Supplementary material (ESI) for Journal of Materials Chemistry

Quantification of quantum dots using phage display screening and assay

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Supplementary information

Quantum Dot Characterization

QDs were characterized through UV-Vis spectrophotometry, fluorescence spectroscopy and gel electrophoresis. Figure 1A, B, C show the absorbance and emission spectra of MAA, MUA and BSA-coated QDs respectively. Since these QDs have identical core, ZnS capped-CdSe quantum dots made from a single batch, they cannot be differentiated by their spectra.

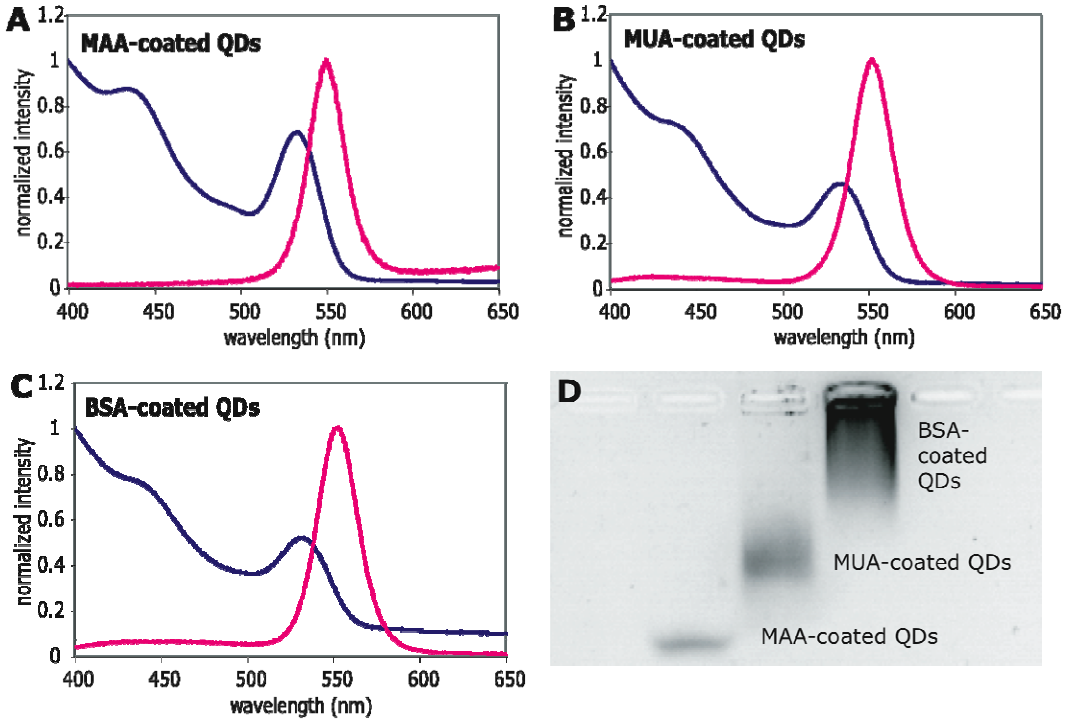

Figure S1 – Spectra and gel electrophoresis of QDs of different surface chemistries. (A) Absorbance and fluorescence spectra of (A) MAA-coated QDs, (B) MUA-coated QDs, (C) BSA-coated QDs are very similar and cannot be used to distinguish the different surface chemistries. (D) Gel electrophoresis of MAA, MUA and BSA-coated QDs indicate that these QDs have different charge to size ratios. (MAA = mercaptoacetic acid; MUA = mercaptoundecanoic acid; BSA = bovine serum albumin)

Gel electrophoresis was done with a 2% agarose gel in 0.5x Tris buffered EDTA (TBE). The gel was subjected to a 100V field in TBE buffer for 30 minutes. The gel was imaged under UV excitation. Figure S1D shows the inverted image

From the image, we can see that MAA-coated QDs traveled furthest, followed by MUA-coated QDs and then BSA-coated QDs. This indicates that MAA-coated QDs have the smallest size to charge ratio,

followed by MUA-coated QDs and then BSA-coated QDs. That the QDs traveled the same direction in the gel indicates that their surface charges are of the same polarity (in this case, negative).

