Supporting information

Effects of Nanoparticle Surface Ligands on Protein Adsorption and Subsequent Cytotoxicity

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Fig. S1 Size histogram of DPA-QDs (A) and MSA-QDs (B) extracted from several TEM images. The QDs fit to a Gaussian size distribution centered at 4 nm with a standard deviation of 0.60 nm.
Fig. S2 FT-IR spectra of DPA-QDs (curve a) and MSA-QDs (curve b).
Fig. S3 (A) Stern–Volmer plots for the quenching of HSA by DPA-QDs (a) and MSA-QDs (b) at different concentration. (B) Lineweaver-Burk plots for the quenching of HSA by DPA-QDs (a) and MSA-QDs (b) at different concentration.
Fig. S4 Fluorescence emission spectra of DPA-QDs (A) and MSA-QDs (B) in the absence (solid line) and presence of HSA (dot line) with 4h incubation time.
Fig. S5 Confocal images of Hela cells after incubation 30 min with DPA-QDs (A), HSA-DPA-QDs (B), MSA-QDs (C) and HSA-MSA-QDs (D). (E) Mean fluorescence intensity of QDs in Hela cells. Scale bar: 30 μm.
Synthesis of CdSe/ZnS Core/Shell QDs:

Briefly, CdO (102.8 mg), stearic acid (1.2 g), trioctylphosphine oxide (TOPO) (3.35 g) and hexadealamine (HAD) (1.65 g) were mixed and heated to 300 °C under Ar flow. A solution of Se (64 mg Se dissolved in 4 mL tributylphosphine (TOP)) was swiftly injected into the hot solution, and aliquots were taken 1-2 mL into cool toluene. The resulting CdSe QDs were purified and precipitated using acetone, and finally redissolved in CHCl₃.

For CdSe/ZnS QDs, zinc acetate and sulfur were used as precursors. The CdSe core solution was mixed with 6 mL OLA and heated to 150-170 °C. Then, the Zn and S precursors were added dropwise to reaction mixture and stirring 15 min. The resulting CdSe/ZnS QDs were purified and precipitated with acetone, and finally stored in CHCl₃.

Surface Ligand Exchange with DPA and MSA:

In order to synthetize water-soluble QDs, a 40 mg of DPA (or 80 mg of MSA) was dissolved with 15 mL of 2-propanol (15 mL of a 1:1 (v/v) methanol/dioxane for functionalization with MSA) and the pH was adjusted to 12-13 with tetramethylammonium hydroxide pentahydrate (TMAHP). Then 0.25 mg of OLA-coated CdSe/ZnS QDs was added and the reaction mixture was left stirring at 60-70 °C under a nitrogen atmosphere for 10-20 min. After the reaction was stopped, the exchanged hydrophilic QDs were purified with ethyl acetate. The pellet was centrifuged out and redispersed in PBS.

Calculations of ligand density per QDs

Packing density $\sigma_{PD}$ was calculated based on the ratio of the mass fractions of S and Zn and defined as:

$$\sigma_{PD} = \frac{n_{LP}}{n_{NP}SA_{NP}}$$ (S1)

where $n_{LP}$ and $n_{NP}$ are the number densities of ligands and QDs, respectively, and $SA_{NP}$ is the surface area per QDs. Since the ligand contains one S atom, the value of $n_{LP}$ is obtained from the followig:
\[ n_{LP} = \frac{w_s(LP)\rho_{\text{sample}}N_A}{M_s} \]  

(S2)

where \( \rho_{\text{sample}} \) is the density of the sample suspension, \( N_A \) is the Avogadro constant, and \( M_s \) is the molar mass of S, \( w_S \) (LP) is the S mass fraction of the ligand, which is obtained from the following equation:

\[ w'_s(LP) = w'_s(T) - \frac{w_{Zn}(NP)M_{Zn}}{w_{Zn}(NP)M_{Zn}} = \frac{w'_s(T)M_{Zn} - w_{Zn}(NP)M_{Zn}}{M_{Zn}} \]  

