Supplementary Information

Selective inclusion of anionic quantum dots in coordination network shells of nucleotides and lanthanide ions

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1. Experimental

Adenosine 5'-monophosphate (AMP) disodium salt was purchased from Sigma-Aldrich Inc. Gadolinium(III) chloride hexahydrate ($GdCl_3$) was obtained from Wako Pure Chemical, Ltd. Aqueous dispersions of surface modified CdSe/ZnS quantum dots (8 μM, QDs), carboxyl-QDs_{525} (Qdot® 525 ITK™ carboxyl quantum dots), amino-QDs_{525} (Qdot® 525 ITK™ amino (PEG) quantum dots), carboxyl-QDs_{605} (Qdot® 605 ITK™ carboxyl quantum dots), and amino-QDs_{605} (Qdot® 605 ITK™ amino (PEG) quantum dots) were purchased from Invitrogen Co. Dextran (from *Leuconostoc mesenteroides*, Mw 35,000–45,000) was purchased from Sigma-Aldrich Inc. 2-[4-(Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) was purchased from DOJINDO laboratories and 0.1 M HEPES buffer (pH 7.4) was prepared from the aqueous mixtures of HEPES and sodium hydroxide. All chemicals were used as received. Water was purified through a Direct-Q system (Millipore Co.). Transmission electron microscopy (TEM) was performed by a JEOL JEM-2010 (acceleration voltage, 120 kV). High-resolution TEM and energy dispersive X-ray (EDX) microanalysis were performed on a Tecnai 20 (acceleration voltage, 200 kV). For TEM measurement, an aqueous suspension of QDs@nucleotide/lanthanide core-shell nanoparticles was placed on carbon-meshed copper grid. After 30 s, the excess suspension was removed by adsorbing to filter paper. The resultant grid was dried in vacuum and observed by electron microscopy without staining. Emission spectra were recorded by a HITACHI F-4500 spectrophotometer at room temperature (20 °C) using quartz cells with 1-mm path length. Absolute photoluminescence quantum yields were determined by an Absolute PL Quantum Yield Measurement System (HAMAMATSU, C9920-02) using quartz cells with 1-cm path length.

2. Preparation of QDs@nucleotide/lanthanide core-shell nanoparticles

The core-shell nanoparticles were prepared by mixing aqueous solutions of AMP, QDs and $GdCl_3$ as follows. First, aqueous solutions of QDs (8 μM, 4 μL) and AMP disodium salt in HEPES buffer ([AMP] = 10 mM, 0.1 M HEPES, pH 7.4, 100 μL) were mixed with aqueous dextran (10 mg/mL, 1.8 mL). To this aqueous mixture, aqueous $GdCl_3$ (10 mM, 100μL) was further added (final concentrations of AMP, $GdCl_3$ and QDs: [AMP] = [$GdCl_3$] = 0.5 mM, [QDs] = 0.016 μM, [HEPES] = 5.0 mM). The resultant aqueous suspension was kept for 1h before the preparation of TEM specimens. Emission spectra were measured for these suspensions after ultrasonic treatment (BRANSON 2510 (YAMATO), for 1 min).

To confirm encapsulation of carboxyl-QDs_{605} in the coordination shell of AMP and Gd^{3+} ion, elemental composition of core-shell nanoparticles observed in TEM was determined by TEM-EDX microanalysis (Fig. S1a). Peaks assignable to P and Gd are found together with those of Cd, Zn, Se, and S, indicating the presence of AMP and Gd^{3+} ions together with carboxyl-QDs_{605} in core-shell nanoparticles. This was further confirmed by high-resolution TEM observation (HR-TEM, Fig. S1b), where lattice fringes of crystalline QD core were found in the center of amorphous shell layers (Fig. S1b).