Adsorption of QDs onto polystyrene plate

Affinity-based selection of binding clones from a phage display library necessitates an affinity matrix. Generally, this is a solid substrate onto which the target antigen is affixed. The phage library is incubated with the affinity matrix, allowing clones with desired binding properties to bind. Non-binding clones are then washed away.

Several coatings were tested to enhance the adsorption of QDs onto NUNC microtitre, polystyrene 96-well plate. For gelatin coating, 0.1% [w/v] gelatin solution in water was incubated in each well for 10-15 minutes and removed. For poly-lysine coating, polylysine (Sigma P4707) was incubated in each well for 5 minutes and then aspirated. The plate was then rinsed thoroughly with water and allowed to dry for at least two hours. For BSA-coating, 0.5% BSA in 0.1 sodium bicarbonate buffer, pH 8.6 was incubated in each well for 1 hour at 4°C and then removed.

After coating, the wells were incubated with ~1μM of QDs in bicarbonate buffer (0.1M sodium bicarbonate, pH 8.6) at 4°C. The plates were then washed 6 times with TBST (0.1 % TWEEN) and the fluorescence of each well was measured in a fluorescence plate reader. Since the gelatin-coated plate resulted in the highest fluorescence signals from bound QDs, gelatin was used to coat the plate for all subsequent phage panning and characterization experiments.

