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Supplementary Information

Quantum dot display enhances activity of a phosphotriesterase trimer

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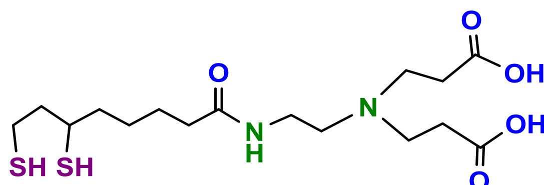
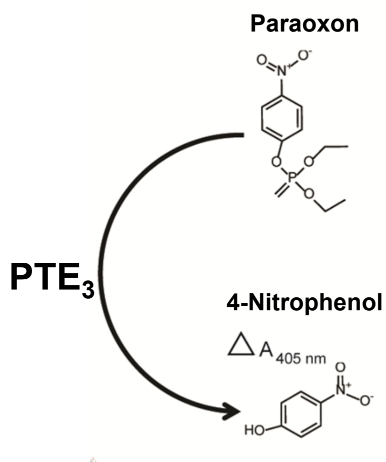
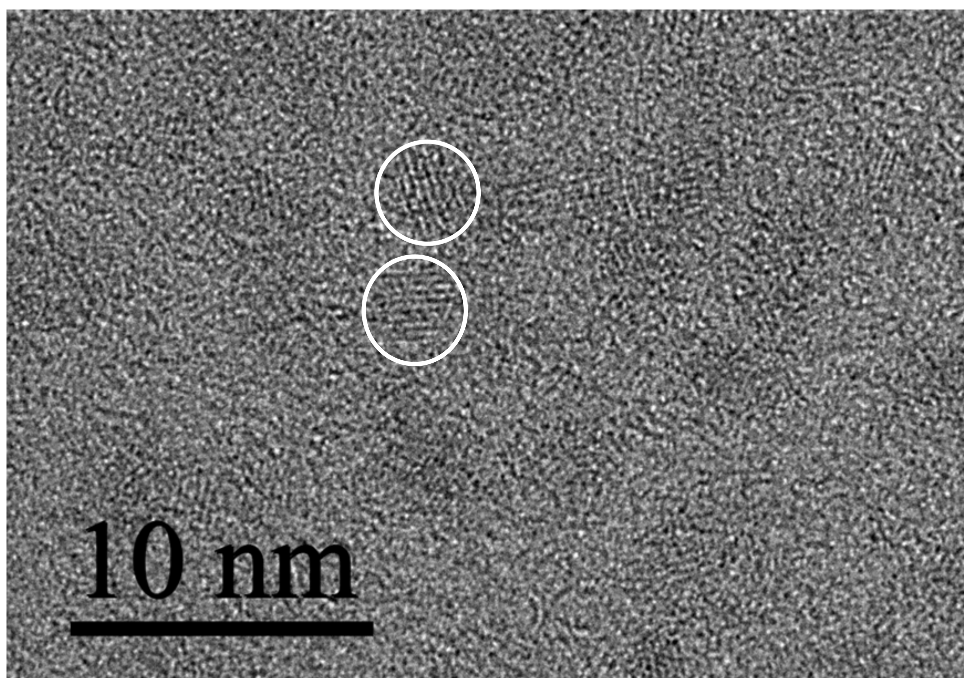
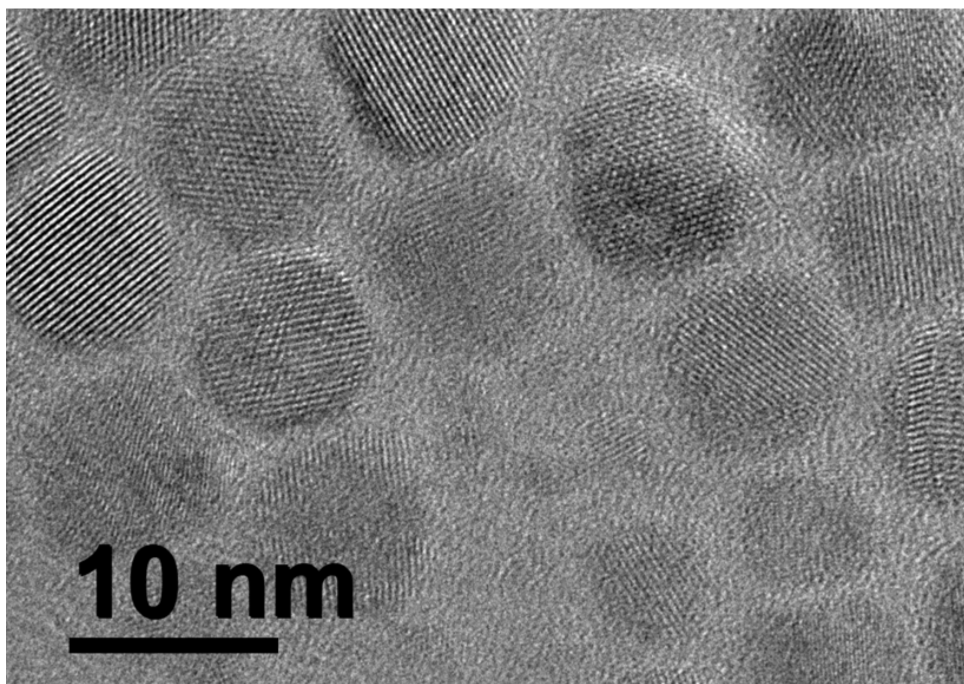
Supplementary Scheme 1. Structure of the DHLA-CL4 ligand used to solubilize the QDs:



