Thermodynamic Implications and Time Evolution of the Interactions of Near-Infrared PbS Quantum Dots with Human Serum Albumin

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ABSTRACT: Near-infrared (NIR)-emitting PbS quantum dots (QDs) are endowed with good stability, high quantum yield, and long lifetime in the body, so they are promising agents in biological imaging. They quickly form the so-called “protein corona” through nonspecific adsorption with proteins in biological fluids once upon exposure to the biological system. Here, PbS QDs and human serum albumin (HSA) were selected as the model system. Fluorescence quenching spectroscopic studies indicated a static quenching process caused by the addition of PbS QDs, which was corroborated by the UV–vis absorption spectroscopy and fluorescence lifetime. Thermodynamic parameters were obtained by the fluorescence quenching method. The enthalpy change and entropy change were well correlated with the “enthalpy–entropy compensation” (EEC) equation summarized in this work. The slope ($\alpha = 1.08$) and the intercept ($T\Delta S_0 = 34.44$ kJ mol$^{-1}$) indicated that the interaction resembled a protein–protein association. The both negative signs of enthalpy change and entropy change were elucidated by a proposed “two-step association–interaction” (TSAI) model. Agarose gel electrohoresis (AGE) and dynamic light scattering (DLS) showed that the binding ratio was roughly 2:1 (HSA/QDs), resembling sandwich-like structures. Furthermore, the secondary structure of HSA depended on the concentration of added QDs and the incubation time. The results preliminarily uncovered the physicochemical properties of QDs in the presence of proteins and elucidated the role of time evolution. These will inspire us to make the fluorescent QDs more biocompatible and use them in a proper way.

INTRODUCTION

Near-infrared light is endowed with deeper tissue penetration than visible light.1,2 In recent years, near-infrared fluorophores, including some small organic molecules3,4 and fluorescent proteins,5,6 have been widely used in the field of biological imaging and even in vivo imaging. In comparison, inorganic semiconductor quantum dots (QDs), including PbS,7 PbSe,8 AgS,9 AgSe,10 etc., have attracted greater interest for their advantages of high photostability and high quantum yields. As to PbS QDs, the syntheses are more mature and simpler, the quantum yields are higher, and the costs are lower. The synthetic methods in organic solvents, however, the quality of the PbS QDs synthesized in the water phase was not good enough, especially in terms of quantum yield and colloidal stability. Therefore, organic phase syntheses followed by phase transfer into aqueous solutions still accounted for the majority of synthetic methods of PbS QDs.

Different from organic molecules, when nanomaterials enter the human body, they cannot be quickly excreted from the kidney due to their nanoscale particle size.21 They can exist in the body for a long time, which is also conducive to the long-term biological imaging. As reported previously,21 when nanoparticles enter the organism, they undergo nonspecific binding with proteins in body fluids within a short period of time to form a “protein corona,” and this binding effect depends on the charges, sizes,22,23,24 shapes,23 and surfaces25 of the nanoparticles. The formed protein corona is critical for the biological effect of nanomaterials after entering the organism.
Therefore, it is of vital importance to study the interactions of nanoparticles with proteins and the formation of protein corona. To date, some studies have reported the interactions between proteins and nanoparticles, such as carbon dots, magnetic nanoparticles, and quantum dots. These articles used various methods, including fluorescence spectroscopy, UV–vis spectroscopy, electrochemistry, etc., to study the process in depth to obtain driving forces, structural influences, and the thermodynamic parameters, which can explain in principle the interactions. These studies found that the interactions depended on surface ligands, surface charges, and particle sizes. Recently, we briefly summarized the interactions of QDs with proteins. Nevertheless, there are still great challenges, e.g., the interpretation of thermodynamic parameters on a molecular basis. Their connections with the driving forces are still lacking. The interaction models are not yet established from a thermodynamic perspective.

To address these challenges, PbS QDs and human serum albumin (HSA) were used as a model system. According to the Vroman effect, nanoparticles first interact with abundant proteins after entering the body, while HSA is the most abundant protein in human blood. Studying the interaction processes between nanoparticles and HSA will help better understand the formation of protein corona and their biological effects. First, we used fluorescence spectroscopy to study the thermodynamic parameters and the driving forces. Next, we used dynamic light scattering (DLS) and agarose gel electrophoresis (AGE) to determine the binding ratio between the two. Finally, we explored the effect of incubation time on the secondary structure of HSA by synchronous fluorescence and circular dichroism (CD) spectroscopies. In addition, according to the reported “enthalpy–entropy compensation” equation for the interactions between small organic molecules and proteins, the related literature studies on interactions between inorganic nanoparticles and HSA or similarly structured bovine serum albumin (BSA) were summarized. We also proposed a “two-step association–interaction” (TSAI) model for the interactions between nanoparticles and proteins, which highly benefited the elucidation of the interaction process and driving forces. It will be of great help to further understand the principle of protein corona formation after the near-infrared nanoparticles enter the organism and to design near-infrared nanoparticles with better biocompatibility for biological imaging in the future.

