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SUPPORTING INFORMATION

Kinetic Enhancement of the Diffusion-Limited Enzyme Beta-Galactosidase When Displayed with Quantum Dots

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SUPPLEMENTARY METHODS

Beta-galactosidase vector construction

The β-galactosidase gene (EC 3.2.1.23) from *E. coli* K12 was synthesized by Genscript (Piscataway, NJ) encoding a 5'- *Not*I site and a 3'- *Xho*I site. The gene was isolated from the shuttle vector and cloned into the pET28b expression vector (Life Technologies). Positive clones following bacterial transformation and selection on antibiotic containing medium were sent to Eurofin MWG Operon for sequencing. Once the correct sequence was confirmed, plasmid DNA was transformed to the *E. coli* expression strain BL21(DE3).

Expression and purification

For each expression, 500 mL of Terrific Broth was inoculated with a 1:100 dilution of overnight starter culture. The culture was incubated for 3 hours at 37˚C, then shifted to 30˚C for an additional hour before induction with 0.25 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). Expression of recombinant protein proceeded overnight at 25˚C. The following morning the cells were pelleted *via* centrifugation at 4000 x g for 20 min. The supernatant was decanted and the pellet stored at -80˚C for a minimum of 1 hour prior to protein harvest.

Cell pellets were resuspended in 30 mL of lysis buffer (0.5x PBS, pH7.4), 1 mM EDTA, 0.1% Triton X-100, 1 mg/mL hen egg white lysozyme). The cell suspension was transferred to a 50 ml screwtop conical tube, placed on ice, and agitated on a platform shaker for 30 m in. Cell lysis and shearing of genomic DNA was accomplished with a Branson sonifier 450 (constant duty cycle, output of 6). Sonication was performed for 3 cycles at 60 seconds each. Insoluble cell debris was pelleted at 4000 x g for 30 min. The supernatant was batched with HisPur Nickel-NTA resin (Thermo Scientific). The slurry was incubated for a minimum for 3 hours at 4˚C rotating on a Dynal rotisserie. Resin was batched washed three times (25 mM phosphate pH 7.0, 300 mM NaCl, 20 mM imidazole) then eluted with the same wash buffer and imidazole at a final concentration of 250 mM. Fractions were pooled and further purified *via* FPLC using a BioRad DuoFlow system. Protein concentration was determined using a Nanodrop 1000 (Thermo Scientific) using the

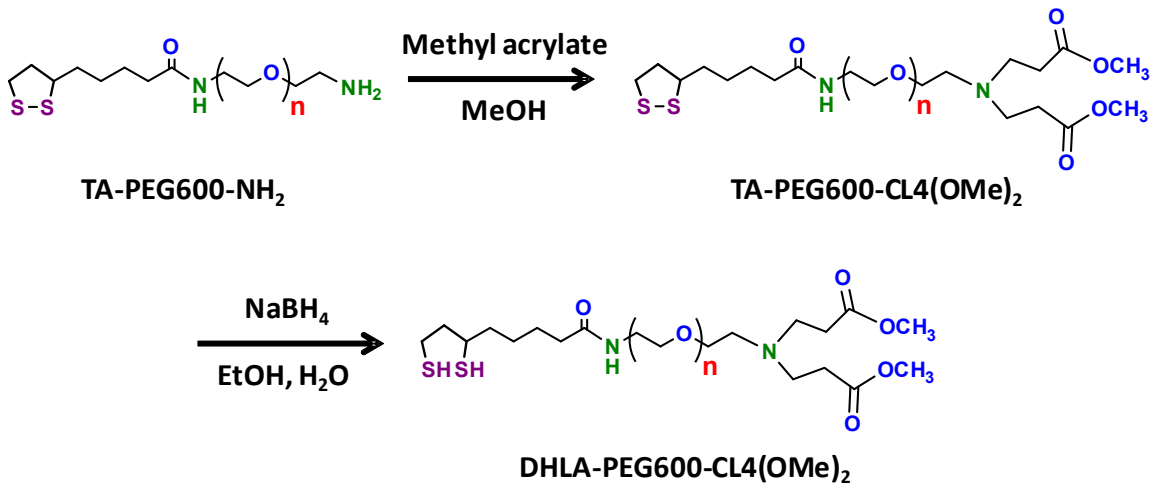
calculated molecular weights and extinction coefficients. Concentrations were confirmed using a BCA assay (Thermo Scientific).

