VIP Water-Soluble Nanocrystals

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Water-Soluble Nanocrystals Through Dual-Interaction Ligands**

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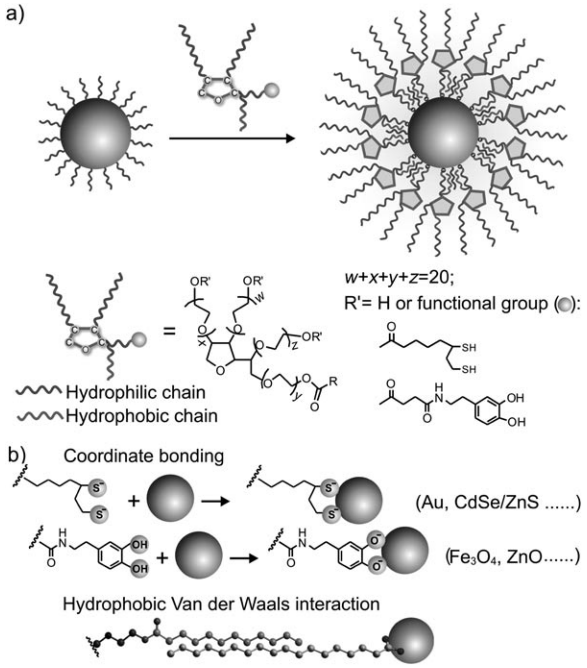
Owing to their unique size-dependent optical, electronic, magnetic, and chemical properties, inorganic nanocrystals are becoming a new class of powerful tools in biological and medical applications for sensing, labeling, optical imaging, magnetic resonance imaging (MRI), cell separation, and treatment of disease.[1–5] These applications, however, require nanocrystals that are soluble and stable in aqueous solutions, thus creating a need to further engineer nanocrystal coatings, because those high-quality nanocrystals are often synthesized in an organic phase and stabilized with hydrophobic ligands.[6] To date, two major approaches have been developed to modify the coatings of hydrophobic nanocrystals using organic ligands.[2,4,5] The first approach is based on coordinate bonding. Functional groups, such as thiols, dithiols, phosphines, and dopamine, are used to directly link hydrophilic groups onto the surface of hydrophobic nanocrystals by replacing their original hydrophobic ligands.[4,5,7–9] The second approach uses hydrophobic van der Waals interactions, through which the hydrophobic tails of amphiphilic ligands interact with, but do not replace, the hydrophobic ligands on nanocrystals, and it leads to the formation of nanocrystal micelles.[10,11] Many types of water-soluble nanocrystals made by these two approaches suffer low stability and/or high nonspecific binding with nontarget biomolecules.[12] Water-soluble nanocrystals coated with PEGylated amphiphilic polymers are proven to have very high stability and low nonspecific absorption levels, but PEGylated polymer shells often produce large hydrodynamic diameters (HDs) on the order of 30-40 nm, which could limit the use of these nanocrystals in applications, such as cell imaging in vivo.[11,12]

Herein, we report an alternative nanocrystal surface-engineering approach that uses a new class of ligands ("dual-interaction ligands") to produce water-soluble nanocrystals of gold, Fe_3O_4, and CdSe/ZnS quantum dots (QDs). These dual-interaction ligands can bind onto the surface of hydrophobic nanocrystals by both coordinate bonding and hydrophobic van der Waals interactions (Scheme 1). The resulting water-soluble nanocrystals have relatively small HDs (e.g., less than 20 nm), and exhibit extraordinary stability of a wide pH range (1–14), salt concentrations, and thermal treatment at 100 °C. In addition, these nanocrystals can be further functionalized with antibodies for monitoring virus–protein expression in cells.



Scheme 1. a) Formation of water-soluble nanocrystals by dual-interaction ligands. R is defined in Scheme 2. b) The two bonding modes.