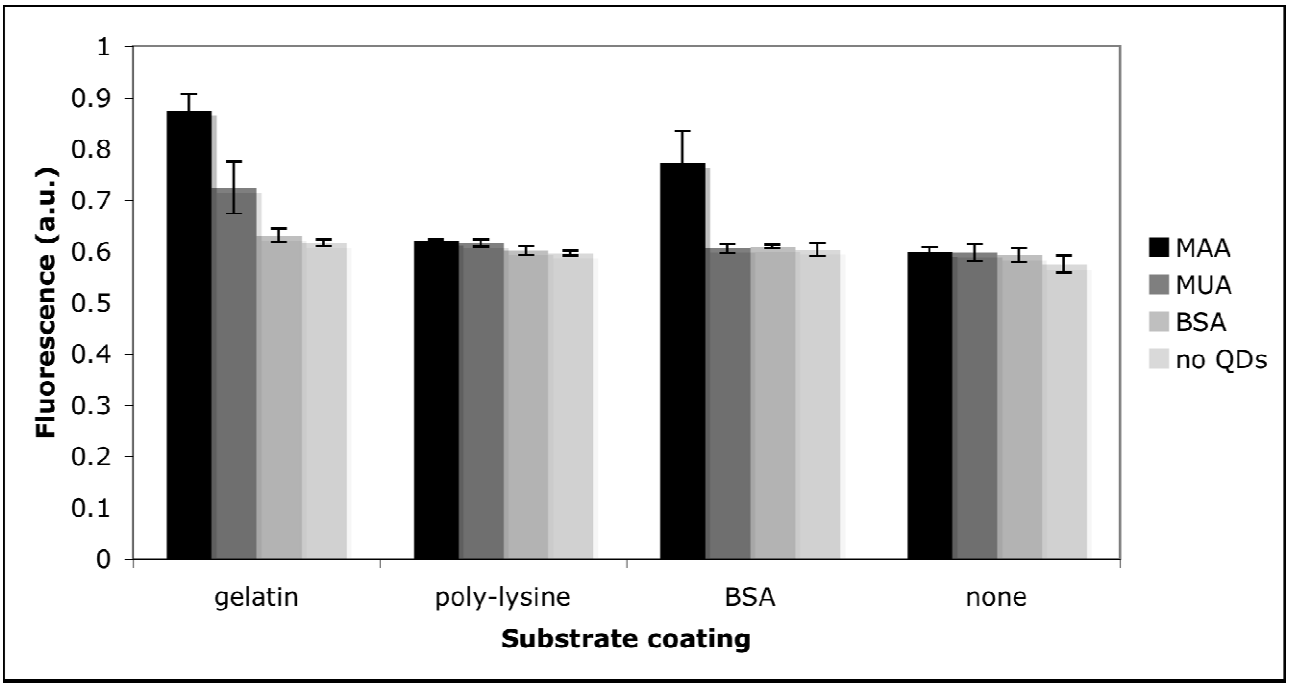

Figure S2 – Fluorescence measurement of MAA, MUA and BSA-coated adsorbed onto coated substrate. Maximum QD adsorption is seen on gelatin-coated plates.

Progress of panning experiment through each round of panning

Table 1 illustrates the progress of the panning experiment on each type of QD. In each round of panning $1x10^{11}$ pfu of phage are incubated with QDs, adsorbed onto a NUNC 96-well plate. Unbound phage are washed away and bound phage are eluted, titered and amplified for use in subsequent rounds. Table 1 shows the phage titer increased significantly between the first and second rounds. This indicates that the population of phage used in the second round of panning had a much higher proportion of phage that bound to the QDs. In the third round of panning, the number of phage eluted decreased in some cases because a more stringent washing technique was used. Table 2 indicates the conditions of the washes used in each round of panning. The length of each wash was increased between round two and three from 10-15 seconds to 1 minute. This likely explains the decrease in the number of phage eluted from MAA-coated QDs and BSA-coated QDs decreasing between the second and third rounds of panning. In all cases, the amount of phage eluted after the third round of panning was at least three orders of magnitude greater than the amount of phage eluted after the first round of panning.

Table S1: # of phage eluted after each round of panning (# input phage = $1x10^{11}$ pfu)

Target	# of phage eluted after 1st round of panning	# of phage eluted after 2nd round of panning	# of phage eluted after 3rd round of panning
MAA-coated QDs	300,000	2,500,000,000	18,000,000
MUA-coated QDs	36,000	900,000,000	3,300,000,000
BSA-coated QDs	40,000	1,030,000,000	95,000,000

Table 2: Conditions of wash used in each round of phage panning.

Conditions	1st round of panning	2nd round of panning	3rd round of panning
Wash buffer	Tris buffered saline*, 0.1% TWEEN-20	Tris buffered saline*, 0.5% TWEEN-20	Tris buffered saline*, 0.5% TWEEN-20
Time for each wash	10-15 seconds	10-15 seconds	1 minute
# washes	10	10	10

*tris buffered saline is 50mM TRIZMA base, 150mM sodium chloride, pH 7.5.

Determination of dissociation constant

The Scatchard plot is used to determine the dissociation constant (K_D) between a receptor and a ligand. In this case, because each bacteriophage has up to five copies of the binding peptide expressed at one tip and the quantum dots have multiple sites at which the peptides might bind, we determined the relative dissociation constant (K_{DRel}) of the phage-QD system. This procedure is modified from the methods described by Dyson et al.

The overall strategy is to mix varying amounts of MAA-QDs and MAA-QD-specific phage and let these mixtures come to equilibrium. This is done in a BSA-coated plate to minimize the binding of free phage to the polystyrene plate. An ELISA is then used to determine the amount of free phage in these equilibrium mixtures. If we know the amount of free phage in each mixture and we know the total amount of phage and QDs that we started with, we can make a Scatchard plot to estimate the relative dissociation constant of the system.

First, a 96-well plate was incubated with a BSA blocking solution (0.5% [w/v] BSA in 0.1M bicarbonate buffer, pH 8.6) at 4°C for at least 1 hour. This coated plate was then washed 6x with tris

buffered saline, 0.1% TWEEN-20 (TBST). In the coated plate, QDs of various concentrations (1nM – 50nM) were incubated with a constant amount of phage (2.5 x 10^{10} pfu/ml) until equilibrium was reached (1 hour at 37°C). These solutions were transferred to plates coated with MAA-coated QDs. Free phage from these equilibrium solutions would bind to the QD-coated plate and be detected by ELISA. To correlate the ELISA signal with an amount of free phage in solution, a calibration curve was made on the same plate.