Quantum dots

Qdot® 625 ITK™ (organic) were a gift from Invitrogen Life Technologies and the 525 nm emitting CdSe/ZnS QDs were synthesized as described previously with some modifications.[1]

Ligand synthesis (Figure 1B)

The compact ligand DHLA-CL4 synthesis procedure was described previously.[2a] The synthesis of the precursors of DHLA-PEG-CL4 is described below. The ligands were then attached to quantum dots *via* the bidentate dihydrolipoic acid (DHLA) anchoring group.



Scheme 1 – Key synthetic steps in the preparation of DHLA-PEG600-CL4. The The terminal methyl ester groups of the ligands were hydrolyzed in 0.1 M NaOH solution after cap exchange to expose carboxyl groups.

TA-PEG600-CL4(OMe)$_2$.

TA-PEG600-NH_2 (2.634 g, ~3.39×10^{-3} mol) and MeOH (30 ml) were added to a 250-mL round-bottom flask equipped with an addition funnel, and the reaction vessel was purged with N_2.[2b] Methyl acrylate (0.67 ml, 7.4×10^{-3} mol) in 10 ml of MeOH was added dropwise over 1h, and the reaction mixture was stirred at room temperature for 3 days under N_2. The solvent and excess methyl acrylate were evaporated. The residue was chromatographed on silica gel with $CHCl_3$:MeOH (15:1). Yield = 2.622 g (~81 % based on 2.634 g of TA-PEG600-NH_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.35 (br s, 1H, N*H*), 3.4–3.8 (m), 3.06–3.25 (m, 2H), 2.82 (t, 4H, *J* = 9.6 Hz, -NC*H*$_2$CH$_2$CO-), 2.67 (t, 2H, *J* = 8.4 Hz, -OCH$_2$C*H*$_2$N-), 2.40–2.53 (m, 1H), 2.45 (t, 4H, *J* = 9.6 Hz, -NCH$_2$C*H*$_2$CO-), 2.20 (t, 2H, *J* = 9.8 Hz, -C*H*$_2$CO-), 1.85–2.00 (m, 1H), 1.55–1.84 (m, 4H), 1.41–1.53 (m, 2H).

DHLA-PEG600-CL4(OMe)$_2$.

TA-PEG600-CL4(OMe)$_2$ (0.515 g, ~5.43×10^{-4} mol), EtOH (1.0 ml) and H_2O (4.0 ml) were added to a 50-mL round-bottom flask. $NaBH_4$ (32.4 mg, 8.6×10^{-4} mol) was added portionwise to the reaction mixture, which was stirred at room temperature for 1.5 h under N_2. 1M HCl solution was slowly added to neutralize the reaction mixture. The product was extracted with $CHCl_3$ (3 times). The combined organic layers were dried over Na_2SO_4. The inorganic solid was filtered off, and the solvent was evaporated to obtain the product as transparent oil. Yield = 0.489 g (~95 % based on 0.515 g of TA-PEG600-CL4(OMe)$_2$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.26 (br s, 1H, N*H*), 3.4–3.8 (m), 2.86–2.99 (m, 1H), 2.82 (t, 4H, *J* = 9.6 Hz, -NC*H*$_2$CH$_2$CO-), 2.60–2.78 (m, 2H), 2.67 (t, 2H, *J* = 8.4 Hz, -OCH$_2$C*H*$_2$N-), 2.45 (t, 4H, *J* = 9.6 Hz, -NCH$_2$C*H*$_2$CO-), 2.20 (t, 2H, *J* = 9.8 Hz, -C*H*$_2$CO-), 1.84–1.98 (m, 1H), 1.40–1.81 (m, 7H), 1.36 (t, 1H, *J* = 10.6 Hz, -S*H*), 1.31 (d, 1H, *J* = 10.0 Hz, -S*H*).

Cap exchange

The hydrophobic QDs were made hydrophilic by exchanging the native ligands with customized ligands containing dihydrolipoic acid (DHLA) using either a biphasic mixture method for DHLA-CL4 or a premetallation method for DHLA-PEG-CL4 as previously reported.[2, 3, 4] The terminal methyl ester groups of the ligands were hydrolyzed in 0.1 M NaOH solution after cap exchange to expose carboxyl groups. [2, 3]

Structural simulation (Figure 1A)

The simulations were essentially undertaken as previously described.[5, 6] Simulations utilized PDB entry 1DP0. On the C-terminus, the His_6 sequence was added as an anti-parallel beta-sheet. Torsion angles in the C-terminal region were then adjusted so that the poly-His region was oriented to enable binding of the QD. Based on a model of DHLA-CL4, a 17 Å layer was put around each 9.3 Å (diameter) QD. QD's were then docked with the poly-His region of the enzyme.