The dual-interaction ligands described herein are synthesized through simple modifications of polyethylene glycol (PEG) sorbitan fatty-acid esters (Scheme 1 and 2). These esters have 20 ethylene glycol units distributed among their four branches. Depending on the type of their fatty-acid tail, these esters are commercially also named Tween 20, Tween 40, Tween 60, and Tween 80 (Scheme 2). Because of their relative nontoxicity, these Tween compounds are often used in the food industry as food additives.[13] More importantly, these Tween compounds are widely used as protein stabilizing and blocking agents to minimize nonspecific binding in immunoassays, such as western blotting and ELISA (enzyme-linked immunosorbent assay).[14] These properties make Tween compounds unique for coating water-soluble nanocrystals for use in biomedical applications. However, these compounds cannot be directly used to stabilize hydrophobic nanocrystals in water because the hydrophobic van der Waals interactions between their fatty-acid tails and the hydrophobic nanocrystal surface are relatively weak. To overcome this difficulty, we here intro-

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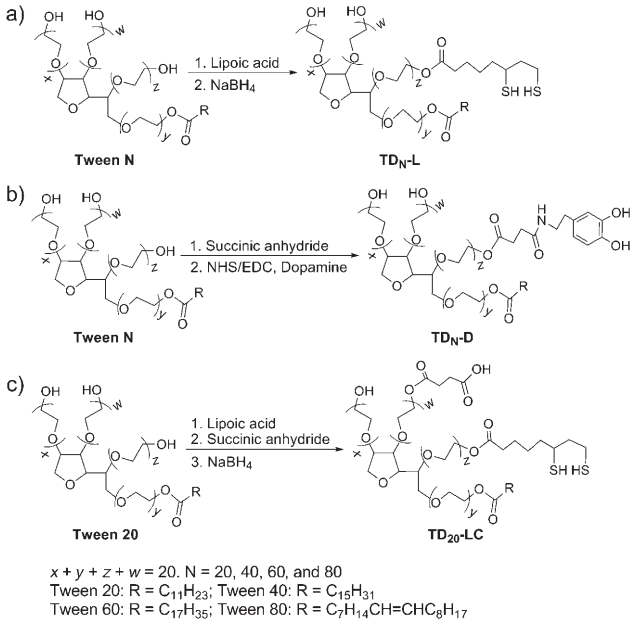

Scheme 2. Synthesis of dual-interaction TD ligands: a) lipoic-acid-functionalized TDs: TD_N-L, b) dopamine-functionalized TD_N-D, and c) carboxyl-group-functionalized TDs: TD_{20}-LC.

duce a coordinating function group into these compounds through one of their hydroxy groups (Scheme 2). The resulting Tween derivatives (TDs) are expected to show affinity to the surface of hydrophobic nanocrystals through both coordinate bonding and hydrophobic van der Waals interactions (Scheme 1).

Two types of coordinating groups are used to functionalize Tween compounds for the surface engineering of different types of nanocrystals (Scheme 2). For noble metal particles and semiconductor QDs,[4,5,7,8] we introduced a dithiol coordinating group into Tween compounds. The synthesis includes two steps: 1) a lipoic acid group is attached to a Tween compound through a mild esterification reaction; and 2) the product is reduced with sodium borohydride to convert the lipoic acid group into a dihydrolipoic acid group. It yields final products named TD_N-L (Scheme 2).[15] For nanocrystals of transition metal oxides, such as Fe_3O_4, we used a dopamine group to modify Tween compounds through a succinic acid cross-linker: 1) a succinic acid group is incorporated into a Tween compound by an esterification reaction with succinic anhydride, and 2) a dopamine group is coupled with the succinic acid cross-linker through a 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC)-mediated amide-formation reaction. It yields final products named TD_N-D (Scheme 2).[9] Moreover, an additional carboxyl group can be attached onto these TDs, and the resulting products allow protein attachment through a mild EDC-mediated coupling reaction.[12] As an example, we have synthesized succinic acid-functionalized TD_{20}-L (TD_{20}-LC, Scheme 2), which can covalently link antibodies onto the surface of QDs or noble metal nanocrystals.[15] The typical yields of these reactions are higher than 80%.[15] The structures of the resulting TD ligands are confirmed using ^{1}H NMR spectroscopy, but the exact positions of the attached functional groups in the TD ligands are not identified herein.[15]