Varying concentrations of phage were incubated on an MAA-QD coated plate for an hour. The plate washed 6 times with TBST. The plate was then incubated for 1 hour at room temperature with a HRP-anti-M13 MAb conjugate, diluted 1:5000 in BSA blocking solution. The plate was again washed and then developed with the chromogenic substrate, 3,3',5,5'-tetramethylbenzidine. The reaction was stopped with 2 M sulfuric acid. A plate reader was used to measure the absorbance at 450nm minus the absorbance at 570nm.

The relationship between the optical density obtained from the ELISA (OD 450-570nm), and the amount of phage in solution is shown in Figure S3. This plot was used in the second part of the experiment to determine an unknown free phage concentration based on an optical density measurement made through ELISA.

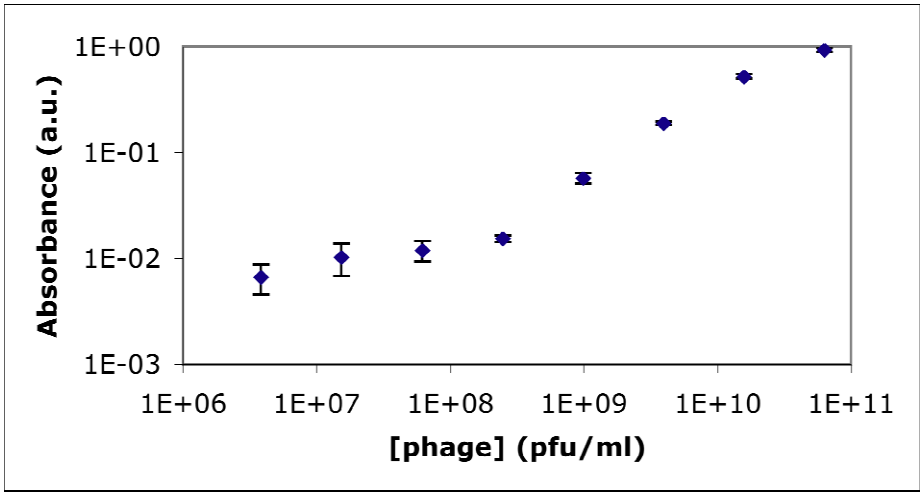

Figure S3 – Standard curve. Correlation of absorbance from ELISA and concentration of free phage in solution. This curve is later used to determine the amount of free phage in various phage-QD mixtures. Error bars indicate the standard deviation (n = 3) (pfu = particle forming units)