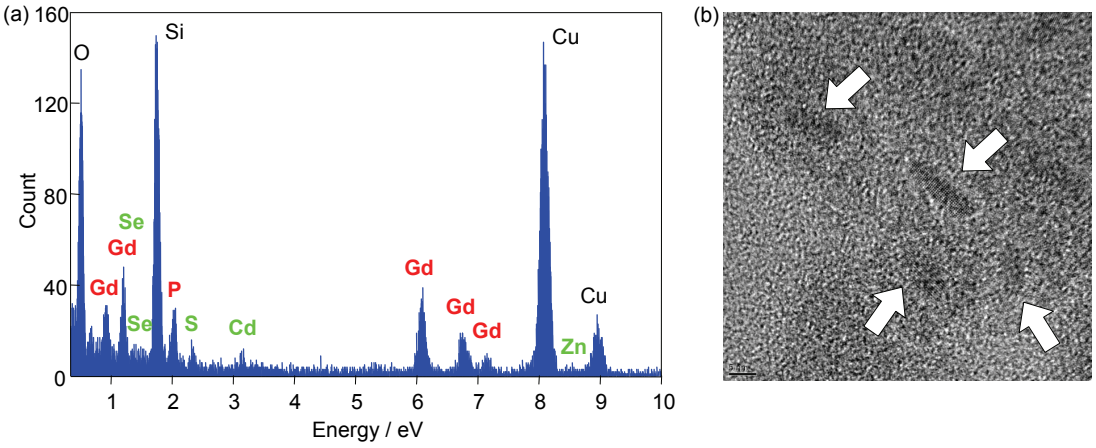

Figure S1. (a) A TEM-EDX spectrum and (b) a HR-TEM micrograph of core-shell nanoparticles obtained by mixing aqueous solutions of AMP, carboxyl-QDs_{525} and $GdCl_3$.

The thickness of AMP/Gd^{3+} coordination shell was controllable by changing the amount of QDs. Figure S2 shows TEM micrographs of QDs@AMP/Gd^{3+} core-shell nanoparticles prepared at different concentrations of carboxyl-QDs_{605}. When the concentration of carboxyl-QDs_{605} was increased from 0.016 μM to 0.064 μM in the final reaction mixture, thickness of shell decreased from 8 nm (Fig. S2a) to 4 nm (Fig. S2b).

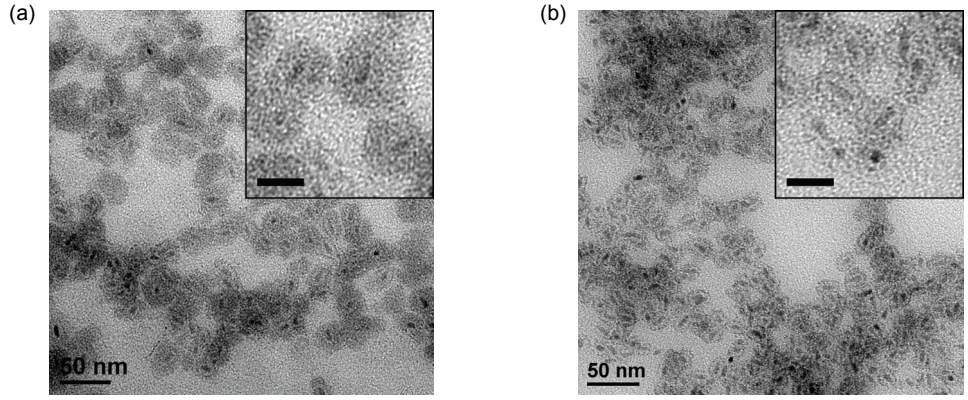

Figure S2. TEM micrographs of carboxyl-QDs_{605}@AMP/Gd^{3+} core-shell nanoparticles prepared in the presence of (a) 0.016 μM and (b) 0.064 μM of carboxyl-QDs_{605}. Final concentrations: (a) [AMP] = [$GdCl_3$] = 0.5 mM, [carboxyl-QDs_{605}] = 0.016 μM and (b) [AMP] = [$GdCl_3$] = 0.5 mM, [carboxyl-QDs_{605}] = 0.064 μM in 5.0 mM HEPES buffer solution containing 10 mg/mL aqueous dextran. Inset: higher-magnification TEM images of carboxyl-QDs_{605}@AMP/Gd^{3+} core-shell nanoparticles (scale bar = 20 nm).

