Supporting information:

Quantum Dot Encapsulation Using a Peptide-Modified Tetrahedral DNA Cage

Divita Mathur[†⊥]‡, Anirban Samanta[†⊥]‡, Eunkeu Oh[‡∫], Sebastián A. Díaz[†ϕ], Kimihiro Susumu[‡∫], Mario. G. Ancona[≠], Igor L. Medintz[†]*

Materials

DNA: Peptide-modified DNA strand T1-pep was obtained from Biosynthesis™ (Lot Number SP1997-1). Remaining strands (T2-T5 as well as T1 without the peptide "handle") were obtained from Integrated DNA Technologies, Coralville IA under lyophilized conditions. Strands were used after reconstituting to a concentration of 100 uM without further purification or chemical modification.

QDs: CdSe/ZnS core−shell QDs with emission maxima centered at ~525 nm were synthesized and solubilized with DHLA-CL4 as previously described.[1]

Methods

Electrophoresis: Electrophoresis experiment (Figure 3) was performed in 1.5% (w/v) agarose in 1x TAE (Tris-acetate EDTA) buffer at 70 V. Gel was pre-stained with 2x GelRed™. Each lane was loaded with 2% glycerol. Gel was scanned under UV excitation using a BioRad™ transilluminator.

Fluorescence experiments: All fluorescence experiments were performed at ambient conditions on a Tecan M1000 plate reader, wherein all samples were observed at a volume of 100 µL in individual wells of a 96-well plate and varying concentrations according to the experimental objectives. QDs were excited at 400 nm.

Dynamic light scattering: Dynamic light scattering (DLS) measurements were carried out using ZetaSizer™ NanoSeries instrument equipped with a HeNe laser source (λ=633

nm) (Malvern Instruments Ltd., Worcestershire, UK) and analyzed using Dispersion Technology Software (Malvern Instruments Ltd.).[2] Each sample, depending on the constituents, contained [QD] = 100 nM and [cage] = 175 nM in 1x TAE buffer (pH 8.3) with 12.5 mM Mg^{2+}. Samples were loaded in disposable cells and data was acquired at 25°C. 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 create number *versus* hydrodynamic size profiles for each sample studied.

Assembly protocols for each configuration:

T1-pep QD conjugation: 200 µl of 1 µM QD was mixed with T1-pep at varying concentrations according to the experiment. The sample was covered with aluminum foil and incubated for 30 min at room temperature. The sample was used without further purification.

Cage assembly: Each oligonucleotide, stored at a stock concentration of 100 uM was used by creating working stocks at 20 uM each in molecular biology grade water. The cage oligonucleotides were mixed at equimolar concentrations in 1x Tris-Acetate EDTA and 12.5 mM Mg^{2+} according to the concentration required in various experiments. Cage assembly was performed at 90℃ for 2 min, followed by cool down to room temperature in ambient conditions and stored at 4℃.

Route 1 assembly of cage+/-QD complex:

1. Complete cage assembly: Individual cage oligonucleotides (except for T3+/-Cy3) were mixed at equimolar concentration to aim for a final reaction concentration of 175 nM (1.75x of QD concentration). The ratio of T3-Cy3 and T3-unlabeled was altered from 0 to 1.75 based on the experimental requirements in order to perform fluorescence assays.
2. Addition of QD: QD was mixed with assembled cage samples to bring the final concentration of QD to 100 nM and each cage sample concentration to 175 nM with varying concentration of Cy3 (0 nM to 175 nM). Samples were incubated at

55℃ for 3 min, followed by cool down to room temperature under ambient conditions (~ 30 min) and stored at 4℃ or immediately measured for fluorescence. Samples that did not contain QD were supplemented with equal volume of water.

