold	Ibid	No	−30%	Decreases	
20	Glucose oxidase	Colloidal gold	Ibid	No	Between −20% and −30%	Decreases	

[a] N-hydroxysulfosuccinimide (NHS).
[b] 1-ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC).
[c] Tris(2-carboxyethyl) phosphine hydrochloride (TCEP).