3. The formation of core-shell structures is selective to anionic QDs.

The formation of AMP/Gd^{3+} coordination shells on anionic QDs is a general phenomenon, as confirmed for QDs of different sizes. Here we used smaller QDs – carboxyl-QDs_{525} and amino-QDs_{525} (diameter ~ 3 nm) – compared to the QDs_{605} series. Similarly to the case of anionic QDs_{605}, carboxyl-QDs_{525} were spontaneously surrounded by AMP/Gd^{3+} coordination shells, giving core-shell structures (Fig. S3a). In contrast, such encapsulation was not observed for amino-QDs_{525}. The amino-QDs_{525} were mostly observed in the bulk solution separately and were not incorporated in AMP/Gd^{3+} CNPs (Fig. S3b).

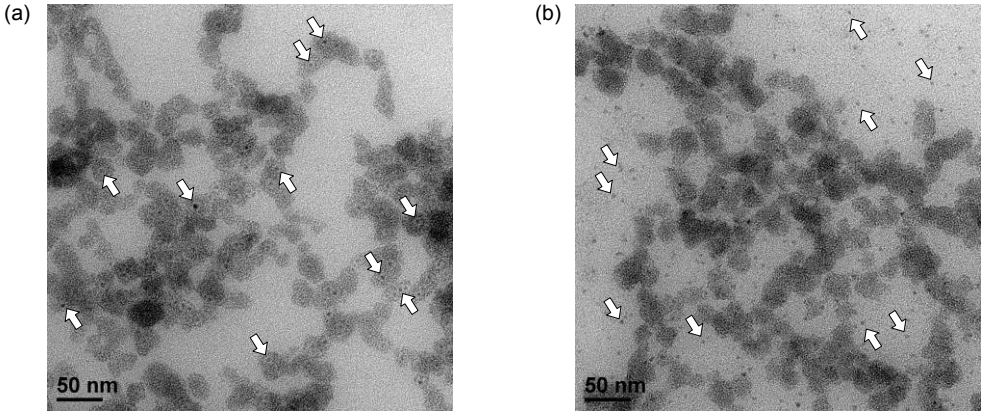

Figure S3. TEM micrographs of AMP/Gd^{3+} CNPs prepared in the presence of (a) carboxyl-QDs_{525} and (b) amino-QDs_{525}. Final concentration: [AMP] = [$GdCl_3$] = 0.5 mM, [QDs_{525}] = 0.016 μM in 5.0 mM HEPES buffer solution containing 10 mg/mL aqueous dextran. Some QDs_{525} are indicated by arrows for clarity.

4. Separation of anionic QDs from cationic QDs by taking advantage of selective encapsulation.

4-1. Luminescence spectra of supernatants

Encapsulation of carboxyl-QDs_{605} and amino-QDs_{605} by coordination networks of AMP and Gd^{3+} ion was investigated by measuring luminescence intensity of supernatant solution after centrifugation. To avoid the non-specific absorption of amino-QDs_{605} to glass vial surface, Eppendorf tubes (Safe-Lock Tubes 1.5 mL) were used as mixing vessels. Nanoparticles were prepared by mixing aqueous solutions of AMP which contain carboxyl-QDs_{605} or amino-QDs_{605} and aqueous $GdCl_3$. The resultant suspensions were centrifuged and emission spectra of the supernatants were measured (Fig. S4). When aqueous $GdCl_3$ was added to the aqueous mixture of AMP and carboxyl-QDs_{605}, emission intensity of QDs in the resultant supernatant solution was almost negligible (Fig. S4a). In contrast, centrifugation of the aqueous mixture containing AMP, Gd^{3+} and amino-QDs_{605} gave a supernatant solution whose luminescence intensity was almost the same as that observed for the equimolar aqueous solutio of n amino-QDs_{605} (Fig. S4b). These results confirm that anionic, carboxyl-QDs_{605} are quantitatively encapsulated in AMP/Gd^{3+} CNPs whereas amino-QDs_{605} do not bind to AMP/Gd^{3+} CNPs. It indicates that coordination of amino groups on the surface of amino-QDs_{605} to lanthanide ions does not occur under the experimental condition employed.