RESULTS AND DISCUSSION

Synthesis and Characterization. First, we synthesized oleic acid (OA)-capped PbS QDs. By controlling the reaction temperature and time, we successfully obtained quantum dots with fluorescence emission wavelengths ranging from 1200 to 1600 nm (Figure S1). The maximum emission wavelength was limited by the detection limit of the instrument. In addition, according to the literature, the stability of QDs with too large particle sizes would be poor. Among them, the absolute quantum yield of the as-synthesized QDs was between 25 and 55% (Table S1), and the quantum yield increased with decreasing particle size, which was consistent with the previous literature studies. In addition, there was a vibrational absorption peak of water at ∼1450 nm, which would affect the biological imaging effect. Based on the above considerations, we used PbS QDs with an emission maximum at ∼1300 nm for subsequent experiments.

The hydrophobic QDs can be transferred into the aqueous solutions by a phase-transfer process (Figure S2). The size distribution and particle size of the PbS QDs dispersed in the organic phase and the aqueous phase were characterized by TEM (Figure 1). The particle size of OA-modified PbS QDs was relatively uniform, with an average particle size of 4.79 ± 0.36 nm (Figure 1a,b); the average particle size of GSH-modified PbS QDs was 4.23 ± 0.39 nm (Figure 1c,d). The difference might be caused by a small amount of etching on the surface during the phase transfer. In addition, according to the
HRTEM results, it could be seen that the crystal structure of the QDs was not greatly affected during the ligand exchange process, and the (100) lattice of the GSH-modified PbS QDs was still clearly visible (inset in Figure 1c).

In addition, we also recorded the absorption and emission spectra of OA- and GSH-modified PbS QDs (Figure 2). These two spectra were similar, except for a slight emission peak shift from 1300 to 1280 nm, presumably due to the reduction of the particle size caused by etching. This was corroborated with the TEM results. Based on the above, it could be proved that the phase transfer had less influence on the structure of the QDs. According to the FTIR spectra of GSH QDs (Figure S3), the peak of the sulfhydryl group disappeared, indicating that most of the excess free GSH was removed during purification by ultrafiltration.

Both OA-modified PbS QDs and GSH-modified PbS QDs showed good colloidal and optical stability. The absorption and fluorescence spectra of the two QDs did not change significantly within several weeks, and no visible precipitation occurred. According to the zeta potential results (Figure S4), it could be seen that the GSH-capped PbS QDs had no obvious changes within 2 weeks, which proved that they had good stability. OA-capped PbS QDs had even better stability, and they could be stored for months.43 The good stability of PbS QDs was probably due to the surface passivation by excess Cl ions incorporated during synthesis, which was consistent with the previous studies.17,44,45

**Visualization of the Interaction of PbS QDs with HSA.** After negatively staining HSA after incubation with PbS QDs, we could observe the complex by TEM (Figure 3). After adding QDs, the protein particles became larger, exhibiting the sign of agglomeration. From the area indicated by the yellow arrows in Figure 3, it could be seen that most of the proteins were distributed around the PbS QDs, which resembled a sandwich-like structure. In addition, free QDs without protein adsorption were still found. According to our studies and the previous literature, the binding constants of smaller nanoparticles with proteins were smaller than those of larger nanoparticles, and the nonspecific adsorption would be less.28,29,36,46 In addition, we used the AFM to directly visualize the complex after the interaction (Figure 4). The heights of well-dispersed QDs and HSA were ~4 and ~3 nm, respectively (Figure 4b,d). In contrast, the height increased to ~10 nm in the mixture suspension of QDs and HSA (Figure 4f). This also indicated that the protein had more obvious agglomeration after adding PbS QDs, confirming the TEM results.

**Thermodynamics of the Interactions of PbS QDs with HSA.** In order to study the driving forces of the interactions between PbS QDs and HSA, we first obtained the thermodynamic constant of the process in the hope of analyzing the process from a more basic thermodynamic perspective. First of all, when PbS QDs were gradually added to HSA, the fluorescence of HSA appeared to be quenched significantly (Figure 5a). However, excess GSH (150-fold) had almost no effect on the intrinsic fluorescence of HSA (Figure S5), indicating that the fluorescence quenching was caused by
the QDs rather than the free GSH. In addition, according to the component content of fetal bovine serum (FBS), we knew that the content of serum albumin was about 0.024 g mL\(^{-1}\). Therefore, we performed another experiment to measure the

**Figure 4.** AFM images of QDs (a), HSA (c), and complex (e). (b, d, f) Corresponding height differences. [HSA] = 0.2 \(\mu\)M; [PbS QDs] < 0.1 \(\mu\)M.

**Figure 5.** (a) Quenching of protein fluorescence by PbS QDs at 298 K. The fluorescence quenching results at 302, 306, and 310 K are presented in Figures S7−S9, respectively. (b) Stern−Volmer plots. (c) Modified Stern−Volmer plots. (d) Van’t Hoff plots.
Figure 6. (a) UV–vis absorption spectra of HSA and [HSA + PbS QDs]-PbS QDs. [HSA] = 2 μM; [QDs] = 2 nM. (b) HSA and complex fluorescence lifetimes. [HSA] = 2 μM; [QDs] = 1.5 μM.

