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Are Bidentate Ligands Really Better than Monodentate Ligands For Nanoparticles?

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Abstract

Coordinating ligands are widely used to vary the solubility and reactivity of nanoparticles for subsequent bioconjugation. Although long-term colloidal stability is enhanced by using bidentate coordinating ligands over monodentate ones, other properties such as non-specific adsorption of target molecules and ligand exchange have not been quantified. In this study, we modified a near infrared dye to serve as a highly-sensitive reporter for non-specific binding of thiolated target molecules to nanoparticle surfaces that are functionalized with monodentate or bidentate coordinated ligands. Specifically, we analyzed non-specific binding mechanisms to quantum dots (QDs) by fitting the adsorption profiles to the Hill equation and the parameters are used to provide a microscopic picture of how ligand density and lability control non-specific adsorption. Surprisingly, bidentate ligands are worse at inhibiting adsorption to QD surfaces at low target:QD ratios, although they become better as the ratio increases, but only if the nanoparticle surface area is large enough to overcome steric effects. This result highlights that a balance between ligand density and lability depends on the dentate nature of the ligands and controls how molecules in solution can coordinate to the nanoparticle surface. These results will have major implications for a range of applications in nanobiomedicine, bioconjugation, single molecule spectroscopy, self-assembly and nano(photo)catalysis where both non-specific and specific surface interactions play important roles. As an example, we tested the ability of monodentate and bidentate functionalized nanoparticles to resist non-specific adsorption of IgG antibodies that contained free thiol groups at a 1:1 QD:IgG ratio and found that QDs with monodentate ligands did indeed result in lower non-specific adsorption.

Keywords

Non-specific binding; biocompatible nanoparticles; quantum dots; ligand exchange; surface chemistry; protein labeling

A range of ligands to render nanoparticles water soluble and biocompatible have been developed over recent years, which has led to significant extension of their applications – particularly for colloidal quantum dots (QDs) as fluorescent labels in biophysics and molecular biology.^{1–3} The two most common formulations to render QDs water soluble involve using coordinating thiolated ligands^{1, 4–6} or amphiphilic polymers,^{7–9} although other methods have also been reported.^{10–12} There are advantages and disadvantages to each method and have been extensively discussed in the literature.^{13, 14} The primary advantages

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Supporting Information Available. Mass spectra showing the modification of the dye, absorption and PL spectra of the QD samples before and after ligand exchange, separation of QD-dye conjugates from free dye by size exclusion chromatography, cumulative probability and probability distribution functions for the dyes binding to QDs. This material is available free of charge via the Internet at <http://pubs.acs.org>.

of polymer-functionalized QDs are their long-term colloidal stability and reduced effects of the environment on their optical properties,¹⁵ while thiol-functionalized QDs are usually cheaper and easier to make, require less work-up and, most importantly, result in a smaller colloidal size.¹⁶ This latter property makes ligand-exchanged QDs attractive platforms for advanced biolabeling applications where probe size is a critical issue.

One difficulty in using coordinating ligands is that they can be labile or exchanged with other molecules that coordinate to the QD surface leading to both heterogeneous attachment of biomolecules as well as eventual aggregation of the QDs.^{4, 6} Using thiolated ligands with bidentate or multidentate as opposed to monodentate thiol functionality has been shown to improve the colloidal stability,^{17–19} but other important properties such as non-specific surface adsorption of target molecules have not been as well-studied. Of particular importance is the adsorption of thiol groups to the nanoparticle surface, since cysteine residues are primary targets used for site-specific fluorescence labeling of biomolecules,^{20, 21} but the same reactive group is also the coordinating groups that are used in the water-solubilizing ligands. In this report, we modified a near infrared dye to serve as a highly-sensitive reporter for non-specific adsorption of thiols to nanoparticle surfaces. Monodentate (mercaptopropanoic acid, MPA) and bidentate (dihydrolipoic acid, DHLA) functionalized QDs are used to investigate the effect of ligand coordination configuration on non-specific adsorption of thiols. We performed these experiments with two core-shell QDs that have the same optical properties but with different shell thicknesses to investigate the effects of particle surface area.