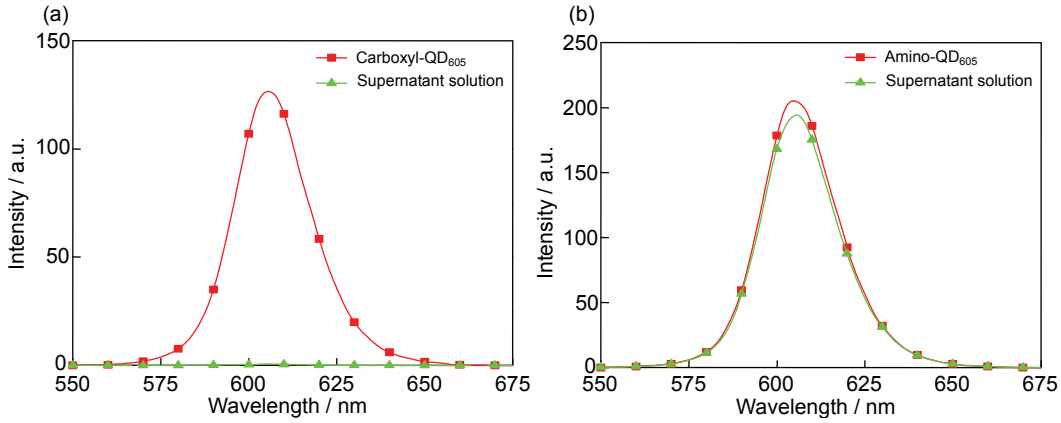

Figure S4. (a) Luminescent spectra of aqueous carboxyl-QDs_{605} ([QDs] = 0.016 μM, square) and supernatant solution after centrifugation of aqueous mixture containing AMP, Gd^{3+} ion and carboxyl-QDs_{605} ([AMP] = [$GdCl_3$] = 0.5 mM, [QDs] = 0.016 μM, triangle). (b) Luminescent spectra of aqueous amino-QDs_{605} ([QDs] = 0.016 μM, square) and supernatant solution after centrifugation of the aqueous mixture of AMP, Gd^{3+} and amino-QDs_{605} ([AMP] = [$GdCl_3$] = 0.5 mM, [QDs] = 0.016 μM, triangle). Condition: 5.0 mM HEPES buffer solution containing 10 mg/mL aqueous dextran, excitation wavelength, 450 nm. Quartz cells with 1-mm path length were used.

4-2. Luminescence spectra of precipitated nanoparticles

The selective encapsulation of carboxyl-QDs in AMP/Gd^{3+} nanoparticles was further confirmed by measuring the luminescence of AMP/Gd^{3+} nanoparticles. Naonoparticles were prepared by adding aqueous $GdCl_3$ to the aqueous mixture of AMP, carboxyl-QDs_{525} and amino-QDs_{605} dispersed in 10 mg/mL dextran. The precipitates formed were collected by centrifugation, which were then re-dispersed in water by ultrasonication. Luminescence spectrum of the aqueous dispersion showed two luminescent bands at 525 nm and 605 nm, which are attributed to that of carboxyl-QDs_{525} and amino-QDs_{605}, respectively (dashed line in blue, Fig S5). The luminescent intensity at 525 nm (carboxyl-QDs_{525}) was almost same as the original aqueous mixture of carboxyl-QDs_{525} (before nanoparticle formation, solid line in red). It clearly indicates quantitative encapsulation of carboxyl-QDs_{525} in AMP/Gd^{3+} nanoparticles. On the other hand, luminescent intensity was also observed at 605 nm (amino-QDs_{605}, dashed line in blue) for the re-dispersed AMP/Gd^{3+} nanoparticles. The intensity at 605 nm amounts to ca. 10 % of the original aqueous mixture (solid line in red). That is, about 10% of amino-QDs_{605} were non-specifically adsorbed in AMP/Gd^{3+} nanoparticles. However, this non-specific binding is quite inefficient by considering the almost complete binding of carboxyl-QDs to AMP/Gd^{3+} nanoparticles. These data confirm selective encapsulation of carboxyl-QDs_{525} in the course of self-assembly.