(S3)

where \( w_{Zn} \) (T) is the total S mass fraction density, which originate in the ligands and QDs, and determined using ICP-AES-MS. \( w_{Zn} \) (NP) is the S mass fraction of QDs determined using ICP-AES-MS. Similar to \( n_{LP} \), the value of \( n_{NP} \) is:

\[ n_{NP} = \frac{w_{Zn}\rho_{\text{sample}}N_A}{M_{Zn}\theta} \]  

(S4)

where \( w_{Zn} \) is the Zn mass fraction, \( \rho_{\text{sample}} \) is the density of the sample suspension, \( N_A \) is the Avogadro constant, \( M_{Zn} \) is the molar mass of Zn, and \( \theta \) is the number of Zn atom per QDs. Substituting Eqs. S2 and Eqs. S4 into Eq. S1 and rearranging yields

\[ \sigma_{PD} = \frac{w_s(LP)M_{Zn}}{w_{Zn}M_sSA_{NP}} \]  

(S5)

where \( w_s \) (LP) is the S mass fraction of ligands, \( w_s \) (T) is the total S mass fraction, \( w_s \) (NP) is the S mass fraction of QDs, \( M_{Zn} \) and \( M_s \) is the molar mass of Zn and S. For DPA-QDs and MSA-QDs, the ratio of packing density of ligand (N) is expressed as:

\[ N = \frac{\sigma_{PD}(DPA)}{\sigma_{PD}(MSA)} = \frac{w'_s(DPA)M_{Zn}}{w_{Zn}(DPA)M_sSA_{NP}} \times \frac{w_{Zn}(MSA)M_sSA_{NP}}{w'_s(MSA)M_{Zn}} \]  

(S6)

where \( \sigma_{PD} \) (DPA) and \( \sigma_{PD} \) (MSA) are the packing density of DPA and MSA ligands, \( w_s \) (DPA) and \( w_s \) (DPA) are the S mass fraction of DPA and MSA ligands, and \( w_{Zn} \) (DPA) and \( w_{Zn} \) (MSA) is the Zn mass fraction of DPA-QDs and MSA-QDs.

Substituting Eqs. S5 into Eqs. S6 and rearranging yields
where \( w_S (T-DPA) \) and \( w_S (T-MSA) \) is the total S mass fraction of DPA-QDs and MSA-QDs, \( M_{Zn} \) and \( M_S \) is the molar mass of Zn and S, \( w_{Zn} (DPA) \) and \( w_{Zn} (MSA) \) is the Zn mass fraction of DPA-QDs and MSA-QDs.

In this work, the values of \( w_S (T-DPA) \) and \( w_S (T-MSA) \) is 72.5 μg g\(^{-1}\) and 89.90 μg g\(^{-1}\), respectively, and the value of \( w_{Zn} (DPA) \) and \( w_{Zn} (MSA) \) is 7.47 μg g\(^{-1}\) and 10.11 μg g\(^{-1}\), determined using ICP-AES-MS. The following values can be used in Eq. S7:

\[
N = \frac{w_z (T - DPA) M_{Zn} - w_{Zn} (DPA) M_S}{M_{Zn}} \times \frac{w_{Zn} (MSA)}{w_z (T - MSA) M_{Zn} - w_{Zn} (MSA) M_S} \times w_{Zn} (DPA) \\
= \left[ \frac{w_z (T - DPA) M_{Zn} - w_{Zn} (DPA) M_S}{w_z (T - MSA) M_{Zn} - w_{Zn} (MSA) M_S} \times w_{Zn} (DPA) \right]
\]

The DPA/MSA ratio on the surface of QDs is around 1.09, equal to 1.0, indicating that the amount of DPA and MSA grafted on QDs is the same. Hence, it is reasonable to compare the two systems.