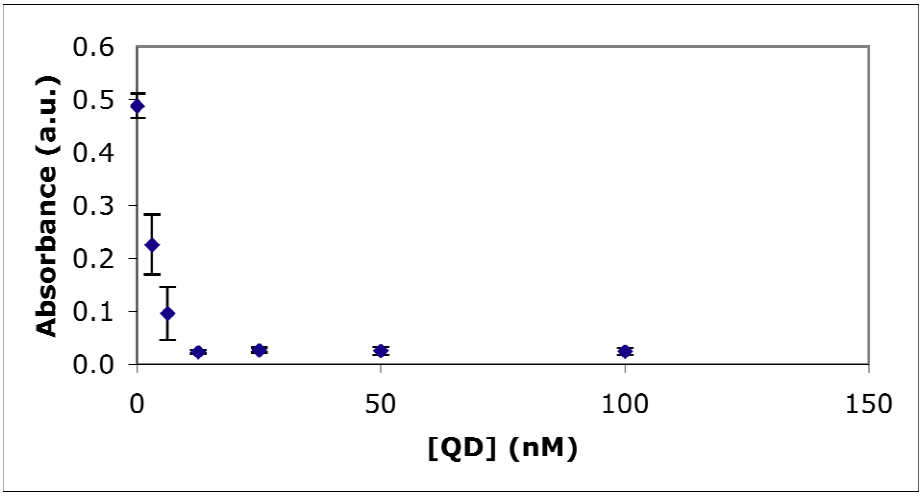

Figure S4 – Competitive assay of phage and QDs. A constant concentration of phage (5 x 10^9 pfu/ml) is incubated in solution with QD solutions of various concentrations (depicted on x-axis). The free phage from these solutions are captured onto QDs adsorbed to a substrate and measured in an immunoassay (reflected in the absorbance measurement on the y-axis). As expected, as the QD concentration increases, more phage are bound to QDs, and less phage are free to bind to the QD-coated plate, leading to a lower OD signal. Error bars indicate the standard deviation (n = 3)

Figure S4 shows the optical density from ELISA obtained from the competitive assay QD-phage solutions. The optical density is related to the amount of free phage in solution, which is inversely related to the QD concentration in the equilibrium phage-QD solutions. At higher QD concentrations, more phage will be bound to the QDs in solution and less phage will be free to bind to the plate.

Using the standard curve on Figure S3, we determine the concentrations of free phage, p, in each solution. The concentration of bound phage, x, is calculated from the mass conservation equation below where p_t is the total phage concentration.

$$x = p_t - p \qquad (1)$$

Assuming that each phage binds to one QD, the number of free QDs, q, is again determined from the mass conservation equation, where q_o is the total QD concentration.

$$q = q_o - x \qquad (2)$$

The Scatchard equation can be written as

$$x/p_t q = (1/K_d) - (x/K_d p_t) \qquad (3)$$

where the slope of the plot of $x/p_t q$ against x/p_t is equal to $-1/K_d$.

Figure S5 shows the plot of $x/p_t q$ against x/p_t. Only three points from figure S4 were transformed and plotted in S5. All other points were too low (in the flat part of the competitive binding curve) or at the y-intercept, where [QD] = 0. The apparent or relative dissociation constant derived from this graph is 5nM ($-1/K_{dRel}$ = - 2 x 10^8, therefore K_{dRel} = 5 x 10^{-9}).

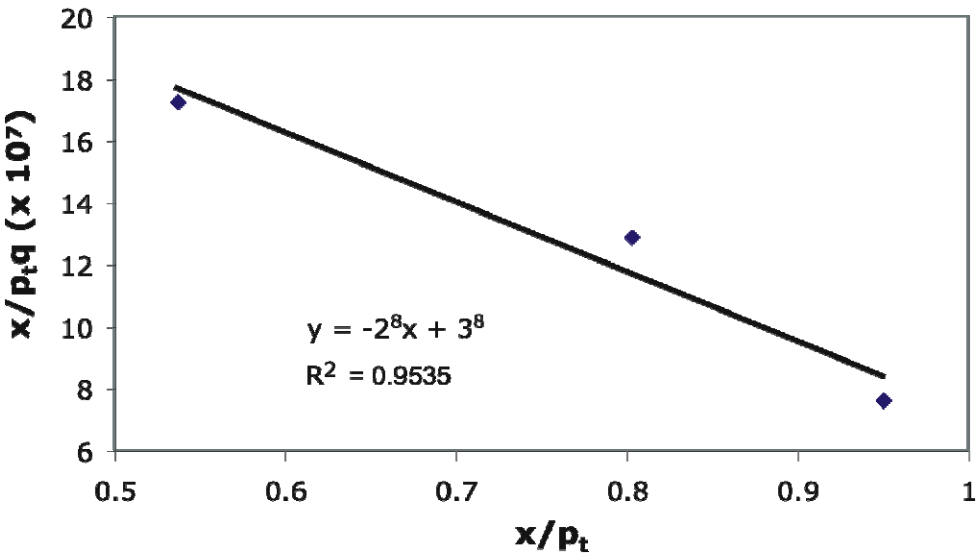

Figure S5 – Scatchard plot. The slope of the Scatchard plot is the negative inverse of the relative dissociation constant. From this plot, $K_{dRel} = 5 \times 10^{-9}$. (x = concentration of bound phage, p_t = total phage concentration, q = concentration of free QDs)