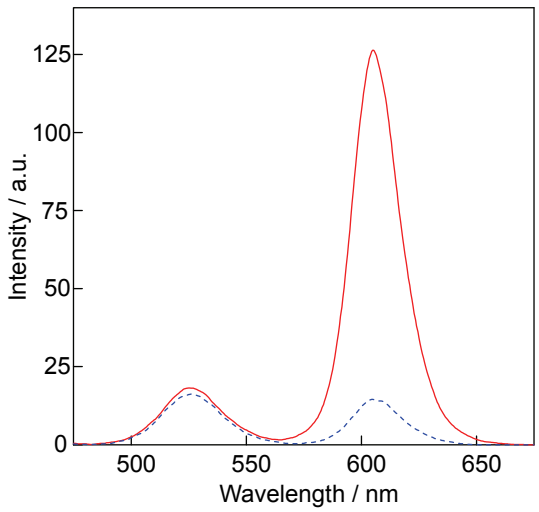

Figure S5. Luminescence spectra of aqueous mixture containing carboxyl-QDs_{605} and amino-QDs_{605} ([carboxyl-QDs_{525}] = [amino-QDs_{605}] = 0.016 μM in 5.0 mM HEPES, solid line in red). The precipitated QDs-containing AMP/Gd_{3+} nanoparticles were re-dispersed in pure water (dashed line in blue). excitation wavelength, 450 nm. Quartz cells with 1-mm path length were used.

4-3. Determination of absolute quantum yield for QDs enfolded in AMP/Gd^{3+} NPs

Luminescence properties of carboxyl-QDs_{605} encapsulated in AMP/Gd^{3+} CNPs (carboxyl-QDs_{605}@AMP/Gd^{3+} NPs) and its absolute quantum yield were investigated. Luminescent spectrum of carboxyl-QDs_{605}@AMP/Gd^{3+} NPs showed emission intensity and wavelength almost identical to aqueous carboxyl-QDs_{605} (Fig. S6a). Quantum yields of aqueous carboxyl-QDs_{605} (solid line in red) and carboxyl-QDs_{605}@AMP/Gd^{3+} NPs (dashed line in green) were determined as 54 % and 59 %, respectively (Fig. S6b). Thus, basic luminescent property of carboxyl-QDs_{605} is maintained after encapsulation by the supramolecular shell of AMP/Gd^{3+}.