A commercially-available amino-functionalized near-infrared dye, Atto 700 amine (Atto-Tec GmbH, Germany), was converted to a thiolated dye (dye-SH) by reaction with SATA (*N*-Succinimidyl S-Acetylthioacetate, Pierce, Thermo, Rockford, IL) (Scheme 1). 0.304 mmol of the amine dye was added to SATA (in 1:20 and 1:100 molar ratios) in 1.5 ml phosphate buffered saline (PBS, Amresco Solon, OH) buffer at pH 7.2, and left to react for 30 minutes. The reaction was monitored by reverse-phase HPLC (Shimadzu Prominence, Kyoto, Japan) on a Supelco Discovery C-18 column (Sigma Aldrich, St. Louis, MO) and MALDI-TOF mass spectrometry (Bruker UltraflexII, Billerica, MA). In figure 1, we show the HPLC traces for Atto 700 before and after the reaction with SATA. The more polar amino-dye (Atto700-NH₂) elutes from the column sooner than the less polar protected thiol (Atto700-S-COCH₃). Mass spectra of the reactant and product are shown in the supporting information. It was found that a molar ratio of 1:100 (dye:SATA) was required to completely convert Atto700 into the protected thiol; the reaction has only ~55% yield at a 1:20 ratio (the ratio recommended by the manufacturer). The dyes were stored in protected form and deprotected immediately prior to use to limit disulfide bond formation in solution. Deprotection of the thioester was performed by reaction with hydroxylamine and Ethylenediaminetetraacetic acid (EDTA, EMD Philadelphia, PA) at pH 7.4 followed by evaporation under reduced pressure, as recommended by the manufacturer. Atto 700 was chosen as the model dye ligand to monitor the non-specific adsorption and/or exchange of thiolated molecules to QD surfaces for 2 primary reasons: first, it absorbs at much lower energy than the QD so that the dye spectrum can be easily separated from the QD spectrum, and second, it is a highly water-soluble, zwitterionic dye, which should reduce electrostatic interactions between the molecule and QD surface⁵ to focus on the thiol coordination chemistry. The ligands on the QD are as short as possible relative to the length of the linker between the thiol and the dye to minimize steric hindrance imposed by the ligand layer so that the thiol adsorption processes can be more easily quantified.

Octadecylamine (ODA)-coated CdSe/ZnS quantum dots were purchased from NN-Labs (Fayetteville, AR) and dissolved in toluene. The absorption wavelength of the excitonic peak was 520 nm (see supporting information), corresponding to a core diameter of 2.5 nm.

TEM images of the thin-shell and thick-shell QD samples (Figure 2 (a) and (b), respectively) were obtained on an FEI TECNAI 200kV electron microscope (Hillsboro, OR) to obtain their overall size and size distribution (figure 2 (c)). Using the first exciton peak position of the absorption spectra to estimate core size, and the TEM images to determine overall size, the shell thickness for each sample was found to be ~3 and ~7 monolayers (ML) respectively.

The native ODA ligands were exchanged with mercaptopropionic acid (MPA, Alfa Aesar, Ward Hill, MA), or dihydrolipoic acid (DHLLA), which had been reduced from DL- α -lipoic acid (TCI, Portland, OR) by reaction with NaBH₄ (Alfa Aesar) and NaHCO₃ (EMD),¹⁷ using a general ligand exchange procedure.²² Briefly, ODA-QDs were precipitated from toluene by the addition of acetone (VWR), centrifuged at 1900g (4000rpm on a Clinical 50 centrifuge, VWR) and the supernatant discarded. DHLLA or MPA was dissolved in methanol, and the solution adjusted to pH 10 by the addition of tetramethylammonium hydroxide pentahydrate (Alfa Aesar). The concentration of QDs was determined from the size-dependent extinction coefficients at the band edge.²³ A molar ratio of $1:3 \times 10^5$ QD:ligand was used for the ligand exchange to ensure as complete a ligand exchange as possible under reproducible conditions. The ligand solution was added to the precipitated QDs and stirred under reflux for 3 hours under argon. Then, the QD-methanol solution was precipitated with a mixture of ethyl acetate and acetone, centrifuged and dissolved in 10 mM phosphate buffer made using Millipore (18.2 M Ω .cm) water. Since the molar ratio of ligand to QD used was in such a huge excess and allowed to exchange for 3 hours at elevated temperatures, this results in the ligand exchange being taken to its thermodynamic equilibrium; performing the process overnight did not improve the ligand exchange. Absorption and photoluminescence (PL) spectra of the QDs with thin and thick ZnS shells before and after successful ligand exchanges are shown in the supporting information. There is a decrease in the PL upon ligand exchange, with MPA showing a stronger decrease than DHLLA. Using a thicker shell resulted in less of a decrease in PL than the thin shell. We used several batches of thin-shell QDs, all with the same specifications (2.5 nm core size, 3 ML of ZnS shell), as well as a batch with the same core, but a thicker shell (7 ML). For some batches of thin-shell QDs, it was observed that upon ligand exchange, significant deep trap state emission was evident in the PL spectra (supporting information), suggesting variations in the quality of the ZnS from batch to batch, and highlights the need for thorough QD characterization prior to their use. We did not use the QDs that showed trap emission for any further experiments here.