Table 1. Binding Constants and Thermodynamic Parameters of PbS QDs and HSA

| T (K) | $K_{SV}$ (L mol$^{-1}$) | $R^2$ | $K_0$ (L mol$^{-1}$) | $R^2$ | $\Delta G$ (kJ mol$^{-1}$) | $\Delta H$ (kJ mol$^{-1}$) | $\Delta S$ (J mol$^{-1}$ K$^{-1}$) |
|-------|------------------------|------|----------------------|------|----------------------|----------------------|----------------------|
| 310   | $3.56 \times 10^7$     | 0.999| $2.11 \times 10^7$   | 0.999| $-43.40$            | $-53.55$            | $-32.66$            |
| 306   | $3.60 \times 10^7$     | 0.997| $2.59 \times 10^7$   | 0.999| $-43.56$            | $-43.72$            | $-43.81$            |
| 302   | $3.68 \times 10^7$     | 0.998| $3.75 \times 10^7$   | 0.999| $-43.56$            | $-43.72$            | $-43.81$            |
| 298   | $4.08 \times 10^7$     | 0.997| $4.73 \times 10^7$   | 0.995| $-43.56$            | $-43.72$            | $-43.81$            |

The thermodynamic parameters obtained using eqs 3 and 4 are shown in Table 1. The both negative enthalpy change and
entropic change clearly demonstrated that the interaction was
enthalpically driven. According to the Ross theory,49 it could
be inferred that the main driving force between HSA and PbS
QDs might be hydrogen bonds and/or van der Waals forces.

In order to verify the accuracy of the data, we learned from
the “enthalpy–entropy compensation” (EEC) equation of the
interaction between small molecules and serum albumin38 and
referred many literature studies to summarize the thermody-
amic parameters of the interactions between nanoparticles
and serum albumins. Thermodynamic parameters (ΔH and
ΔS) in 25 references were included for an EEC plot (37 data
points, Table S3). There was a linear relationship between ΔH
and TΔS (Figure 7).

![Figure 7. Enthalpy–entropy compensation between nanoparticles
and serum albumins. T = 298 K.](image)

The linear fitting resulted in eq 5.

$$TΔS \text{ (kJ mol}^{-1}\text{)} = 1.08 \times ΔH \text{ (kJ mol}^{-1}\text{)} + 34.44 \quad (5)$$

Taking ΔH and ΔS in Table 1 into eq 5, the deviation
obtained was within the acceptable range, which further
verified the accuracy of the empirical formula. Therefore, eq 5
would provide a reference for the interactions of nanoparticles
with serum albumins. The slope and the intercept were very
close with the results reported by Rotello et al. (1.07 and 35.2
kJ mol\(^{-1}\)) in their work.50 In Rotello’s work, they found that
the interactions of gold nanoparticles with α-chymotrypsin
were similar to the protein–protein associations. In combina-
tion with this work, the interactions of nanoparticles with proteins mostly resembled the protein–protein associations,
presumably due to the nanoscale sizes of nanoparticles. The
slope and the intercept empirically reflected the confor-
mental change and the desolvation upon complex formation,
respectively. From the EEC equation, it could be expected that
large conformational changes and extensive desolvation would
happen in the nanoparticle–protein associations.

To further explain this thermodynamic process, we proposed a
“two-step association–interaction” (TSAI) model, as
illustrated in Scheme 1. When proteins and nanoparticles
were present in the same circumstances, they were close to
each other due to electrostatic or hydrophobic interactions
and tended to combine to reduce surface energy. This process
would be spontaneous since the nanoparticles were heteroge-
neous, so they preferred association with other species to
reduce surface energy. This nature was quite different from the
protein–ligand interactions. According to the Kauzmann–
Tanford concept of hydrophobic interactions, water near the
surface of hydrophobic groups is more structured than bulk
water, and entropy dominates the favorable free energy of
hydrophobic interactions since association of two nonpolar
surfaces causes the release of structured water molecules near
nonpolar surfaces.51–53 To be noted, the “hydrophobic
interactions” herein mostly represented the desolvation of
nanoparticles and proteins rather than the interactions of
hydrophobic amino acids of proteins with hydrophobic ligands
on the surface of QDs.