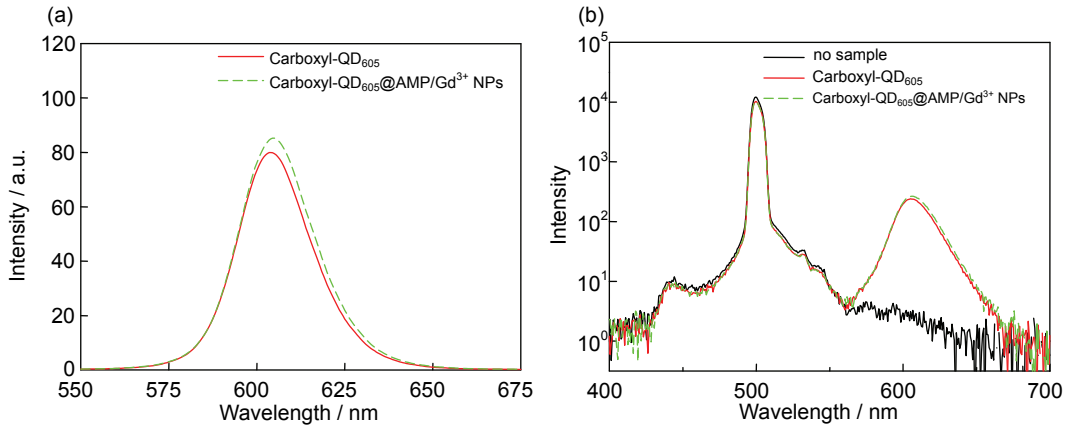

Figure S6. (a) Luminescent spectra of carboxyl-QDs_{605} (solid line in red) and carboxyl-QDs_{605}@AMP/Gd^{3+} NPs (dashed line in green) in aqueous dextran. (b) Luminescent spectra of carboxyl-QDs_{605} (solid line in red) and carboxyl-QDs_{605}@AMP/Gd^{3+} NPs (dashed line in green) in aqueous dextran measured by an Absolute PL Quantum Yield Measurement System (HAMAMATSU). The peaks observed at 500 nm and 605 nm in (b) are unabsorbed excitation light from the light source and emission of carboxyl-QDs_{605}, respectively. Condition: carboxyl-QDs_{605}, [QDs] = 0.016 μM, carboxyl-QDs_{605}@AMP/Gd^{3+} NPs, [QDs] = 0.016 μM, [AMP] = [$GdCl_3$] = 0.5 mM in 5 mM HEPES buffer containing 10 mg/mL dextran. Excitation wavelength, 500 nm. Quartz cells with 1-mm path length and those with 1-cm path length were used for emission spectra and quantum yield determination, respectively.

5. Encapsulation of anionic QDs by varied nucleotides and lanthanide ions.

The formation of core-shell structured QDs/nucleotide-lanthanide NPs was investigated for varied combination of nucleotides and lanthanide ions. In this experiment, cytidine 5'-monophosphate (CMP) disodium salt, guanosine 5'-monophosphate (GMP) disodium salt, and 5'-uridine 5'-monophosphate (UMP) disodium salt were used as nucleotides (Fig. S7a, b, and c). $LuCl_3$ was employed in place of $GdCl_3$ (Fig. S7d). All combinations of nucleotides and lanthanide ions yielded core-shell nanoparticles. These observations evidently indicate the generality of encapsulation phenomena by a wide combination of nucleotides and lanthanide ions.