Non-specific adsorption of thiolated dye molecules onto the monodentate or bidentate-coordinated QDs was studied by exposing the QDs to the dye at QD:dye molar ratios varying from 1:0 to 1:100 in 10 mM PBS buffer at pH 7.2, consistent with typical bioconjugation conditions. After 2 hours, the unreacted dyes were separated from the QD-dye conjugates using size-exclusion chromatography (PD-10 Column, GE Healthcare), which showed excellent separation (see supporting information) allowing pure QD-dye conjugates to be obtained for quantitative analysis. The conjugates were analyzed by immediately measuring the absorption spectra using a Hitachi 3900H spectrophotometer after separation. Bidentate ligands, such as DHLLA are more strongly attached to the QD,^{17, 24} and are thus expected to show lower affinity for the monodentate thiolated dyes compared to the monodentate MPA-QDs at a given ratio of QD:dye. Figure 3 shows a schematic representation of the ligand exchange and thiolated dye reactions as well as the separation of QD:dye conjugates from unbound dye using size exclusion chromatography, which is measured using the absorption of the QD at 520 nm and the Atto 700 dye at 700 nm. Figure 4 (a) shows the absorption spectra of the QD-Atto 700 conjugates after separation. It is clear that there is an increase in the dye absorption relative to the QD absorption for higher QD:dye ratios, as would be expected. For Atto700, the absorption spectra of the QD and the dye have very little overlap, and they can be easily deconvoluted to provide the

concentration of each species (figure 4 (a) inset). A small correction is needed for the absorbance of the dye at the QD λ_{max} in order to accurately measure the QD concentration. There is a small broadening and shifting of the spectrum of the dye following conjugation, but is small enough to not affect the results. The absorption of the dye relative to the QD allows the number of dyes per QD to be calculated as a function of the ratio initially mixed together. In order to calculate the QD:dye ratio, we used the QD extinction coefficients reported by Yu and Peng,²³ although others have also been reported.^{25–27} While the exact ratio of QD:dye will depend on which absorption coefficient is used, as long as the same value is consistently used for all samples, the observed trends will be the same. Figure 4 (b) shows the relationship between the molar excess of dyes added to the QD and the number of dyes that actually bound for thin-shell and thick-shell QDs, each functionalized with either MPA or DHLA. The data were fit to the Hill equation as follows:

$$y = \frac{L_{\text{max}} \cdot x^n}{K^n + x^n} \quad (1)$$

The excellent fit to the data allowed us to determine the maximum number of dye ligands that can bind, L_{max} , relative binding strength, K , and the Hill coefficients, n , which are listed in table 1. Figure 4 (b) and table 1 suggest that the effects of both the ligand coordination geometry and the QD surface area were significant. For thin-shell (lower surface area) QDs, there is surprisingly very little difference of the total number of thiolated dyes that can bind per QD between the MPA-QDs and the DHLA-QDs, saturating at about 8-9 dyes/QD. For thick-shell QDs, this difference is much larger, with MPA-QDs showing a maximum of ~30 dyes:QD while DHLA-QDs bind about half as many, ~15. There are also differences in the binding strengths, with MPA-QDs having higher K values (lower affinities) than the equivalent DHLA-QDs, opposite to our original hypothesis. The Hill coefficients for DHLA-QDs are lower than for MPA-QDs, suggesting mechanistic differences in the binding to MPA-QDs and DHLA-QDs. Figure 4 (c) shows the average fraction of added dyes that bound to each QD, together with the Hill equation fits, providing an alternative view of the data in terms of a binding probability, with the mechanistic differences depending on ligand coordination geometry becoming more evident. The fits of probability are calculated using the Hill equation parameters in table 1 to compute the number of dyes attached as a function of the number of dyes added (varying from 0 to 500) and determining the bound/added fraction. It can be seen that the shapes of the curves in figure 4 (c) depend on the ligand coordination geometry but not on the QD size, and highlights the important role of the thiol coordination for the probability of the thiol to adsorb on the QD surface. The highest probability of binding occurs at low dye:QD ratios for DHLA-QDs while for MPA-QDs, the highest probability is at much higher dye:QD ratios.