Transmission electron microscopy (Figures 2A, S2-4)

Structural characterization of as-prepared QDs was carried out using a JEOL 2200-FX analytical high-resolution transmission electron microscope (TEM) with a 200 kV accelerating voltage. Samples for TEM were prepared by spreading a drop (5~10 µl) of the filtered NPs dispersion (filtered using 0.25 µm Millipore syringe filters) onto ultrathin carbon/holey support film on a 300 mesh Au grid (Ted Pella, Inc.) and letting it dry. The concentration of NPs in the deionized water used was typically ~ 1 µM. Individual particle sizes were measured using a Gatan Digital Micrograph (Pleasanton, CA); average sizes along with standard deviations were extracted from analysis of ~100 or more nanoparticles.

Dynamic light scattering (Figure 2C, SI Table 1)

Dynamic light scattering (DLS) measurements to determine the hydrodynamic size and diffusion coefficient were carried out using ZetaSizer NanoSeries equipped with a HeNe laser source (λ = 633 nm, Malvern Instruments Ltd, Worcestershire, UK) and analyzed using Dispersion Technology Software (DTS, Malvern Instruments Ltd, Worcestershire, UK). 0.1~ 1µM concentration solutions of QDs were loaded into disposable cells, and data was collected at 25°C. All the samples were prepared in 0.1×PBS buffer pH 7.4. For each sample, the autocorrelation function was the average of five runs of 10 seconds each and then repeated about three to six times. CONTIN analysis was then used to number *versus* hydrodynamic size profiles for the dispersions studied.

ONPG kinetic experiments (Figure 3)

2-Nitrophenyl β-D-galactopyranoside (*o*-nitrophenyl β-D-galactopyranoside, product number: 73660 FLUKA), abbreviated ONPG was obtained from Sigma Aldrich (St. Louis, MO). Stock solutions of 2 mg/mL in 1×PBS were prepared. Kinetic reactions were carried out in a 1×PBS.

A 100 µL 6 µM aliquot of β-gal was defrosted on ice and diluted to a 66 nM (22 µL into 1 mL PBS) working stock. From there, the β-gal working stock was diluted further into either QD solution or PBS for the solution-only experiments. The QD solution was made by dilution stock concentrations of QDs into PBS, at a working concentration of 113 nM for 1:1 QD:β-gal ratio. Higher QD ratios had a correspondingly higher amount of stock QD added. B-gal/PBS and β-gal/QD solutions (480 µL β-gal to 279 QD/PBS) were incubated at room temperature for 1 hr. Corning (Corning, NY) 96 well round bottom, transparent plates were used for the reactions. PBS and ONPG solutions were first added to the plates to set up an ONPG concentration profile of 0, 0.1 mM, 0.2 mM, 0.3 mM, 0.5 mM, 1 mM, 2.5 mM and 5 mM. Initial 60s baselines of the solution absorbance at 420 nm were taken at 10s intervals and averaged together to generate a baseline for each well. 25.3µL of the β-gal/PBS and β-gal/QD solutions were then added simultaneously via a multi-channel

pipette. This resulted in a 10 nM final concentration of the β-gal enzyme for each experiment. Another 60s measurement was taken, in 10s intervals, to determine the initial velocity of each substrate concentration. After 20 minutes, the column was again read in 10s intervals for 60s to generate a standard curve to correlate maximum absorbance with substrate concentration. Each β-gal solution-only and β-gal/QD ligand and ratio was run in the presence of an ONPG concentration profile at least three times to generate standard deviations.

ONPG Kinetics Calculations (Table 1)

Statistical analysis of the kinetic experiments was performed in Graphpad (La Jolla, CA) Prism 6 Software. As previously described, each baseline was calculated as an average from seven data points taken before the addition of the βgal enzyme. This baseline constituted the "0" time point for all reactions except those involving the 625 CL4 QD, in which the large increase in background absorbance due to the QDs themselves necessitated that the 0 mM substrate was used as the baseline after the addition of B-gal/QD solution. In the 525 CL4 and PEG-CL4 QDs, the QD itself contributed a negligible amount to the background absorbance. A 30s dead time was accounted for between the baseline and the first absorbance measurement after the addition of β-gal. The data was then baseline-subtracted and transformed using a conversion factor calculated from the 20 min time point.