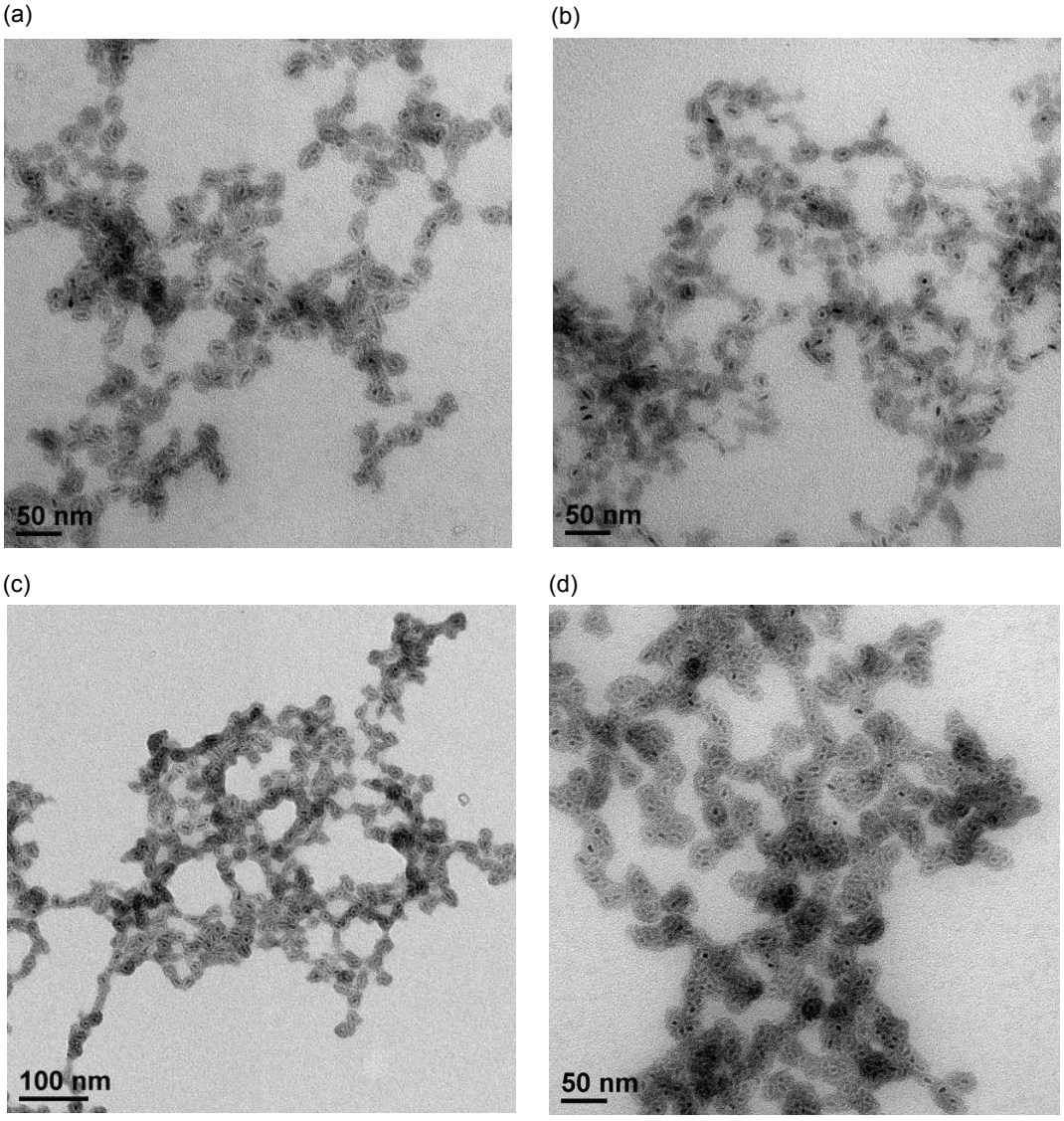

Figure S7. TEM micrographs of carboxyl-QDs_{525} encapsulated by nucleotide/lanthanide coordination networks. Combinations; (a) CMP and Gd^{3+} ion, (b) GMP and Gd^{3+} ion, (c) UMP and Gd^{3+} ion, (d) AMP and Lu^{3+} ion.

6. The effect of dextran and HEPES buffer on the morphology of CNPs.

To confirm intrinsic ability of nucleotides and lanthanide ions to form core-shell structures in the presence of QDs, nanoparticles were prepared in pure water without using HEPES buffer solution and dextran (Fig. S8). Formation of QDs(core)-AMP/Gd^{3+}(shell) structure was confirmed by TEM micrographs. It is seen however that sample prepared in pure water show higher tendency to form network-like aggregates (Fig. S8b). It indicates that dextran adsorbed on the surface of CNPs tend to prevent their agglomeration (Fig. S8a). We have reported previously that polyelectrolytes such as poly(styrene sulfonate) can be also employed to enhance dispersibility of CNPs.[3b]