Route 2 assembly of cage+/-QD complex:

1. T1-pep attachment to QD: T1-pep and QD were mixed as described above.
2. Assembly of partial cage: Cage oligonucleotides (except for T1-pep) were mixed to a final concentration of 175 nM each with varying concentration of Cy3 (as described above) in 1x TAE + 12.5 mM Mg^{2+}. Samples were incubated at 90 ℃ for 2 minutes, followed by cool down to room temperature in ambient conditions.
3. Full complex formation: The above two reaction mixtures (T1-pep+QD and partial cage) were mixed which resulted in a final QD concentration of 100 nM and cage concentration of 175 nM. Reaction was carried out at 55 ℃ for 3 min, followed by cool down to room temperature in ambient conditions (~ 30 min) and stored at 4 ℃ or immediately measured for fluorescence. Samples that did not contain QD were incubated with T1-pep only.

QD TEM:

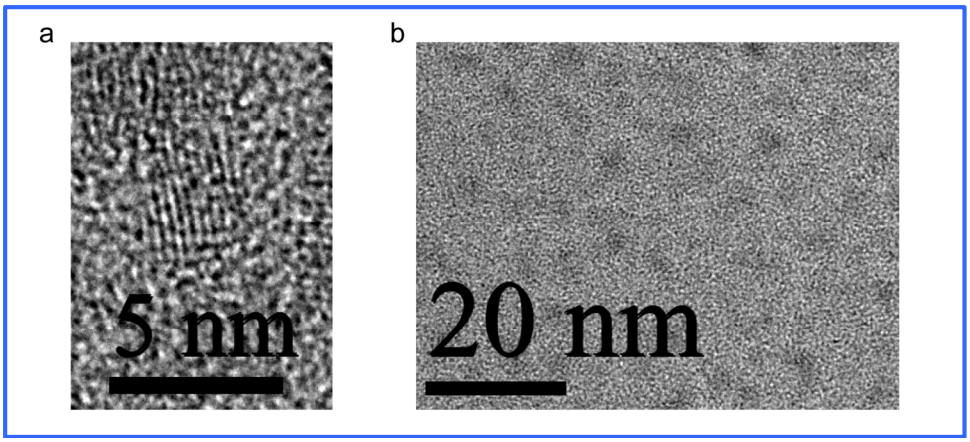

Figure S1. TEM image of CdSe/ZnS core/shell QDs taken using a JEOL 2200-FX analytical high-resolution TEM with a 200kV accelerating voltage. For TEM imaging, 5-10 µL of the QD (at 1 µM in deionized water, 0.25 µm filtered) was deposited onto a 300 mesh Au grid (containing an ultrathin holey carbon support film) (Ted Pella, Inc.) and allowed to air dry. Particle images were visualized and captured using Gatan Digital Micrograph (Pleasanton, CA). The observed average size is 4.3 nm. (a) and (b) represent a high magnification and a low magnification scan respectively of one sample.