In the first step, association and desolvation occurred. In this
process, the proteins and QDs underwent associations by
mutual penetration of their hydration layers, so the hydration
layers on the surface of both of QDs and proteins were
destroyed and the solvent-accessible surface areas were buried,
leading to a positive enthalpy change (ΔH\(_f\) > 0). Meanwhile,
this would lead to the disorder of structured water molecules
and thus a positive entropy change (ΔS\(_f\) > 0). So, step I was an
entropically driven process. In the following step II, when the
two species got closer, the combination of the two was more
stable presumably due to the van der Waals and possible
hydrogen bonding interactions. Moreover, Chen et al. reported
that gold nanorods (AuNRs) and two-dimensional MoS\(_2\)
nanosheets (MoS\(_2\) NSs) could interact with proteins via
metal–sulfur bonds by synchrotron radiation X-ray absorption
near-edge structure (XANES) and X-ray absorption spectro-
copy (XPS) characterizations.54,55 Hence, since the proteins
contained –SH, –S=–S=, –COOH, –NH\(_2\) etc., metal–S,
metal–O, or metal–N bonds might be formed from the

**Scheme 1. Two-Step Association–Interaction (TSAI) Model**

\[\text{A: Individually Hydrated Species} \quad \text{B: Associated Complex} \quad \text{C: Interacted Complex}\]

This scheme does not depict the real sizes and conformations of proteins and nanoparticles. Semiconductor quantum dots are usually smaller than most of proteins. For clarification, this scheme only shows the possible interaction of one nanoparticle with one protein. The interacted complex (C) will probably undergo structural rearrangements and conformational changes in the evolution time period. However, the time scale (>12 h) exceeds the experimental time for thermodynamic studies (1–3 h). Thus, the time evolution of C is not presented here but it is investigated and discussed in the following sections.
In contrast to the covalent bonds mentioned above, previous studies showed that hydrogen bonds could be formed in the buried region from which solvent molecules were excluded. In addition to the covalent bonding and nonbonding interactions (van der Waals and possible hydrogen bonding), the translational and rotational motions of the ligands on the surface of QDs would be restricted. These all led to a negative entropy change (ΔS < 0). Therefore, for the entire process, ΔH = ΔH₁ + ΔH₂, and ΔS = ΔS₁ + ΔS₂. For instance, if ΔH₁ outweighed ΔH₂, it resulted in a negative ΔH; if ΔS₂ outweighed ΔS₁, it resulted in a negative ΔS. This was suitable for the explanation of both negative ΔH and ΔS in this work. The contributions to the favorable Gibbs free energy were attributed to both steps, namely, favorable ΔS₁ and ΔH₁. Herein, the favorable enthalpy was offset by the unfavorable entropy in step II. The positive or negative signs of ΔH and ΔS depended on the types of proteins, surface ligands, sizes of nanoparticles, ionic strength, etc. As to the molecular basis, the −COOH and −NH₂ groups of GSH on the surface of QDs and the −SH, −S−S−, −COOH, and −NH₂ groups of proteins should play vital roles in the interactions like the formation of metal−X (X = S, O, and N) covalent bonds, hydrogen bonds, and van der Waals interactions (dipole−dipole force, dipole-induced dipole force, and London dispersion effect).

According to preliminary experiments, we knew that when QDs were gradually added to HSA, their hydrodynamic diameter changed to different degrees and finally reached stability. The Hill equation was often used to calculate the binding constant. The hydrodynamic diameters were brought into the modified Hill equations as follows.

\[
\frac{d - d_0}{d_{\text{max}} - d_0} = \frac{1}{1 + \left(\frac{K_D}{[\text{QDs}]_0}\right)^n}
\]  

(6)

Herein, \(K_D\) represents the dissociation constant. \(n\) represents the Hill coefficient, which was an important indicator of association synergy. \([\text{QDs}]\) represents the concentration of PbS QDs. \(d\) represents the hydrodynamic diameter measured at a given QD concentration, and \(d_{\text{max}}\) represents the maximum hydrodynamic diameter obtained by adding excess PbS QDs. By fitting with eq 6, the binding constant \(1/K_D\) obtained was \(5.17 \times 10^6\) L mol\(^{-1}\) (Figure 8). Taken together with the binding constant obtained by the fluorescence quenching method, it could be concluded that the binding constant was at a magnitude of \(10^5\)−\(10^7\) L mol\(^{-1}\). The deviation was likely caused by different techniques. In our previous work, we found that the binding constant of Au nanoclusters (\(\sim 2\) nm) to HSA was at a magnitude of \(10^5\) L mol\(^{-1}\), clearly demonstrating the size dependence of nanoparticles when interacting with proteins. Larger nanoparticles would have more surface ligands engaging in the interactions with proteins. In addition, the Hill coefficient \(n > 1\) indicated that the interaction was a positive synergistic process.

**Binding Ratio of PbS QDs to HSA.** The agarose gel electrophoresis (AGE) and dynamic light scattering (DLS) were further used to determine the binding ratio of PbS QDs to HSA. Agarose gel electrophoresis is based on the difference in molecular weight and charge, so species can be separated under the action of an electric field. The components with smaller molecular weight move faster, while the components with larger molecular weight move slower. The molecular weight becomes larger after the QDs are tightly bound with the protein, which is obviously different from the unbound components. We first formulated QDs and proteins of known concentrations and prepared different mixtures according to different concentration ratios. When QDs were incubated with HSA for 3 h, their affinity with HSA was low, evidenced by the still presence of free QDs when the ratio of [HSA]/[QDs] even reached 4:1 (Figure 9a). As indicated in the literature, the interactions of nanoparticles with proteins highly depended on the incubation time, so the incubation time for AGE was extended to 12 h (Figure 9b). In the presence of increasing concentrations of HSA, the electrophoresis speed of QDs slowed down. When the ratio of [HSA]/[QDs] reached 2:1, almost all the free QDs were combined with HSA, demonstrating a binding ratio of 2:1 (HSA/QDs).