Supplementary Scheme 2. PTE_3-paraoxon (parathion) reaction:







Supplementary Figure 1. Representative TEM micrographs of the 525 nm emitting (Top, diameter 4.3 ± 0.5) and 625 nm emitting QDs (Bottom, diameter 9.2 ± 0.8) utilized in this study.

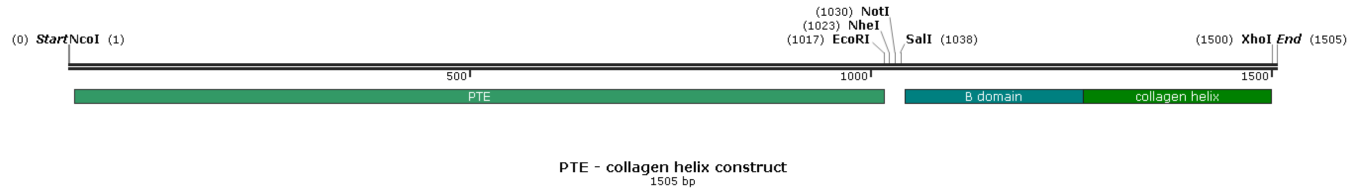
PTE_3 protein design and expression. The phosphotriesterase (PTE) gene from *Brevundimonas diminuta* [1] and the collagen-like triple helix domain from *Streptococcus pyogenes* [2,3] were synthesized by Genscript with flanking *Nco*I and *Xho*I sites to facilitate cloning into the bacterial expression vector pET28 (Novagen). The sequence of the expression construct was confirmed prior to transformation of the *Escherichia coli* expression strain BL21 (DE3) (New England Biolabs).

Expression of the PTE triple helix construct was conducted in shake flask cultures. Briefly, 500 ml of Terrific Broth (Fisher Scientific) was inoculated with 5 ml of an overnight culture and incubated for 3-4 hours at 37˚C until mid-log stage as determined by optical density at 600 nm (OD_{600} = 0.6 – 1.0). The temperature was then adjusted to 30˚C and expression induced with isopropyl-β-D-pyranogalactoside (IPTG) at a final concentration of 0.25 mM. Expression continued overnight (~20 hours). Cells were pelleted via centrifugation (wet weight 6 – 8g) then stored for a minimum of 3 hours at -80˚C to facilitate cell lysis.

Cell pellets were resuspended in 30 ml of ice cold lysis buffer (1/2 x phosphate buffered saline (PBS), 1 mM ethylenediaminetetraacetic acid (EDTA), 0.01% Triton X-100, 1 mg/ml lysozyme) and mixed to homogeneity. The cell suspension was sonicated using a Branson sonifier (output 7 at a constant duty cycle) three times at one minute intervals to ensure cell lysis and shearing of chromosomal DNA. The lysate was centrifuged for 30 min at 8,000 x g to pellet cellular debris and insoluble material. The soluble fraction was decanted to a 50 ml conical tube and batched with a 500 µl bed volume of Ni-NTA resin (GE Healthcare) for 3 hours at 4˚C with constant rotation on a Dynal rotisserie. The Ni-NTA resin was batch washed three times with a 60x bed volume of wash buffer (20 mM phosphate (pH 6.5), 400 mM NaCl, 0.05% Tween-20, 25 mM imidazole). The resin was then transferred to a chromatography column and the PTE triple helix protein eluted with wash buffer containing 300 mM imidazole. Protein concentration of individual fractions was approximated based on optical density at 280 nm measured using a NanoDrop 1000 instrument (Fisher Scientific). Protein containing fractions were pooled and stored at 4˚C for 48 hours to allow the tertiary structure to form. The triple helix complex was then separated from monomer and dimer forms of the protein via size exclusion chromatography on an ENrich EC650 column and DuoFLow system (both from Bio-Rad Laboratories).