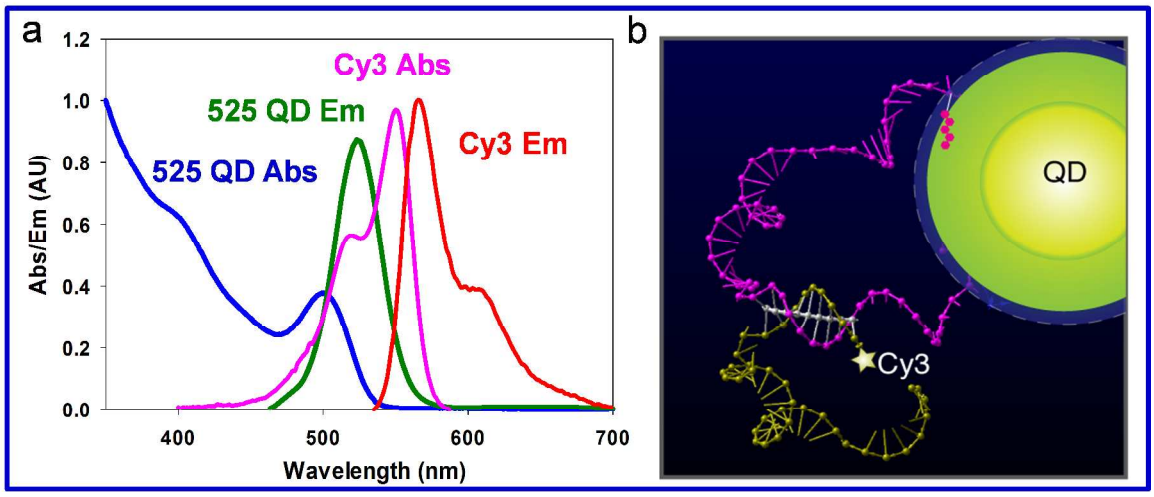

Figure S2. (a) Absorption and emission spectra of the 525 nm QD donor and Cy3 acceptor. (b) Schematic highlighting the correlation of QD-Cy3 FRET interaction with complete DNA cage formation. The cage design allows for T1-pep and T3-Cy3 strands to partially hybridize with each other, which could result in FRET when assembled to the QD irrespective of complete cage formation and QD encapsulation. So we tested this by annealing T1-pep and T3-Cy3 in the presence of QD but without the other constituent strands of the cage. **Figure 5b** shows that only complete cage formation yields highest FRET compared to T1-pep-T3-Cy3 duplex attachment to QD.

FRET to verify non-specific QD attachment to DNA cage. In order to demonstrate that the peptide "handle" on the DNA cage is key to QD binding and encapsulation into the DNA cage, we assembled the cage-QD complex in the absence of $(His)_5$-peptide tag (using T1 without peptide modification along with T2-T5). Figure S3 shows the fluorescence output of samples when excited at 400 nm. In all cases, no Cy3 emission was observed, indicating the absence of non-specific interaction between the QD and cage.

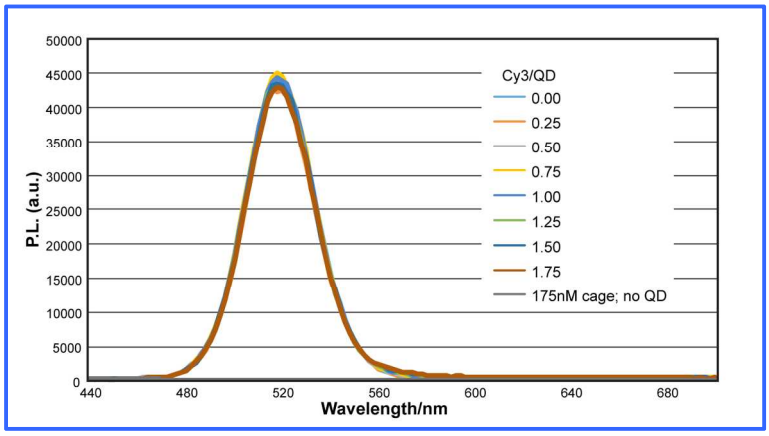

Figure S3. Fluorescence analysis of cage+QD assembly (520 nm CL4 QDs used here) in the absence of $(His)_5$-peptide handle. The series of curves represent increasing ratio of Cy3/QD, where [QD] = 100 nM. Samples were assembled following Route 1 protocol.

Additional data: Fluorescence output of cage-QD assembly following Route 1. In addition to the data shown in Figure 4, Figure S4 shows fluorescence results when the samples were annealed using Route 1 protocol as a function of varying Cy3/QD concentration.