The hydrodynamic diameter referred to the particle size with the hydration layer when the nanoparticles entered the aqueous solutions. According to the formulas developed by Röcker et al. and Dominguez-Medina et al., the following formula was proposed.

\[
V_{\text{HSA}} = kV_{\text{QDs}}
\]

(8)

Here, \(V_{\text{QDs}}\) represents the measured hydrodynamic diameter when different concentrations of QDs are added into the HSA solution, \(d_{\text{QDs}}\) represents the hydrodynamic diameter of PbS QDs, and \(k\) is the ratio of the hydrodynamic volume of HSA and PbS QDs determined by dynamic light scattering. \(V_{\text{HSA}}\) and \(V_{\text{QDs}}\) represent the hydrodynamic volumes of HSA and QDs, respectively. The hydrodynamic diameters of HSA and QDs are shown in Figure S10. The experimental results are shown in Figure 10. Based on the above experimental results, the binding ratio of quantum dots to proteins was 1:2, which agreed well with the AGE result. When \([\text{QDs}]_0/\text{[HSA]}\) approached \(-0.5\), the hydrodynamic diameter reached saturation, which also confirmed the binding ratio. Therefore, statistically speaking, one PbS QD was combined with two HSA molecules.

**Conformational Changes of Proteins in Time Evolution.** In order to investigate the effect of quantum dots on the conformation of proteins, we performed synchronous fluorescence and circular dichroism experiments. We could study the effect of quantum dots on the microenvironment of amino acid residues in proteins by synchronous fluorescence. The difference between the
emission wavelength and the excitation wavelength (Δλ) was set as 15 and 60 nm, respectively, to focus on the luminescent amino acid residues in the protein. First, HSA was titrated with QDs within the time duration of ∼1 h ([PbS QDs] = 0−0.011 μM). When Δλ was 15 nm, it indicated the influence on the microenvironment around tyrosine residues, but there was only a very minor shift of ∼2 nm, which also indicated that QDs had little impact on the microenvironment of tyrosine residues of HSA in the presence of a small amount of PbS QDs (Figure S11a). When Δλ was 60 nm, it indicated the effect on the microenvironment around tryptophan residues. The results showed that the peak position had almost no change, indicating that QDs had essentially no effects on the microenvironment around tryptophan residues in the protein (Figure S11b). Next, HSA was incubated with QDs overnight (∼12 h). At this time, the concentration of QDs was increased to 0.45 μM ([HSA] = 5 μM). This concentration indicated a high ratio of [QDs]/[HSA] (∼1:10) since it was normally in the range of 1:1000−1:100 in the biomedical applications (the concentrations of QDs and HSA are usually in the scale of nM to μM and mM, respectively). When the concentration of QDs and the incubation time increased, we found that the microenvironment of amino acid residues changed to a certain extent (Figure 11). In detail, the emission maximum with Δλ = 15 nm was redshifted for ∼5 nm (Figure 11a and the blue solid line in Figure 11c), indicating that the influence on the microenvironment around tyrosine residues was obvious. On the contrary, the emission maximum with Δλ = 60 nm was still not remarkably shifted (Figure 11b and the yellow solid line in Figure 11c), indicating that QDs would not affect the microenvironment around tryptophan residues. Then, we used molecular simulations to analyze the surface conformation and charge distribution of HSA (Figure S12). Since the QDs were negatively charged, they should be prone to bind to the positively charged regions of the protein. According to previous reports on the interactions between nanomaterials and HSA,63,64 the binding site was likely to be located at the triangular face of HSA.

Circular dichroism spectroscopy could analyze the changes in the secondary structures of proteins, which provided more accurate information about the effect of PbS QDs on the conformation of proteins. For the circular dichroism spectrum of HSA, the results were consistent with the results reported in the literature.65 When HSA was incubated with QDs overnight (∼12 h), the secondary structure was altered (Figure 12a). In addition, further studies demonstrated that it was concentration-dependent (Figure 12b). A ratio of [QDs]/[HSA] below 1:100 caused no effects. The negative peak at 208 and 222 nm represented the two characteristic peaks of α helix due to the Cotton effect. With the increase in the concentration of QDs and the incubation time, the α helix in HSA gradually decreased, and the corresponding β sheets and random coils would increase to a certain extent. It would lead to structural changes in HSA. This reminded us that we can obtain high biocompatibility of QDs using as low concentrations as possible in their biomedical applications. In addition, less circulation time in vivo would result in higher biocompatibility as well.