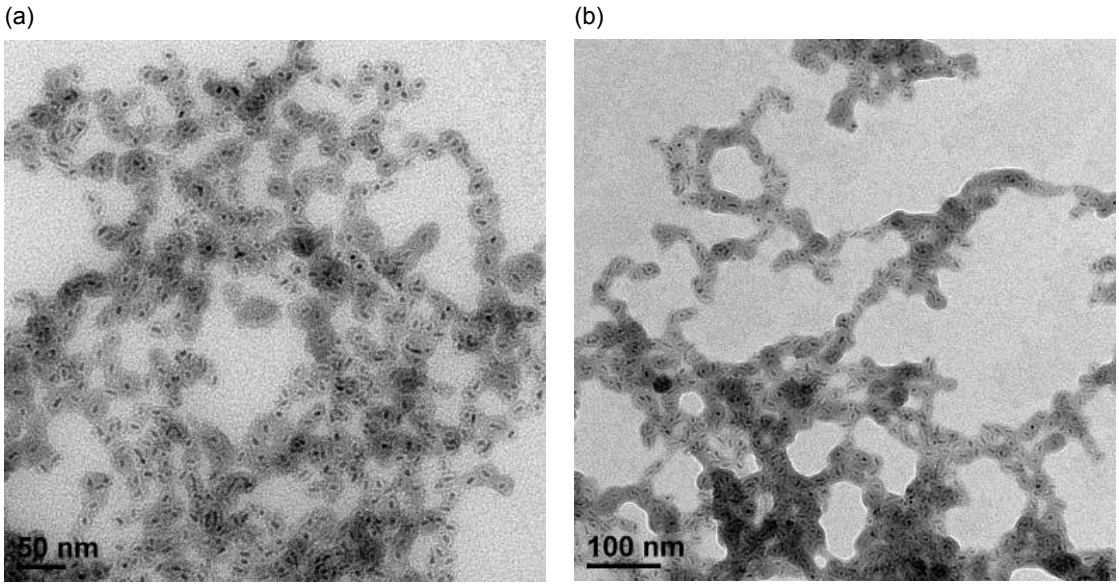

Figure S8. TEM micrographs of carboxyl-QDs_{525}@AMP/Gd^{3+} core-shell nanoparticles prepared by mixing aqueous AMP containing carboxyl-QDs_{525} and aqueous $GdCl_3$ in (a) aqueous dextran (10 mg/mL) and in (b) pure water.

7. Selective formation of coordination networks on the surface of anionic QDs.

As described in the manuscript, when carboxyl-QDs_{525} and amino-QDs_{605} were mixed with AMP and Gd^{3+} ion, coordination networks of AMP and Gd^{3+} ion were selectively formed on the surface of carboxyl-QDs_{525} (Fig. 4). The selective growth of coordination networks is ascribed to selective accumulation of Gd^{3+} ions on the surface carboxyl-QDs_{525}. Formation of AMP/Gd^{3+} CNPs were also tested in aqueous mixtures containing carboxyl-QDs_{605} and amino-QDs_{525} (Fig. S9). TEM picture clearly showed that larger carboxyl-QDs_{605} were selectively encapsulated by the coordination networks, whereas such coordination network shell structures were not formed on smaller amino-QDs_{525}.

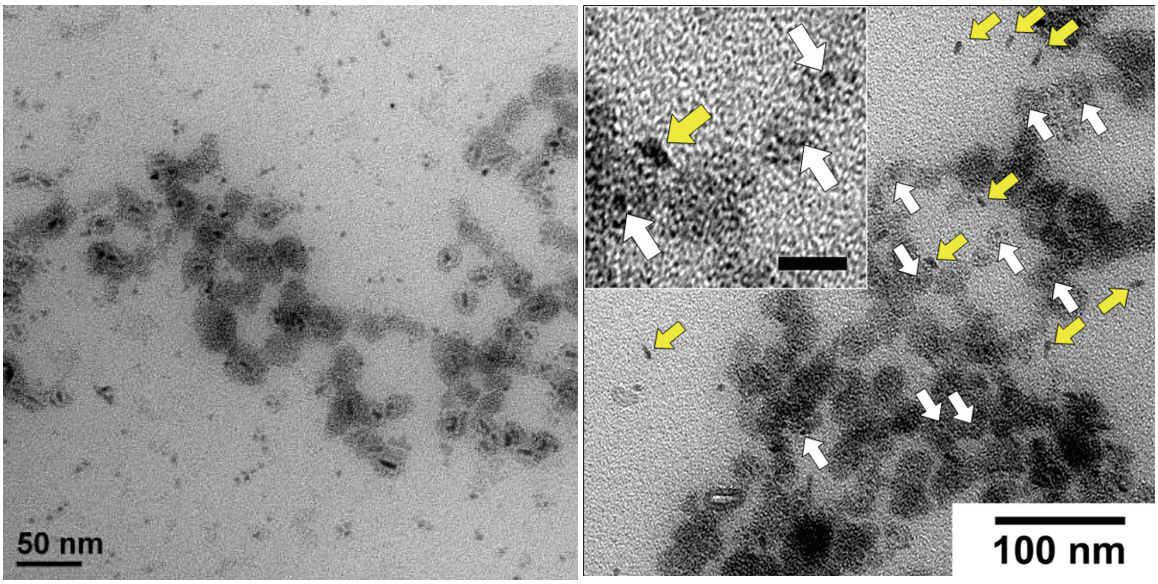

Figure S9. TEM micrographs of AMP/Gd^{3+} CNPs prepared in the aqueous mixture of carboxyl-QDs_{605} and amino-QDs_{525}. Right inset: A high-magnification TEM image (scale bar = 20 nm), in which unwrapped amino-QDs605 and carboxyl-QDs525 confined in AMP/Gd^{3+} shell are indicated by yellow and white arrows, respectively.