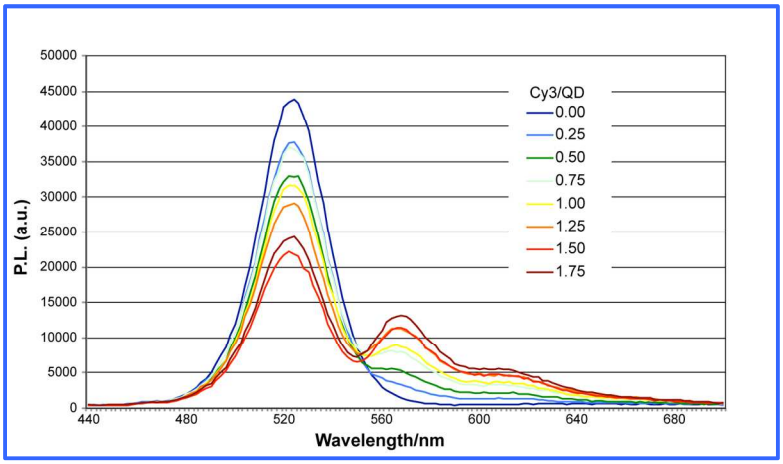

Figure S4. FRET output of cage-QD assembly *via* Route 1. Various curves represent increasing values of Cy3/QD concentration, where [QD] = 100 nM.

Estimating the QD-to-dye distance in the encapsulated cage conformation:

The QD to dye distance was determined by utilizing the following equations and assumptions:

$$E_{app} = \frac{n*(\frac{R_0}{r_{DA}})^6}{1+n*(\frac{R_0}{r_{DA}})^6} \qquad \text{Eq. S1}$$

Where E_{app} is the measured FRET efficiency, n is the number of Cy3 per QD, R_0 is the Forster distance, and r_{DA} is the center-to-center QD-dye distance. Due to the size of the DNA cage and QDs we assume that they would be limited to 1-1 interactions. This eliminated the need for any Poissonian corrections. It is important to distinguish between the E_{app} and the E_{ideal}, which would correspond to a system of 100% encapsulated QDs, by using:

$$E_{app} = n * f * E_{ideal} \qquad \text{Eq. S2}$$

The f factor is the formation efficiency of the cages filled with the desired QD guest. As determined by our AFM images we utilized a value of 0.3 for f. The r_{DA} value determined for the ideal case (QD encapsulated with DNA cage) was 3.9 ± 0.3 nm, which is well in line with our physical estimate which considers each component (~4.7 nm = 2.1 nm for the QD radius + 2.2 nm for dsDNA width + 0.4 nm for the dye linker). Additionally we

obtained the value free from assumptions (f =1) of 6.0 ± 0.1 nm, which is closer than both the no-cage control (see Figure S2, r_{DA} of 6.6 nm) and the geometric limitations imposed by the external binding possibility. This supports the conclusion that a considerable fraction of the structures are fully encapsulated QDs.

AFM analysis of cage only sample. All AFM imaging was performed on Multimode™ under dry tapping mode conditions. On a freshly-cleaved piece of mica (which was mounted on a metal puck), 7 µL of the sample was deposited and allowed to adsorb for 45 sec. Following this, the mica was rinsed by dipping into molecular biology grade water and dried under a stream of Nitrogen gas. Particle height analysis was performed using the Section tool in the Nanoscope™ software. Histogram (Figure S5) was generated using the software R.

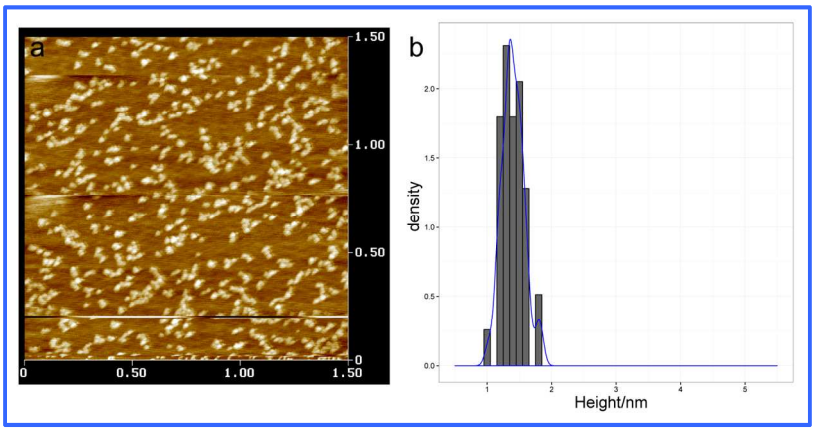

Figure S5. Height analysis of cage only sample. (a) A representative AFM image of cage only sample showing a homogeneous population of particles. (b) Histogram representing particle density across height of particles. N = 40.