As indicated in the previous literature, time evolution of protein corona took place after the association of proteins with nanoparticles.60 There might be a consecutive process in the formation of hard corona and soft corona. It was found that the hydrodynamic sizes of the QDs/HSA system were gradually increased when the incubation time was extended from 0 to 12 h (Figure 13). When the ratio of QDs/HSA was 1:100, protein agglomeration (or protein clusters) might form (Figure 13a), consistent with the TEM and AFM characterizations (Figures

![Figure 9](image-url) Agarose gel electrophoresis image after incubation for (a) 3 h and (b) overnight (∼12 h). The red numbers indicate the ratio of HSA to PbS QDs. [QDs] = 0−6.5 μM; [HSA] = 0−26 μM.

![Figure 10](image-url) Hydrodynamic diameters of HSA in the presence of QDs. N means that N HSA molecules are bound to one PbS QD. [HSA] = 5 μM; [QDs] = 0−4 μM.

![Figure 11](image-url) Synchronous fluorescence spectrum of HSA incubated with QDs for 12 h with Δλ = 15 nm (a) and Δλ = 60 nm (b). The emission maximum of HSA in the presence of different concentrations of PbS QDs with Δλ = 15 nm and Δλ = 60 nm (c). The solid lines represent titration without incubation. [PbS QDs] = 0−0.45 μM; [HSA] = 5 μM.
3 and 4, respectively). In this condition, another possible reason was attributed to the hard corona and soft corona (vide infra). Given the spherical shape of QDs, the intensity \( I \) of light scattering was proportional to the radius \( r \) of QDs to the sixth power, i.e., \( I \propto r^6 \). Thus, the DLS intensity would obviously increase in the case of a small amount of protein agglomeration induced by even 1% QDs. In contrast, when the ratio of QDs/HSA was 1:1, it seemed that the protein agglomeration was alleviated (Figure 13b). When QDs and proteins were mixed, they were first randomly combined together by an intrinsic property of lowering surface energy of colloidal nanoparticles, stimulated by the so-called hydrophobic and possible electrostatic interactions (proteins, as a whole or the subunits, and colloidal nanoparticles are essentially charged). Then, there were covalent bonding, relatively weak van der Waals, and possible hydrogen bonding interactions (depend on the surface ligands) at the interface between the QDs and proteins. The above process is depicted in Scheme 1 in a thermodynamic perspective. In the time evolution (incubation overnight for instance), the interactions became stronger and thus resulted in structural changes and conformational rearrangements. Previous studies reported that conformational rearrangement was much slower than the association process,\(^{66,67}\) which was also found in the time evolution herein. The binding mode was similar to the three-step model reported in the previous literature.\(^{68}\) As a result, hard corona was formed. In addition, the hard corona might be surrounded by free proteins, namely, the soft corona.\(^{21}\) The interaction process is illustrated in Scheme 2. The binding ratio of HSA/QDs was statistically determined as 2:1 (Figures 9 and 10). This did not exclude other stoichiometries, e.g., 3:2. In fact, the PbS QDs (4–5 nm) were smaller than HSA (\( \sim 8 \) nm). The conventional model of protein corona was no longer suitable since it essentially depicted that a large nanoparticle (>10 nm) was associated with tens to hundreds of proteins. Therefore, the QDs and proteins should form protein complexes, in which one protein molecule might be associated with >1 QD.\(^{28}\) Although the binding ratio between HSA and QDs was 2:1, resembling the sandwich-like complex (Scheme 2), this can still be included in the concept of the protein complex as well.\(^{68}\) As illustrated in Figure 3, there were QDs surrounded by proteins but also free QDs. As the sizes of nanoparticles increase, the association constants of nanoparticles with proteins will increase, along with greater

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**Figure 12.** (a) Circular dichroism spectra of HSA incubated with QDs for 2, 6, and 12 h. [HSA] = 5 \( \mu \)M; [PbS QDs] = 0.1 \( \mu \)M. (b) Circular dichroism spectra of HSA (5 \( \mu \)M) incubated with different concentrations of QDs (0–0.1 \( \mu \)M).

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**Figure 13.** DLS results with different incubation times. Each line is the mean of three parallel experiments. (a) [HSA] = 5 \( \mu \)M; [QDs] = 50 nM. (b) [HSA] = 2.5 \( \mu \)M; [QDs] = 2.5 \( \mu \)M.

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**Scheme 2. Schematic Diagram of the Interaction Model of HSA and PbS QDs\(^{a}\)**

“This scheme does not depict the real sizes and conformations of HSA and PbS QDs. As indicated by our experimental results, the binding ratio was approximately 2:1 (HSA/QDs), resembling the sandwich-like complex. However, this scheme does not exclude other binding ratios.
influences on the secondary structures of proteins.\textsuperscript{29,33,69} It inspired us to make the biomedical nanomaterials as small as possible in the consideration of alleviating the nonspecific protein adsorption.