As with overexpression of the standard PTE enzyme, the triple helix construct was somewhat toxic to the cell culture. The optimized expression protocol yielded 8 – 10 mg of protein per liter of cell culture (12 – 16 g of wet cell pellet). Additionally, initial expression/purification efforts did not account for the slow formation of the triple helix and samples were immediately subjected to FPLC following IMAC purification. In these instances, the monomer fraction was the predominant product. The 48 hour incubation was determined empirically which led to the triple helix being the primary product following FPLC.

Protein and nucleotide sequences:



PTE - collagen helix construct
1505 bp

Cloning sites

PTE

V-domain

Collagen repeats

Amino acid sequence of construct:

MGSIGTGDRINTVRGPITISEAGFTLTHEHICGSSAGFLRAWPEFFGSRKALAEKAVRGLRRARAAGVRTIVDVSTFDIGR
DVSLLAEVSRAADVHIVAATGLWFDPPLSMRLRSVEELTQFFLREIQYGIEDTGIRAGIIKVATTGKATPFQELVLKAAAR
ASLATGVPVTTHTAASQRDGEQQAAIFESEGLSPSRVCIGHSDDTDDLSYLTALAARGYLIGLDHIPHSAIGLEDNASASA
LLGIRSWQTRALLIKALIDQGYMKQILVSNDWLFGFSSYVTNIMDVMDRVNPDGMAFIPLRVIPFLREKGVPQETLAGIT
VTNPARFLSPTLRASEFASAAAVDADEQEEKAKVRTELIQELAQGLGGIEKKNFPTLGDEDLDHTYMTKLLTYLQEREQA
ENSWRKRLLKGIQDHALDGPRGEQGPQGLPGKDGEAGAQGPAGPMGPAGERGEKGEPGTQGAKGDRGETGPVGP
RGERGEAGPAGKDGERGPVGPA LE

Nucleotide sequence of construct:

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ccatgggcAGCATTGGCACCGGCGATCGCATTAACACCGTGCGCGGCCCGATTACCATTAGCGAAGCGGGCTTTACC
CTGACCCATGAACATATTTGCGGCAGCAGCGCGGGCTTTCTGCGCGCGTGGCCGGAATTTTTTGGCAGCCGCAAAGC
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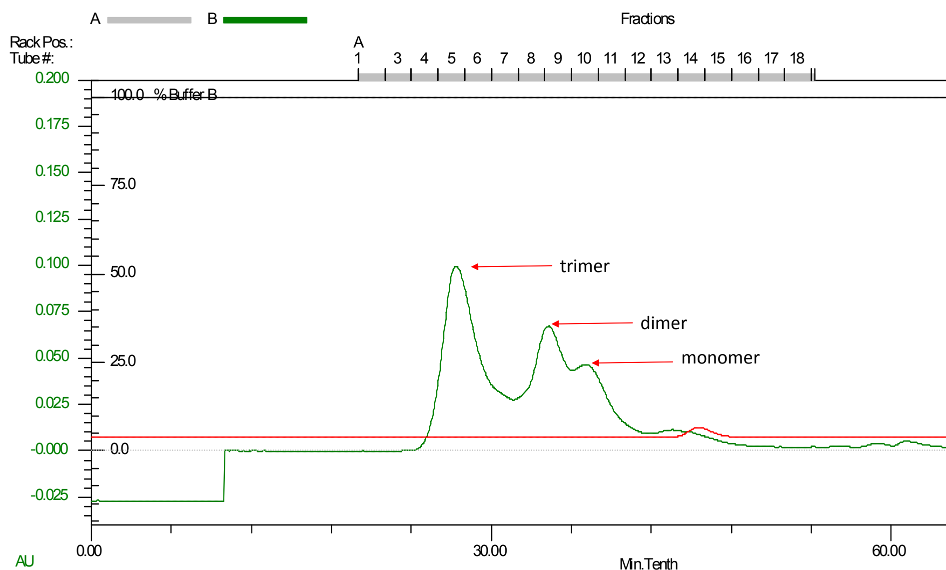

Supplementary Figure 1. Representative FPLC chromatogram showing the presence of PTE3 trimer, dimer and monomers during purification.