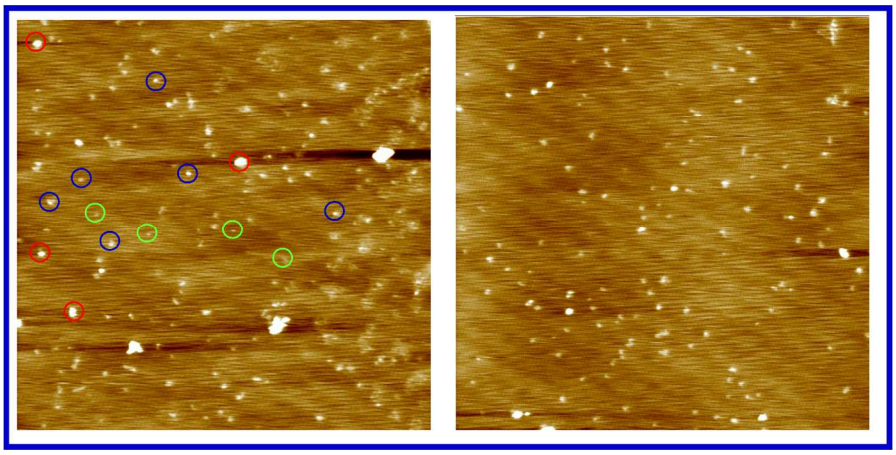

Figure S6. Additional AFM images of Route 1 cage-QD complex assembly. In the left image, particles encircled in red are examples of QD aggregates that were not taken into consideration during height analysis. These correspond to free QDs in the solution-phase ensemble. Particles encircled in blue are a few that were part of the height analysis. The far lighter particles in green circles in the image represent empty cages. Images are 3 µm by 3 µm in size.

Initial QD binding to cage. QDs were exposed to the Cage or Cage controls at different ratios and separated by electrophoresis to visualize changes in mobility based upon binding and confirm binding by the $(His)_5$ motif.