\textbf{CONCLUSIONS}

In this work, PbS QDs with good stability and fluorescence properties were synthesized in the organic phase and were successfully transferred to the aqueous phase by ligand exchange. The as-synthesized GSH-capped PbS QDs had an emission at \( \sim 1300 \, \text{nm} \) in the NIR-II window. The interaction of the PbS QDs with HSA was studied by the fluorescence quenching method. The obtained thermodynamic parameters were well correlated with the “enthalpy–entropy compensation” (EEC) equation in the nanoparticle/serum albumin system. The negative enthalpy change was offset by the negative entropy change, demonstrating the favorable enthalpy contributed to the free energy. To elucidate the thermodynamic implications of the interactions, a “two-step association interaction” (TSAI) model was proposed. (i) Step I: association and desorption were driven by the hydrophobic and electrostatic interactions. (Notably, “hydrophobic” here was referred to the dehydration at the interface between a nanoparticle and a protein molecule.) (ii) Step II: the interaction was driven by the covalent bonding, van der Waals, and hydrogen bonding forces. The specific interaction types depended on both of the nanoparticles (especially the surface ligands) and the proteins. Based on the both negative signs of enthalpy change and entropy change, the interaction of PbS QDs with HSA was probably attributed to the covalent bonding, van der Waals, and hydrogen bonding forces. According to the fluorescence spectroscopic and DLS techniques, the binding constant was found to be of magnitude \( 10^{6} \text{–} 10^{7} \, \text{L} \, \text{mol}^{-1} \), larger than the binding constant of AuNCs (\( \sim 2 \, \text{nm} \)) to HSA (\( \sim 10^{3} \, \text{L} \, \text{mol}^{-1} \)), owing to the larger size of PbS QDs (4–5 nm). In addition, the binding ratio of HSA/QDs was approximately 2:1 as determined by gel electrophoresis and dynamic light scattering. According to synchronous fluorescence and circular dichroism spectroscopic studies, it was found that the QDs had an impact on the secondary structure of HSA with high concentrations of QDs and the extension of the incubation time. However, the secondary structure of HSA was essentially not influenced by low concentration of QDs and short incubation time, providing a fundamental guidance to strengthen the biocompatibility of QDs. This work will contribute to the synthesis, applications, and biocompatibility of QDs.

\textbf{EXPERIMENTAL SECTION}

\textbf{Materials.} All reagents were used without further purification. Sublimed sulfur (S, 99.5%), sodium hydroxide (NaOH), hydrochloric acid (HCl), ethanol, and \( n \)-hexane were purchased from Sinopharm Chemical Reagent Co. (China). Lead chloride (PbCl\(_2\), 99%) was obtained from Shanghai Shishewei Chemical Co. HSA was obtained from Sigma-Aldrich. Oleic acid (OA, 85%), oleylamine (OAm, 80–90%), and glutathione were purchased from Aladdin. Ultrapure water with 18.2 M\( \Omega \) cm (Millipore Simplicity) was used in all experiments.

\textbf{Synthesis and Phase Transfer of Oleic Acid-Modified PbS QDs.} In this experiment, we used a simple synthetic method reported by Weidman et al.\textsuperscript{43} with a slight modification. Sulfur powder (0.08 g) and OAm (7.5 mL) were added to a 10 mL centrifuge tube, and the mixture was ultrasonically dispersed for 15 min. PbCl\(_2\) (10.0 g) in OAm (30 mL) was added into another flask. The Schlenk method was used to maintain the vacuum environment. The reaction solution was heated to 100 \(^\circ\)C and cooled down to 80 \(^\circ\)C after 10 min, and then 5 mL of the as-prepared S precursor was injected into the flask. After heating for 10 min, 80 mL of cold hexane was added into the flask, and then it was cooled down to room temperature with an ice-water bath. Twice the volume of OA was added and centrifuged. Finally, the precipitate was dissolved in hexane. The product was purified twice by centrifugation by adding half the volume of ethanol and stored at room temperature.

The phase-transfer process followed a method reported by Deng et al.\textsuperscript{42} The OA-PbS QD solution was prepared in chloroform. The concentration of GSH aqueous solution was adjusted to 0.3 M, and the pH was adjusted to 5.0 with sodium hydroxide. The two solutions were mixed and thoroughly stirred in a glass bottle. The phase transfer was completed after 10 min, and the process could be visibly observed (Figure S2). Then, the water phase was purified by ultrafiltration twice with an ultrafiltration tube with a cutoff molecular weight of 10,000 and stored in a refrigerator at 4 \(^\circ\)C in the dark.

\textbf{Characterization Methods.} All images of transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM) were recorded using a JEOL JEM-2100 (HR) electron microscope. The Fourier transform infrared (FTIR) spectrum was recorded by the KBr method using a 5700 Fourier infrared spectroscopy analyzer (Thermo Company, USA). Atomic force microscopy (AFM) was recorded on an NT-MDT NTEGRA spectrometer (NT-MDT, Russia).