Dynamic Light Scattering and Zeta-Potential. Dynamic light scattering (DLS) measurements were carried out using a CGS-3 goniometer system equipped with a HeNe laser illumination at 633 nm and a single-photon counting avalanche photodiode for signal detection (ALV, Langen, Germany). The autocorrelation function was performed by an ALV-5000/EPP photon correlator (ALV, Langen, Germany) and analyzed using Dispersion Technology Software (DTS, Malvern Instruments Ltd, Worcestershire, UK). QDs (625 nm; 20-50 nM in 0.1×PBS buffer, 525 nm; 50-100 nM in 0.1×PBS buffer, pH 7.4) and enzyme (1.5-3.0 µM) were prefiltered through 0.25 µm syringe filters prior to DLS measurements to remove dust or impurities in the sample. To measure the effect of enzyme conjugation on the hydrodynamic size of NPs, we used the prefiltered QD solution mixed with the desired amount of enzyme (4-30 times that of QD concentration) with and without substrates (~10 µM). Sample temperature was maintained at 20°C. For each sample, the autocorrelation function was the average of three runs of 10 sec each and then repeated at different scattering angles (within 80° and 110°). CONTIN analysis was then used to extract number *versus* hydrodynamic size profiles for the dispersions studied. For Zeta-Potential (ζ-potential) measurement, Laser Doppler Velocimetry (LDV) measurements were performed using a ZetaSizer NanoSeries equipped with a HeNe laser source (λ = 633 nm) (Malvern Instruments Ltd, Worcestershire, UK) and an avalanche photodiode for detection,

controlled with DTS software. Micromolar concentration solutions of QDs or QD-bioconjugates were loaded into disposable cells, and data were collected at 25°C. Three runs of the measurements were performed for each sample to achieve the zeta potential. All the samples were prepared in 0.1×PBS buffer pH 7.4 with similar concentration as DLS measurement.

Supplementary Table 1. Hydrodynamic diameters, diffusion coefficients and ζ-potential of select PTE_3-QD conjugates

Sample	Hydrodynamic diameter (nm)	Diffusion coefficient (μ^2/s)	ζ-potential (mV at pH 8.5)
525 QD only	**14.4 ± 0.7**	**29.1 ± 1.4**	**-37.7 ± 0.7**
PTE_3:525 QD (2:1)	**16.7 ± 0.7**	**25.1 ± 1.0**	**-27.8 ± 0.4**
PTE_3:525 QD (4:1)	**20.4 ± 1.2**	**20.5 ± 1.2**	**-23.9 ± 0.8**
PTE_3:525 QD (8:1)	**22.2 ± 0.8**	**18.9 ± 0.7**	**-21.3 ± 12.2**
PTE_3:525 QD (16:1)	**32.8 ± 1.2**	**12.8 ± 0.5**	**-19.4± 0.9**
625 QD only	**16.8 ± 0.1**	**24.9 ± 0.2**	**-33.1 ± 1.3**
PTE_3:625 QD (2:1)	**18.4 ± 1.2**	**22.8 ± 1.4**	**-26.6 ± 1.3**
PTE_3:625 QD (4:1)	**18.4 ± 1.1**	**22.8 ± 1.4**	**-20.5 ± 0.5**
PTE_3:625 QD (8:1)	**24.6 ± 2.6**	**17.0 ± 1.8**	**-19.6 ± 0.4**
PTE_3:625 QD (16:1)	**34.4 ± 1.6**	**12.2 ± 0.6**	**-17.9 ± 1.4**
PTE_3:625 QD (32:1)	**39.3 ± 0.7**	**10.6 ± 0.2**	**-16.2 ± 1.2**
PTE_3	**10.1 ± 0.7**	**41.3 ± 2.7**	**-9.8 ± 1.7**

Standard deviation was calculated from repeated measurements (n = 3-5). H_D was obtained from Dynamic Light Scattering (DLS) experiments and ζ-potential was obtained from Laser Doppler Velocimetry (LDV) measurements. 525 QD diameter 4.3 ± 0.5 nm/625 QD diameter 9.2 ± 0.8 nm.

PTE_3 Structural Simulation. Models were constructed using coordinates available in the PDB and short peptides constructed using tools in Chimera 1.7 [4]. For domains with no published structure (*i.e.* the V-domain) a search was conducted on the PDB using the FASTA algorithm to identify structures with similar sequences. Domains that were constructed or modified were energy minimized in Chimera using built in features including ANTECHAMBER (version 1.27) and the AM1-BCC method of calculating charges [5].

The simulated protein complex consists of four domains: a phophotriesterase domain (PDB entry 1PSC [6]), a peptide linker (constructed as an extended peptide using tools in Chimera 1.7), the V-domain (modeled starting from PDB entry 4NSM selected based on sequence homology with the target sequence [7]), the collagen domain (constructed from PRB entry 1K6F [8]), and a poly-histidine tail. After assembling the phophotriesterase-linker-D-domain onto one of the collagen chains, it was noted that the two alpha-helices of the V-domain could be re-oriented to lay along the collagen helix. Since the V-domain is required for proper assembly of the collagen triple helix, torsion angles in the region where the V-domain is joined to the collagen were adjusted to create this orientation. While there is no direct evidence to support this arrangement, the fact that the V-domain is required for proper folding of the collagen helix supports some kind of interaction between these two domains.

References:

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