After 20 minutes, the completed reaction absorbance was monitored again over seven data points and averaged together. Due to a decay in the absorbance over time, the highest two substrate concentrations were not used in the standard curve calculation. Using the absorbance values from 0-1 mM substrate concentration range, a linear relationship between absorbance and substrate concentration was obtained. The linear slopes of all reactions were all very similar, with no bearing on whether it was a QD or solution-only experiment. These values were then averaged together and used as a single transformation factor to convert arbitrary units of absorbance into units of molarity.

The baseline-subtract initial rate data was converted to molarity using the aforementioned transformation factor and a linear fit was performed to determine the initial velocity of each substrate concentration. Each reaction condition was determined with the replicates from all performed experiments under that condition, and each experiment was performed with at least three independent replicates. As the β-gal solution-only was performed as a control for all experiments, it has the highest *N* of 24. For ratios tested in a single experiment (*i.e.* 525 CL4 QD 2:1), the *N* was 3. The initial velocities and standard deviations were then plotted versus substrate concentration and fit using the built-in Michaelis-Menten nonlinear regression. These fits provided the results and error for the V_{max} and K_m. k_{cat} was calculated using Equation 1:

Equation 1: $$V_{max} = k_{cat}[E]$$

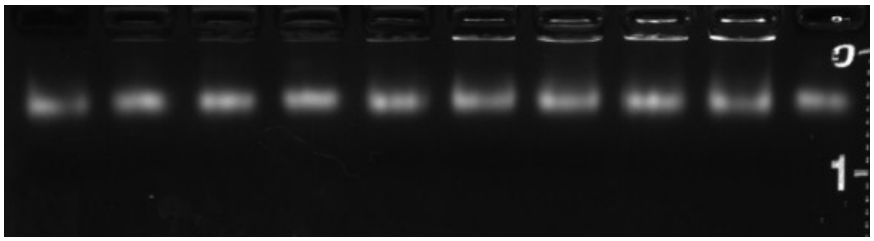
The enzyme efficiency ratio was calculated as k_{cat}/K_m. Errors for k_{cat} and K_m were calculated as the addition of the ratios of the indeterminate errors between the two measurements (Equation 2).

Equation 2: $$\frac{\Delta R}{R} = \frac{\Delta A}{A} \times \frac{\Delta B}{B}$$

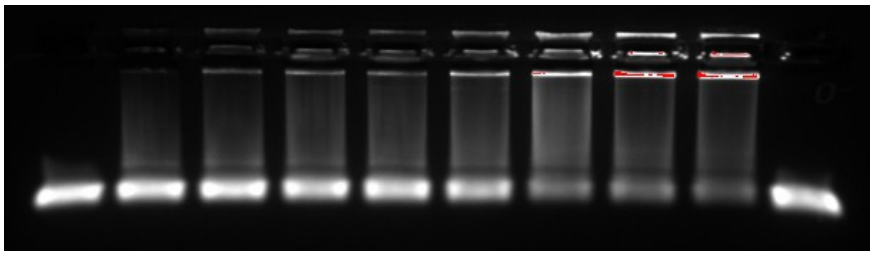
Here, the relative error of the R measurement (in the first case, k_{cat}) comes from the addition of the ratio of error of the V_{max} and [E]. As we only have an error for V_{max}, the ratio of the error to the value for k_{cat} is the same as V_{max}. The error for the enzyme efficiency ratio is the addition of the relative errors of k_{cat} as well as K_M. Thus, in the case of β-gal solution-only, the relative error of V_{max} (and therefore k_{cat}) was ~1.5% (0.06/4.1), while the relative error of K_M was ~5.8% (10/172), and therefore the additive propagation of the error to k_{cat}/K_M was ~7.3%. Representative traces (Figure 3) are derived from a single experiment of three replicates, graphed with a Michaelis-Menten curve fit.