\textbf{UV–Visible Absorption Spectroscopy.} A UV-3600 was used to record the absorption spectra of the QDs in a 1 cm quartz cell, and the measurement range was 800–1500 nm. A Cary UV–vis spectrophotometer was used to measure the UV–visible absorption spectra of HSA with and without QDs. To facilitate the thermodynamic studies, the absorbance at 400 nm was also measured to determine the concentration of PbS QDs according to the reported empirical formula, eqs \textsuperscript{9} and \textsuperscript{10}.

\begin{equation}
\epsilon_{400} \left( \text{cm}^{-1} \cdot \text{M}^{-1} \right) = 0.0233d^3 \left( \text{nm} \right)
\end{equation}

\begin{equation}
A_{400} = \epsilon_{400}bc
\end{equation}

Here, \( A_{400} \) represents the absorbance at 400 nm, \( \epsilon_{400} \) represents the molar absorption coefficient at 400 nm, \( b \) is the side length of the cuvette (1 cm), \( c \) is the concentration of the PbS QDs, and \( d \) is the average value of the particle sizes determined by TEM.

\textbf{Fluorescence Spectroscopy, Quantum Yield, and Fluorescence Lifetime.} The fluorescence emission spectra of QDs and the fluorescence lifetime of HSA were recorded using an FLS 1000 steady-state/transient spectrometer (Edinburgh Instruments, UK). The sample was measured in a 1 cm quartz cell. The scanning range of fluorescence emission spectra was 800–1600 nm. The absolute quantum yield \( \eta \) was calculated using this instrument. The laser at 785 nm was used to excite the sample and the blank solution. The integrals of the scattering spectrum at 760–810 nm (\( S_{\text{blank}} \)) and the integrals of the emission spectrum at 1000–1600 nm (\( E_{\text{blank}}, E_{\text{sample}} \)) were obtained. The selection of the
integral regions, the calculation of the integrals, and the final calculation of η were handled by Fluoracle software with eq 11. The fluorescence spectra of HSA were recorded using an LS-55 spectrometer (PerkinElmer). The temperature was kept constant with a circulating water bath. QDs were added by a microsyringe. Synchronous fluorescence spectra were obtained by controlling the difference between excitation wavelength and the emission wavelength to Δλ = 15 or 60 nm. The fluorescence lifetime was fitted by eq 12, and the average lifetime was calculated by eq 13.

\[ \eta = \frac{N_{\text{em}}}{N_{\text{abs}}} = \frac{E_{\text{sample}} - E_{\text{blank}}}{S_{\text{blank}} - S_{\text{sample}}} \]  

\[ I(t) = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2} \]  

\[ \tau_{\text{average}} = \frac{A_1 \tau_1^2 + A_2 \tau_2^2}{A_1 \tau_1 + A_2 \tau_2} \]

Here, \( \tau_1 \) and \( \tau_2 \) represent the two lifetime components and \( A_1 \) and \( A_2 \) represent the corresponding fractions.

**Dynamic Light Scattering (DLS) and Zeta Potential.**
Zetasizer Nano ZS (Malvern, UK) was used to record the hydrodynamic diameter and zeta potential of QDs, HSA, and their complexes. The concentration of HSA was 5 μM.

**Agarose Gel Electrophoresis (AGE).**
Tris–HCl (0.01 M, pH = 7.4) was used as a solvent to configure an agarose gel, and it was also used as the electrophoresis solution to perform electrophoresis at different ratios of HSA to QDs. The electrophoresis experiments were conducted on a Bole electrophoresis apparatus (USA) (80 V, 20 min). The mixture was incubated for 3 and ~12 h before electrophoresis.

**Circular Dichroism (CD).**
The circular dichroism spectra were recorded using a circular dichroism photomultiplier (Applied Photophysics Limited, UK). In each measurement, the signals of the added QDs were subtracted to remove the effect of the QDs themselves. Each data line was the average of three scans. The test sample was placed in a quartz cell with a thickness of 0.1 cm, and the scanning range was 190–260 nm.

### ASSOCIATED CONTENT

**Supporting Information**
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c05974.

Tunable fluorescence spectra of PbS QDs (Figure S1); quantum yields of PbS QDs with different emission maxima (Table S1); photos of the phase-transfer process (Figure S2); FTIR spectrum (Figure S3); zeta potential (Figure S4); fluorescence spectra of PbS QDs in excess GSH (Figure S5); fluorescence spectra of PbS QDs in excess HSA or water (Figure S6); quenching of protein’s fluorescence by PbS QDs at 302 K (Figure S7), 306 K (Figure S8), and 310 K (Figure S9); fluorescence lifetime (Table S2); thermodynamic parameters for the “enthalpy–entropy compensation” equation (Table S3); hydrodynamic diameters of PbS QDs and HSA (Figure S10); synchronous fluorescence of HSA in the presence of a small amount of PbS QDs within short time duration (Figure S11); and surface charge distribution of HSA and the possible binding sites (Figure S12) (PDF)

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**Notes**
The authors declare no competing financial interest.

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