Figure 4 showed that the maximum number of thiolated dyes that can bind to QDs (L_{max}) only becomes significantly different between monodentate- and bidentate-functionalized QDs for larger surface area QDs. For DHLA-QDs, the K values and the Hill coefficients, n , are similar whether small or large QDs are used, while they are different for MPA-QDs. It has been discussed that, as the number of potential binding sites increases, the Hill coefficient increases above 1 for both sequential and independent binding mechanisms, and whether the binding shows positive, negative or no cooperativity.²⁸ Comparing L_{max} to n allows us to postulate the possible mechanistic differences in thiols binding to DHLA-QDs compared to MPA-QDs. n approaches L_{max} only when the binding is sequential and there is high positive cooperativity. For example, it was found that for 10 binding sites, n never exceeds 2.1 for sequential binding or 1.4 for independent binding when there is no cooperativity, and is even less when there is negative cooperativity.²⁸ It must also be noted that when L_{max} is larger than about 6 and the binding is independent, even positive

cooperative binding shows a Hill coefficient less than 2 and decreases weakly with the number of binding sites. For MPA-QDs, $n = 3.14$ and $L_{max} = 8.97$ for smaller QDs and $n = 2.08$, $L_{max} = 29.70$ for larger QDs indicates a degree of sequential binding with some positive cooperativity, which is stronger for the smaller QDs than for larger QDs. For DHLA-QDs, the Hill coefficient between 1.14 and 1.45 and L_{max} between 7.70 and 14.65 is more indicative of negative cooperativity, although it is more difficult to distinguish between sequential and independent binding. At this point, it is important to make a cautionary note on the difference between the values of K in table 1 and the often-reported dissociation constants, K_d , for ligand binding. For $n > 1$, in the case of marked strong cooperativity, $K_d = K^n$. However, since this is not the case here, one must be extremely careful in extracting K_d values for binding of coordinating species to QD surfaces and, likely, for nanoparticles in general.

The mechanistic differences in binding were particularly evident from plotting the probability of dyes to bind as a function of the number of dyes added (figure 4(c)). It is clearly seen that the probabilities are strongly dependent on the monodentate or bidentate nature of the initial QD ligands, while they are not so dependent on the QD size. The probabilistic aspects of the Hill equation in physicochemical equilibrium applications has been previously examined,²⁹ and is further explored in the context of QD ligand exchange in the supporting information. Taken together, these data allow to us postulate a microscopic view of the binding and exchange mechanisms present for each type of ligand coordination. The fact that thiolated dyes bind to DHLA-QDs more readily at low dye:QD ratios than MPA-QDs may be related to the non-linear geometry of DHLA over linear MPA resulting in a lower packing density of DHLA on the QD, thus allowing the first thiolated dye molecules to bind without having to remove the original ligands. As the surface area increases, dye ligands can bind even easier at lower dye:QD ratios from having more potential binding sites available. For the more densely-covered MPA-QDs, the ligands must undergo an exchange process even at low ratios of dye:QD. As more dye ligands are added to the QDs, both types of thiol ligand must now be exchanged, which is easier for the monodentate MPA-QDs than the bidentate DHLA-QDs. However, once the first dyes have bound to the MPA-QD, the positive cooperativity highlights that subsequent dye ligands can bind more easily. This suggests that the binding of the first dyes opens up additional binding sites by facilitating the dissociation of additional MPA ligands, possibly by rotational collisions of the large, bound dye with other MPA ligands, followed by surface ligand rearrangement which opens up additional binding sites and thereby increases the probability of additional dyes to bind. These mechanistic differences are highlighted in figure 5.