SUPPLEMENTARY FIGURES

(A) 525 PEG-CL4 QD



(B) 625 CL4 QD



0 10 6.66 5 4 2.5 2 1.33 1 0 QD to B-gal ratio

Figure S1 – Agarose gels of (A) 525 DHLA-PEG-CL4 QD and (B) 625 CL4 QD. The 625 CL4 QD shows formation of a non-mobile conjugate and reduced mobility at lower QD-β-gal ratios, similar to that of the 525 CL4 QD, although the effect is attenuated here due to the far larger loading capacity of these QDs.[7] The 525 DHLA-PEG-CL4 shows little mobility change across all ratios due to the strong influence of the PEG.

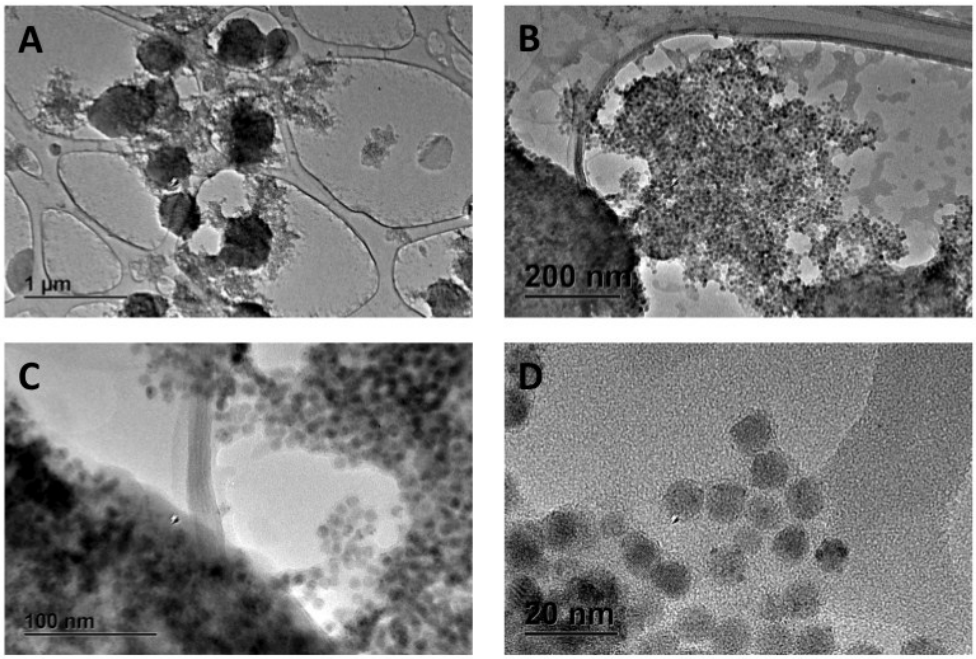


Figure S2 – TEM images showing aggregation of QDs at 1:1 ratio of 625 DHLA-CL4: QD-β-gal at various magnifications