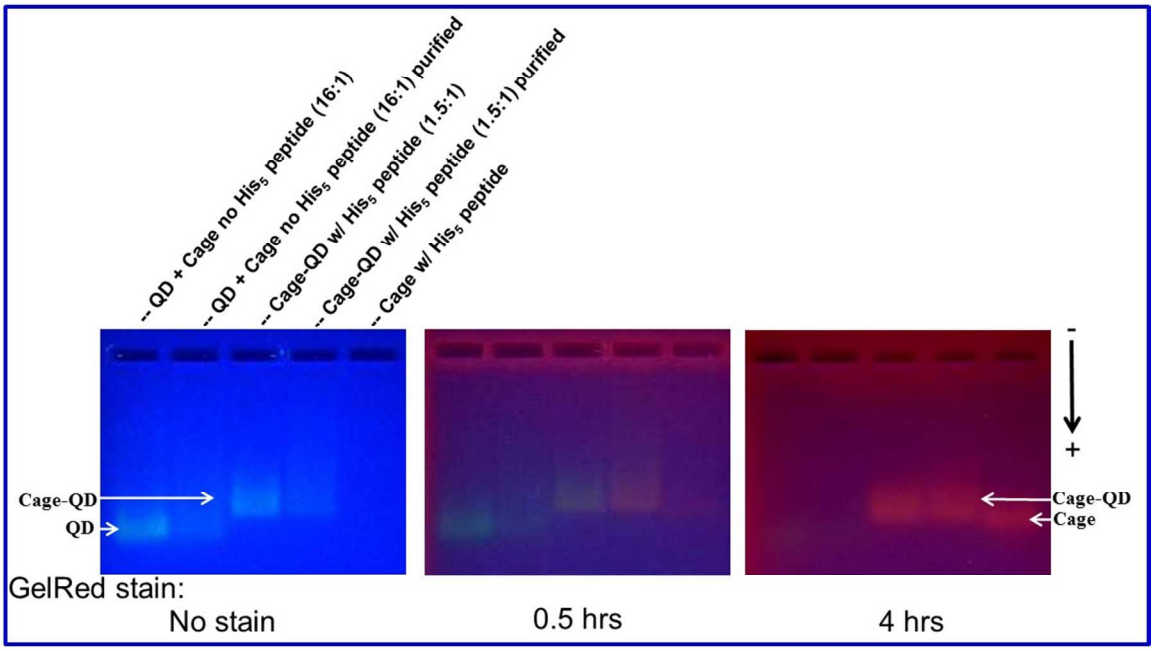

Figure S7. QD binding to the Cage. Gels (2% agarose gel, 1×TAE/12.5mM Mg^{2+}, 4ºC) were run with QDs exposed to Cage with a T1-pep that had the $(His)_5$ or a control DNA only. Samples also underwent purification with a 100 kDa centrifugal filter tube. Gels were imaged by QD PL directly or following GelRed staining for the indicated time periods. Images are identical. The Cage-QD complex has a much slower migration than the QD or cage itself. Direct QD PL was lost after 4 hrs of staining. These results also show that the Cage-QD can be purified with a centrifugal filter tube with the loss of some product.

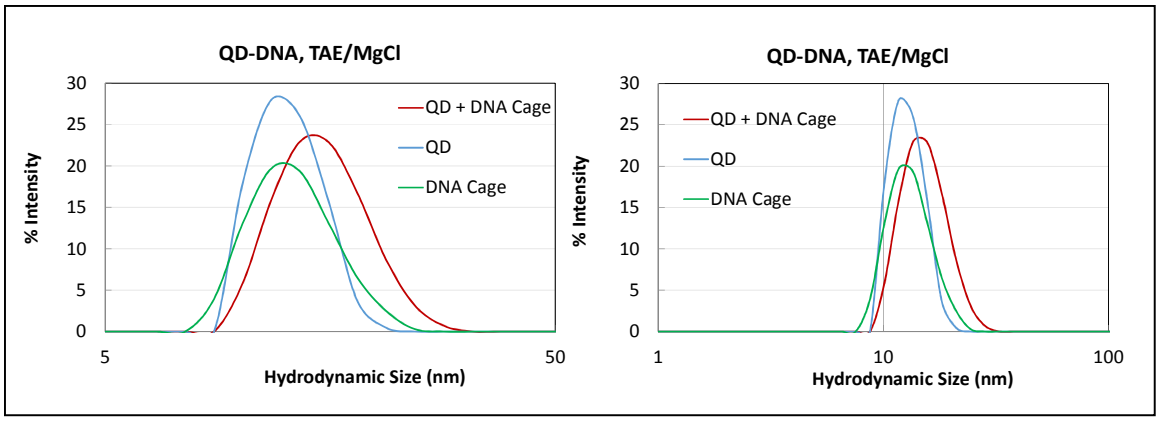

Figure S8. H_D size distribution of cage +/- QD and QD only samples *versus* intensity. Hydrodynamic size is plotted in logarithmic scale and two different sets of scaling are used.

References:

1. Susumu, K.; Oh, E.; Delehanty, J. B.; Blanco-Canosa, J. B.; Johnson, B. J.; Jain, V.; Hervey, W.J.; Algar, W. R.; Boeneman, K.; Dawson, P. E.; Medintz, I.L. Multifunctional Compact Zwitterionic Ligands for Preparing Robust Biocompatible Semiconductor Quantum Dots and Gold Nanoparticles. *J. Am. Chem. Soc.* **2011**, *133*, 9480-9496.
2. Oh, E.; Fatemi, F.K.; Currie, M.; Delehanty, J.B.; Pons, T.; Fragola, A.; Leveque-Fort, S.; Goswami, R.; Susumu, K.; Huston, A.L.; Medintz I.L. PEGylated Luminescent Gold Nanoclusters: Synthesis, Characterization, Bioconjugation, and Application to One- and Two Photon Cellular Imaging. *Particle & Particle Systems Characterization*. **2013**, *30*, 453-466.