To examine the function of these TD ligands, three types of hydrophobic nanocrystals were used: gold nanocrystals (6.6 nm diameter, with a standard deviation $\sigma = 7.0\%$), Fe_3O_4 (5.8 nm diameter, $\sigma = 6.0\%$), and CdSe/ZnS QDs (5.6 nm diameter, $\sigma = 8.0\%$) Transmission electron microscope (TEM) images are shown in Figure 1 a–c. In the first set of

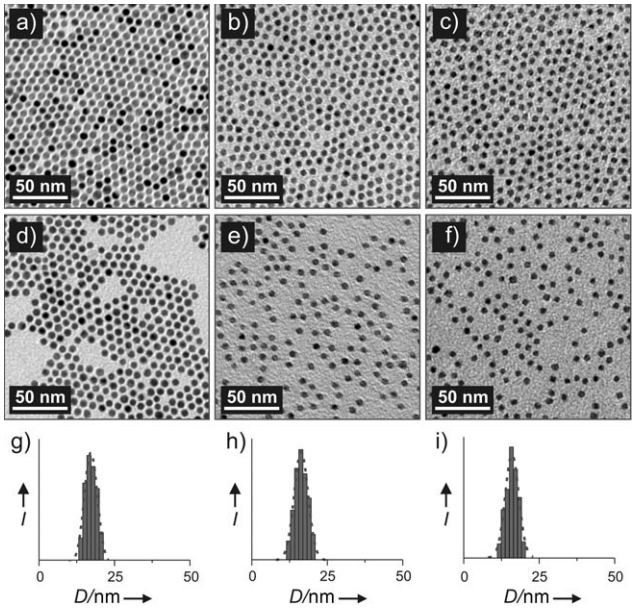

Figure 1. TEM images of hydrophobic nanocrystals:[15] a) 6.6 nm gold, b) 5.8 nm Fe_3O_4, and c) 5.6 nm CdSe/ZnS core/shell nanocrystals. Hydrophilic counterparts, which are functionalized with TD_{20} ligands:[15] d) gold, e) Fe_3O_4, and f) CdSe/ZnS nanocrystals. DLS data for these hydrophilic nanocrystals: g) gold, h) Fe_3O_4, and i) CdSe/ZnS nanocrystals.

experiments, TD_{20}-L (or TD_{20}-D) was used to functionalize the gold and CdSe/ZnS (or Fe_3O_4) nanocrystals, respectively.[15] The ligand-exchange reactions were performed in chloroform for 20 min. After the chloroform is removed by evaporation, the resulting hydrophilic nanocrystals are highly soluble in water, with a transfer yield of nearly 100%. The extra ligands in the hydrophilic nanocrystal solutions were removed by washing them through a spin filter four times.[15]

TEM measurements show that the TD-functionalized hydrophilic nanocrystals have nearly identical sizes and shapes compared to their hydrophobic counterparts (Figure 1 d–f). Dynamic light scattering (DLS) measurements show that the HDs of these nanocrystals are 17.1 nm for the TD_{20}-L-functionalized gold particles, 16.3 nm for the TD_{20}-D-functionalized Fe_3O_4 nanocrystals, and 15.9 nm for the TD_{20}-L-functionalized CdSe/ZnSe QDs (Figure 1 g–i). After subtracting the HDs from their respective core sizes, we obtain a nearly identical shell thickness of about 5.2 nm for all three types of nanocrystals. The shell thickness is very close to the average length of these TD ligands (ca. 4.9 nm). This result shows that only one monolayer of TD ligands is functional-