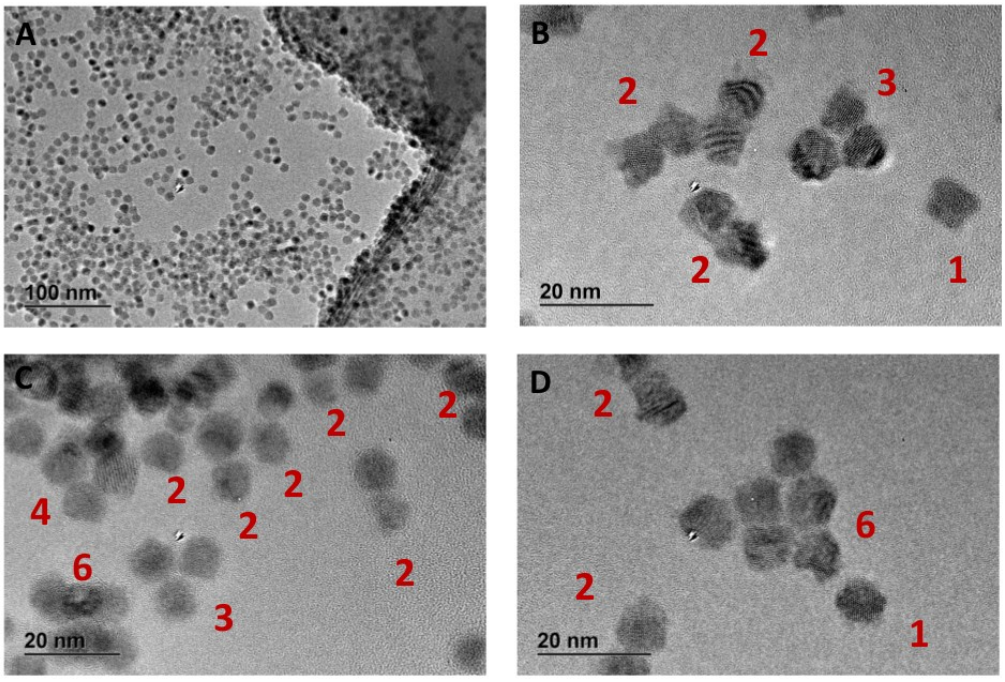


Figure S3 – TEM images showing resolution of small 625 DHLA-CL4 QD clusters at 4:1 QD:β-gal ratio at various magnifications.

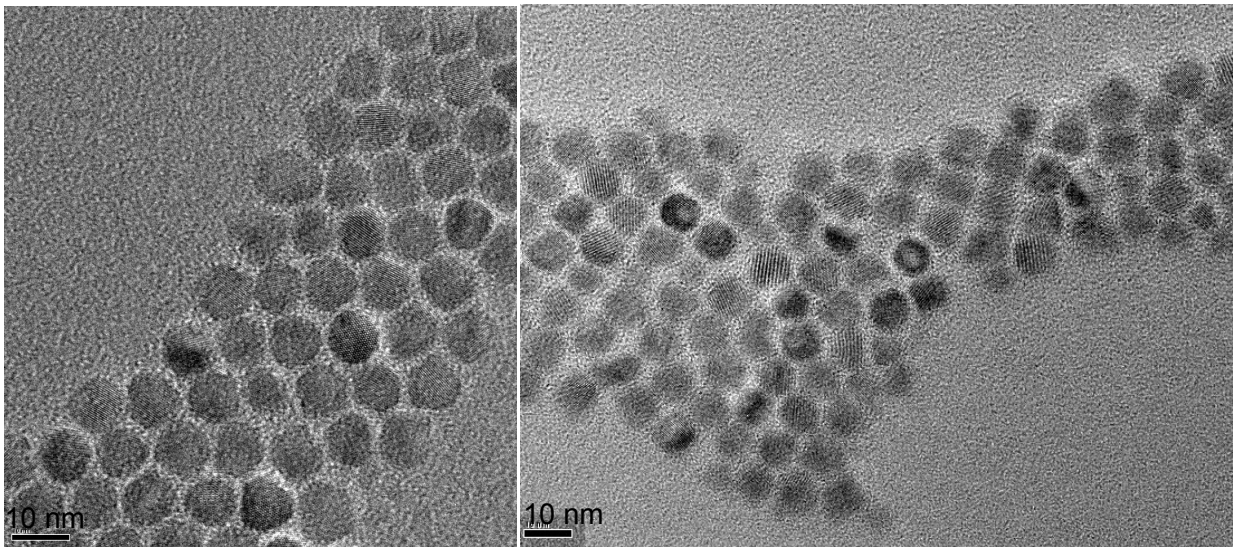


Figure S4 – Representative TEM images showing water soluble 625 QD without being subjected to any chemical modification or enzyme linkage. Note how this QD pattern is quite distinct from those above and in the main text as well.

Table S1. Dynamic Light Scattering Analysis of β-gal Assemblies with QDs

Ratio of β-gal-QD	D_H (nm)	Diffusion coefficient (μm²/s)
B-gal alone (100 nM)	**16.4 ± 1.76**	**25.5 ± 2.73**
525 nm CL4 QD		
0	11.5 ± 1.2	36.5 ± 3.75
0.25	17.6 ± 0.64	23.8 ± 0.87
1	25.7 ± 1.53	16.3 ± 0.97
2	34.5 ± 2.27	12.2 ± 0.80
525 nm PEG-CL4 QD		
0	18.0 ± 1.02	23.3 ± 1.33
0.25	19.7 ± 1.85	21.3 ± 2.01
1	22.5 ± 2.23	18.6 ± 1.83
2	26.5 ± 0.53	15.8 ± 0.32
625 nm CL4 QD		
0	15.5 ± 1.25	27.0 ± 2.17
0.25	18.9 ± 3.92	22.1 ± 4.58
1	129.5 ± 11.73	3.2 ± 0.29

All values are averaged with standard deviations collected from at least 3 independently assembled experiments.

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