As the QDs reach the maximum number of dyes that can bind, L_{max} , the bidentate nature of DHLA limits the total ligands exchanged, although this difference is small for thin-shell QDs that have a total average diameter of 4.3 nm (surface area 58.1 nm²). For thick-shell QDs, with a total average diameter of 6.8 nm (surface area 145 nm²) the difference in L_{max} is significant; twice as many thiolated dye ligands can bind to MPA-QDs than DHLA-QDs. For smaller QDs, the similar saturation level suggests that the limitation is not the number of binding sites available but steric effects from the adsorbed species. The structure of Atto 700 is not published, but the molecular weight of the amine-dye ion, 608 g mol⁻¹, together with the general structure of long wavelength oxazine dyes,³⁰ and taking into account the linker moiety allows us to postulate a conservatively high estimate of the footprint of the dye to be ~6 nm² (i.e. ~ 2 nm × 3 nm). The L_{max} value for the thin-shell QDs of 7–8 dyes per QD is therefore in general agreement with a sterically-limited exchange. Similarly, for the thick-shell QDs, the L_{max} for MPA-QDs is 26, which is also in general agreement with steric limitations. The lower L_{max} for thick-shell DHLA-QDs compared to MPA-QDs, indeed highlights that the bidentate nature of the ligand provides reasonable protection to extensive ligand exchange, but only after the already-available surface sites have been taken up.

Monodentate ligands will more thoroughly exchange with the thiols in solution, until the surface becomes sterically saturated. It is interesting to note that the fact that 2 thiol bonds must be broken to allow a single monodentate ligand to bind reduces the L_{max} by a factor of 2, compared to a simple monodentate-for-monodentate ligand exchange. It will be interesting to see how this scales with larger nanoparticle sizes and thicker shells. One possibility that must be considered is that different shell thicknesses may result in different amounts of Cd-to-Zn on the QD surface, due to imperfect shelling. The surface atom ratio may indeed result in different affinities to ligand functional groups, as previously shown for core-only CdTe.²² Quantifying this ratio for core-shell QDs is more difficult due to the lack of techniques that probe surface atoms without interference from internal atoms, but will lead to a more thorough understanding of non-specific binding mechanisms, and will be the focus of future studies.

A major driving force behind this study was to understand the role of the ligand coordination geometry in preventing non-specific adsorption of thiolated biomolecules in order to optimize site-specific labeling strategies of proteins using QDs. The use of a thiolated dye as a spectroscopic probe allowed us to focus specifically on quantifying the role of coordination geometry in thiol binding and exchange mechanisms. Biomolecules are far more complicated, and the size, shape and pI of the biomolecule and pH of the solution are all expected to play a role. Nevertheless, we tested if our model on the role of monodentate vs bidentate ligands can be directly applied to proteins containing free thiols in the form of reduced cysteine groups. We partially reduced a dye-labeled immunoglobulin G (IgG) antibody containing 3 dyes/antibody (AlexaFluor 700 Goat Anti-mouse IgG, A21036, Life Technologies, Carlsbad, CA) by mixing 13.2 nmol of TCEP with 0.66 nmol of IgG in 50 μ L buffer (100 mM Na₃PO₄, 0.15 M NaCl, 10 mM EDTA, pH 7.2) and reacting for 2 hours at 37 °C. We separated reduced IgG from unreduced IgG using a 100kDa Nanosep centrifugal filter (Pall, Port Washington, NY) for 5 mins at 5000 rpm. The blue-colored solution from the reduced dye-labeled antibody passed through the membrane, while the unreduced antibody (also blue) remained above the filter. This unreduced antibody was re-diluted in additional TCEP/buffer solution (50 μ L), and left to react for 2 more hours at 37 °C. Then, the solution was passed through another round of filtration and the second filtrate was combined with the first. Binding to QDs was evaluated by adding 0.1 nmol of the reduced antibody to 0.1 nmol of MPA-QD or DHLA-QD in 100 μ L of buffer and left for 2 hours at room temperature in the dark. Unbound antibody was separated from QD-antibody conjugates using the 100 kDa centrifugal filter, first by increasing the volume to 220 μ L with buffer then centrifuging for 90 s at 5000 rpm. Approximately 170 μ L of the solution containing free antibody passed through the filter, leaving 50 μ L of the QD-antibody conjugate solution above the filter. This was diluted to 200 μ L and another round of centrifugation was completed, to ensure as much of the unbound antibody as possible was removed. For each sample, the solution was diluted back to 200 μ L and the absorption spectra were measured, as shown in figure 6. We found that performing more than 2 rounds of separation resulted in aggregation of the QDs, particularly the MPA-QDs. However, under these conditions, any free antibody will have been reduced by a factor of ~17 compared to the QD-antibody conjugates, and thus will not interfere with the assay. It is clear from the absorption spectra that the same concentration of MPA-QDs and DHLA-QDs remained in solution but a smaller dye peak (from the dye-labeled antibody) was observed for the MPA-QDs than for the DHLA-QDs. Specifically, DHLA-QDs contained 35% more antibody per QD than MPA-QDs, highlighting that, at low QD:target ratios, our model for thiol binding to monodentate and bidentate-QDs holds for thiolated biomolecules, although other factors such as size and electrostatics are likely to play a role as well.