ized onto the nanocrystal surface. In addition, GC-MS measurements show that these TD ligands do not totally remove the original hydrophobic ligands of these nanocrystals. In a sample of TD_{20}-D-functionalized Fe_3O_4 nanocrystals, oleic acid (the original ligand) was unambiguously identified by GC-MS (Figure S1 in the Supporting Information). Taken together with the results above, this exchange of partial ligands suggests that TD ligands indeed functionalize hydrophobic nanocrystals by both coordinate bonding as well as the hydrophobic van der Waals interactions between the fatty-acid chain in the TD ligands and the hydrophobic ligands on the nanocrystals (Scheme 1). This nanocrystal functionalization (with dual-interaction ligands) is further consistent with the results from the following nanocrystal-stability measurements.

We investigated the stability of these hydrophilic nanocrystals as a function of pH, salt concentration, and thermal treatment at 100 °C (Figure 2).[15] For the TD_{20}-L-functionalized gold nanocrystals, the stability tests were monitored using both DLS and UV/Vis absorption spectroscopy. These gold nanocrystals do not exhibit significant change in their HD after heating in boiling water (pH 6.5) for 4 h, as measured using DLS, or in the position of their absorption peak, as measured by absorption spectroscopy (Figure 2a).[15] The results from pH stability tests show that these hydrophilic gold nanocrystals are stable from pH 2 to 13 for more than one week. At pH 1, the HD of these particles is slightly decreased, but without a change in the position of absorption peaks for more than two hours. At pH 14, the particles show small changes in both HD and absorption peak position, but the nanocrystal solution is stable for more than three days under these conditions (Figure 2b). In addition, the gold nanocrystals are stable almost indefinitely in NaCl solutions having concentrations up to 5 M (Figure 2c). Altogether, these results show that TD_{20}-L-functionalized gold nanocrystals exhibit extraordinary stability in various extreme conditions. It should be noted that such stability is even higher than that of gold nanocrystals heavily functionalized with alkylthiol-capped oligonucleotides, which have been used in commercial biomedical diagnosis because of their high stability in high-concentration salt solutions.[16]

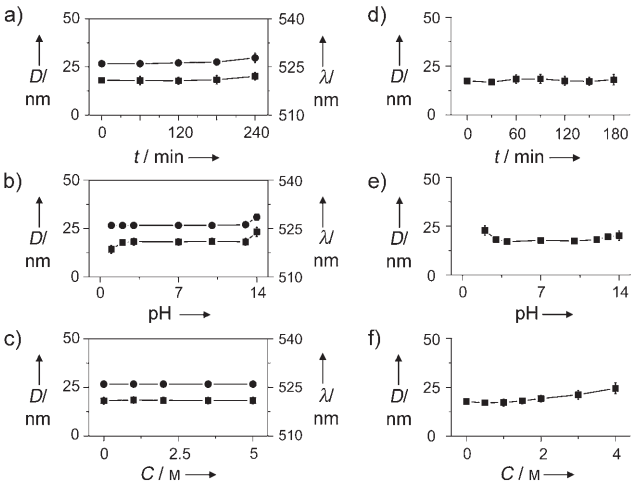

Figure 2. Stability tests of TD_{20}-L-functionalized 6.6 nm gold nanocrystals monitored with DLS (■) and UV/Vis spectra (●): a) Thermal stability test at 100 °C; b) pH stability test; and c) stability as a function of NaCl concentration. d),e), and f) are the respective stability tests for TD_{20}-D-functionalized 5.8 nm Fe_3O_4 nanocrystals.

TD_{20}-D-functionalized Fe_3O_4 nanocrystals also show excellent stability in these tests. These Fe_3O_4 nanocrystals are stable in a solution of boiling water (pH 6.5) for 3 h (Figure 2d), and pH stability tests show that they are stable from pH 3 to 14 for more than one week. At pH 2, the HD of these Fe_3O_4 nanocrystals slightly increases (Figure 2e). Surprisingly, TEM measurements show that after a 2 h treatment at pH 2, the nanocrystals exhibit no measurable change in size and shape compared with the nanocrystals in the control experiment at pH 7 (Figure S2 in the Supporting Information). Moreover, these TD_{20}-D-modified Fe_3O_4 nanocrystals are stable nearly indefinitely in a NaCl solution with concentrations up to 2 M. In a 4 M NaCl solution, they are stable for more than 4 h (Figure 2f). These results show that TD_{20}-D-functionalized Fe_3O_4 nanocrystals exhibit a much higher stability than those Fe_3O_4 nanocrystals functionalized with PEGylated-dopamine ligands, which attach onto the nanocrystal surface by only coordinate bonding.[9b] Therefore, the excellent stability of TD-functionalized hydrophilic nanocrystals should be attributed to the ability of the TD ligands to attach onto the nanocrystal surface through both coordinate bonding and hydrophobic interactions.

To further explore the effect of the hydrophobic van der Waals interactions between TD ligands and the nanocrystal hydrophobic coating, we studied the stability of TD_N-L-functionalized CdSe/ZnS QDs as a function of the fatty-acid chain in TD_N-L ligands (N = 20, 40, 60, or 80). In these experiments, 5.6 nm oleylamine-coated CdSe/ZnS particles were transferred into the aqueous phase by surface functionalization with the four types of TD_N-L ligands. The resulting TD_{20}-L-modified CdSe/ZnS nanocrystals are stable over a pH range of 3.5 to 11 (Figure 3a). This stability is higher than that of CdSe/ZnS particles functionalized with PEGylated-lipoic-acid ligands, which lack hydrophobic interactions with the nanocrystal surface.[12,17] When the length of the fatty-acid chain was increased to 16 and 18 carbon units, TD_N-L-modified CdSe/ZnS nanocrystals (N = 40, 60, or 80) are stable over a wider pH range (1–14), according to DLS measurements (Figure 3a). Such fatty-acid-chain-dependent stability is also observed in the stability tests with NaCl solutions. TD_{20}-L-modified particles are only stable in NaCl solutions up to 0.6 M, whereas TD_{40}-L-modified particles are stable in a 2 M NaCl solution. With a further increase of fatty-acid chain length to 18 carbon units, TD_{80}-L (or TD_{60}-L)-functionalized CdSe/ZnS nanocrystals are stable in an almost saturated NaCl solution (Figure 3b). Taken together, these results demonstrate that the stability of nanoparticles indeed depends on the length of the fatty-acid chains on the TD ligands.

This chain-length-dependent stability suggests that the van der Waals interactions between the fatty-acid chains and the oleylamine coating play a significant role in stabilizing nanocrystals in aqueous solutions. The longer the fatty-acid chain, the greater the van der Waals interactions with the oleylamine coating.[18] In addition, the van der Waals interactions should create a hydrophobic shell on the nanocrystal