In summary, we thiolated a near infrared absorbing dye reporter to use as a spectroscopic probe to examine non-specific binding of thiol groups to monodentate (MPA)- and bidentate

(DHLLA)-coated QDs. Surprisingly, we found that, at low dye:QD ratios, DHLLA-QDs are worse than MPA-QDs at inhibiting adsorption of thiol ligands, but are better as the dye:QD ratio increases. For small QDs, both types of QDs are able to accommodate approximately the same maximum number of thiolated dye ligands per QD, suggesting steric limitations. As the surface area of the QD increases, bidentate ligands limit the number of ligands than can be exchanged, thus preventing excessive non-specific adsorption. Parameters extracted from fitting the data to the Hill equation were used to postulate mechanistic differences in the exchange and binding of thiols to QDs with these two common but different types of surface passivations. We tested this model on a reduced IgG antibody and found that, at 1:1 QD:IgG ratios, monodentate ligands did indeed reduce non-specific binding to QDs compared to bidentate ligands. Therefore, to answer the question posed in the title of this manuscript, if it is required to inhibit binding of single molecules at low concentration, such as for labeling proteins for single molecule assays or the ultrasensitive detection of biomarkers, monodentate ligands such as MPA are likely better ligands to render the QDs biocompatible, whereas if limiting the total non-specific adsorption of biomolecules at high concentrations is the major requirement, such as for labeling overexpressed proteins in cells, bidentate or multidentate ligands such as DHLLA are probably better suited. This conclusion may be applicable to other fields of interest in the nano community. For example, if QDs, or nanoparticles in general, are to be used in catalytic applications, the lower ligand density of bidentate ligands such as DHLLA may allow for more catalytically-active sites to be exposed to the substrate at low concentrations, but for higher conversion rates at higher substrate concentration, the more labile monodentate ligands such as MPA may allow the QD to become more catalytically active, although it may also reduce colloidal stability. Finally, controlling the ligand coordination geometry on nanoparticles together with the concentration of self-assembling ligand connectors added to solution may allow for more control over the final geometry of assembled nanostructures by controlling the number of active sites for such ligand connectors to coordinate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
HPLC chromatograms of the unmodified (amino-functionalized) and modified (thioester functionalized) Atto700 dye.

Figure 2. TEM images of (a) thin shell and (b) thick shell QDs and (c) their size histograms.

Figure 3.

Schematic representation of the ligand exchange of octadecylamine (ODA) QDs with monodentate (MPA) and bidentate (DHLA) ligands and the non-specific binding assay of the thiolated dye to each QD surface, followed by separation of unbound dye from the QD-dye conjugates by size exclusion chromatography. The QD-dye conjugates were eluted from the column within 2–4 ml while the free dye was only eluted after 7 ml.

Figure 4.

(a) Absorption spectra of QD-dye conjugates after separation of the unbound dyes by size-exclusion chromatography. (b) Ratio of dyes bound to each QD as a function of the ratio added to the solution together with fits to the Hill equation. (c) Plot of the fraction of dyes added to the solution that was found to bind to each QD sample.

Figure 5.
Schematic representation of the proposed mechanistic differences in thiolated dye molecules binding/exchanging to DHLA-QDs and MPA-QDs.

Figure 6.

Absorption spectra of partially-reduced IgG, labeled with AlexaFluor 700, non-specifically bound to MPA-QDs (black) and DHLA-QDs (red) that were initially mixed at a 1:1 IgG:QD ratio and separated by ultrafiltration.

Scheme 1.

Table 1

Parameters of thiolated dyes binding to QDs from fitting to the Hill equation

	$L_{\max}$	K	n
Thin-Shell-MPA	8.97 ± 0.15	26.21 ± 0.73	3.14 ± 0.20
Thin-Shell-DHLA	7.70 ± 0.48	19.85 ± 2.66	1.45 ± 0.18
Thick-Shell-MPA	29.70 ± 1.37	52.89 ± 3.08	2.08 ± 0.13
Thick-Shell-DHLA	14.65 ± 0.49	23.55 ± 1.85	1.14 ± 0.05