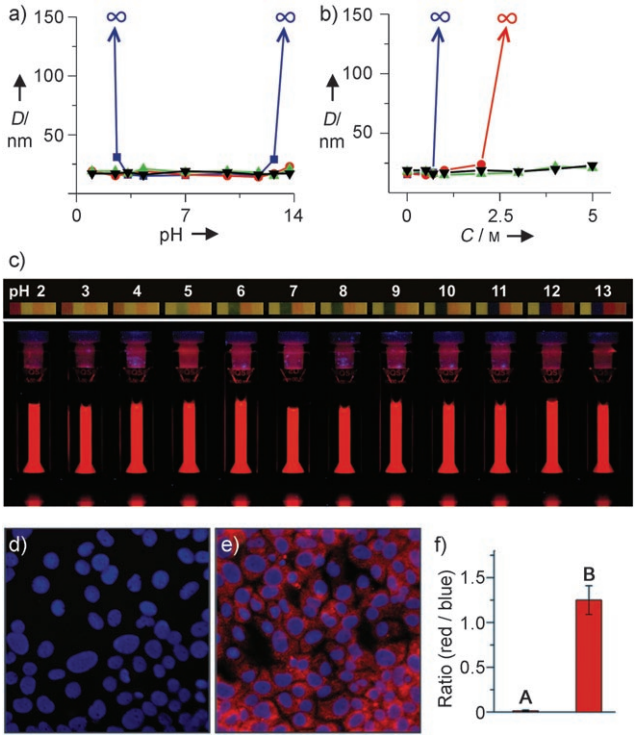

Figure 3. a) pH and b) NaCl stability tests of TD_N-L-functionalized 5.6 nm CdSe/ZnS nanocrystals as a function of the fatty-acid chain on these TD ligands: TD_{20}-L (■), TD_{40}-L (●), TD_{60}-L (▲), and TD_{80}-L (▼). c) Fluorescence images of TD_{80}-L-functionalized 5.6 nm CdSe/ZnS nanocrystals as a function of pH, as indicated by pH test paper. d)–f) Immunofluorescence tests of HCV-NS5A-containing FCA1 cells using TD-functionalized CdSe/ZnS core/shell QDs: d) without and e) with NS5A-specific antibodies. The nuclei of these FCA1 cells were counterstained with DAPI (4′,6-diamidino-2-phenylindole, showing blue fluorescence) as an internal reference. The images were taken under an Olympus fluorescence microscope.[15] f) Fluorescence-intensity ratios of the blue channel (from DAPI) and the red channel (from QDs) were calculated on the basis of the fluorescence images taken from the immunostaining tests. A: control test; B: test with antibody-functionalized QDs.

surface.[4,5,10,11] Such a hydrophobic shell can provide additional protection for the hydrophilic nanocrystals as this shell can prevent hydrophilic reagents (such as H^+) from reacting with the nanocrystal surface.[18] Indeed, we found that the fluorescence quantum yield of TD_{80}-L-functionalized CdSe/ZnS QDs is maintained at about 50% for more than three months in aqueous solutions over a pH range of 3.5–12.5. Furthermore, the fluorescence brightness of these nanocrystals does not significantly change for 2 h in an aqueous solution of pH 2 (Figure 3c).

To demonstrate the suitability of using these water-soluble nanocrystals for biomedical diagnosis, we used TD-modified CdSe/ZnS QDs as fluorescence labels to monitor the expression of a HCV (Hepatitis C virus) protein NS5A inside FCA1 cells.[15,19] In these experiments, 5.6 nm oleylamine-capped CdSe/ZnS QDs were first functionalized with a mixture of TD_{80}-L and TD_{20}-LC (5:1). Anti-HCV NS5A monoclonal antibodies were then attached onto the resulting QDs through an EDC coupling reaction.[15] In a control test, the QDs without NS5A-specific antibodies show very low nonspecific absorption onto NS5A-containing FCA1 cell substrates (Figure 3d). In contrast, the antibody-modified QDs exhibit a very high specific affinity to such substrates (Figure 3e). Significantly, the fluorescence intensity from QDs labels is more than 75 times higher than that in the control test (Figure 3f).

In summary, we have demonstrated an approach of using dual-interaction ligands to convert hydrophobic gold, Fe_3O_4, and CdSe/ZnS nanocrystals into hydrophilic ones. A series of dual-interaction ligands has been synthesized by simple modifications of Tween compounds. The hydrophilic nanocrystals functionalized with these TD ligands exhibit high stability in aqueous solutions with a wide range of pH and salt concentrations, and under thermal treatment at 100 °C. Such extraordinary nanocrystal stability is attributed to the new type of surface functionalization through both coordinate bonding and hydrophobic van der Waals interactions. In addition, we show that TD-functionalized QDs are excellent fluorescence labels for detecting the HCV-NS5A expression in FCA1 cells. Moreover, the new surface-functionalization approach can be readily generalized for nanocrystals with other compositions. Finally, because of their excellent stability, these TD-functionalized nanocrystals should play an important role in a variety of nanocrystal-based biomedical applications.[1–5]

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