Implications of "Trap Emission" Observed from Quantum Dot Nanoparticles Accumulated in Toxicity Test Organism, *Daphnia magna*

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Despite the great achievements in nanosciences and nanotechnologies, there are increasing concerns about their potential harmful effects on our environment and human health.[1] Therefore, increasing number of studies on the toxicities of engineered nanomaterials have been published during last few years and understanding their environmental fates and biological impacts became one of the urgent scientific and social issues to be addressed in near future.

Quantum dots (QDs) are semiconductor nanocrystals (NCs) with size-dependent optical properties, which have great potential in many application areas, such as solar cells, light-emitting devices, biological and medical imaging.[2] However, increasing concerns for potential QD toxicities are raised recently, due to its unknown toxicity as a nanometer-scale material as well as the well-known toxicity of its constituting elements (*i.e.*, Cd, Zn, Se and etc). Although the number of toxicity studies on QDs are rapidly increasing,[3] current understanding on its toxicity mechanisms is fragmented, especially on the chemical changes occurring in QD nanoparticles. For instance, photooxidation reactions at the surface of QDs, their colloidal stability and dissolution mechanism under various biological and environmental conditions have not been completely understood yet. In this short communication, we will present our recent observations on the unique fluorescence spectral features ("trap emission" caused by the formation of surface species[4]) of QDs accumulated in *Daphnia magna*, a small freshwater crustacean commonly called "water fleas" and frequently used as a toxicity test organism.[5] Due to several unique spectral features (*e.g.*, enhanced emission intensities, unusually large red shifts and broadening) of these QD "trap emission" phenomena, we believe that this observation has important implications for better understanding of chemistry involved in the toxicity mechanisms of nanomaterials, such as loss or exchange of capping ligand (*e.g.*, TOPO).

$^{CTAB/TOPO}$QD nanocolloid used in this study was prepared according to the method by Fan *et al.*[6] cetyltrimethyl ammonium bromide (CTAB) was purchased from Sigma-Aldrich and dissolved in deionized water to form 0.01 M solutions. Then, CTAB solution was mixed with well-dispersed ^{TOPO}QD in chloroform (CdSe/ZnSe, obtained from Nanosquare Inc. (Seoul, Korea)). The mixed solution was strongly stirred for 24 hours until the formation of a QD microemulsion, which was then slightly heated up to 60 °C until the complete evaporation of organic solvent. Finally, the QD solution was further filtered with 0.2 μm syringe filter to remove unwanted large particles. The $^{CTAB/TOPO}$QD stock solution has hydrodynamic size of 48.4 nm and [QD] concentration of 0.1 nM, which is similar with the concentration range we used in the parallel QD toxicity test using *D. magna*.[7] Details of surface modification and characterization procedures for similar QDs were previously described.[8,9] *D. magna* were cultured and maintained in the Environmental Toxicology Laboratory of Seoul National University (Seoul, Korea) under the standard conditions outlined by the US EPA.[10] For dosing $^{CTAB/TOPO}$QD on *D. magna* neonates (< 24 hours old), $^{CTAB/TOPO}$QD stock solution was diluted to the desired concentration with moderately hard water (MHW), which was prepared following US EPA guideline.[10]

Fluorescence images and spectra were obtained using Olympus IX51 inverted type microscope equipped with Ocean optics QE65000 scientific-grade spectrometer. Fluorescence spectra collected with excitation energy of 400 nm from $^{CTAB/TOPO}$QD in MHW media and *D. magna* exposed to

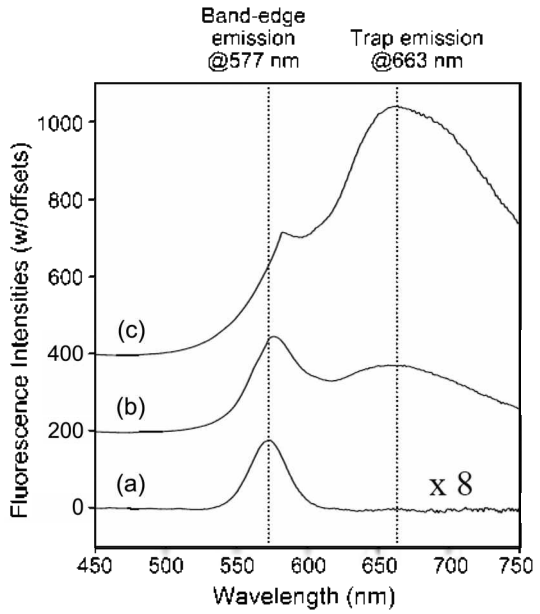

Figure 1. Fluorescence spectra of (a) $^{CTAB/TOPO}$QD in MHW media, (b) and (c) *D. magna* exposed to $^{CTAB/TOPO}$QD.

$^{CTAB/TOPO}$QD solutions are presented in Figure 1. In MHW solution. $^{CTAB/TOPO}$QD has its band-edge emission peak located at 577 nm (see Figure 1(a)). However. as can be seen in Figure 1(b) and (c). fluorescence spectra collected from *D. magna* show quite different spectral features. First of all. significant enhancements (~10 times) of band-edge emission were observed. which probably resulted from accumulation of the $^{CTAB/TOPO}$QD within *D. magna* species as well as fluorescence yield enhancement caused by the exchange of coordinating ligands.[4] Additionally, the fluorescence spectra from *D. magna* have red-shifted. broadened and significantly enhanced (~30 times compared to solution QD band emission) peak located around 663 nm. These additional spectral features were observed repeatedly for several different *D. magna* specimens cultured under the same solution conditions, including those presented in Figure 1(b) and (c). although their relative intensity ratios between the two peaks were varied from specimen to specimen.

Since *D. magna* were repeatedly rinsed with MHW media before the fluorescence measurements and potential autofluorescence contributions from *D. magna* or microalgae (used to feed *D. magna*, *e.g.*, *Selanastrum capricornutum*) were carefully considered and completely subtracted (see Figure 2 for their spectra). it can be assured that the red-shifted and broadened peaks around 663 nm is closely related to the chemical state changes of QDs accumulated within *D. magna*. According to recent literature.[4] wide variation of the quantum yields (QY) and blue or red shits of absorption/emission peak positions were observed depending on the type of organic ligand on the QD surface. For instance. Kalyuzhny and Murray,[4] in their studies on the effects of purification and aging process, suggested that the loss or exchange of capping ligand is primarily responsible for the changes in photoluminescence during the purification and aging process. However. the extent of red-shift and broadening observed in Figure 1(b) and (c) is unusually large. therefore simple ligand exchange or loss mechanism can not explain our observation in *D. magna*. In Kalyuzhny and Murray's work. they also reported another interesting phenomena in their fluorescence spectral features. which is very similar with our observations shown in Figure 1(b) and (c). They observed a new broad photoluminescence peak located around 700 nm (peak maximum varies widely between 650-750 nm. depending on the ligands). which is called as "trap emission" and its intensity increased as the loss of the surface ligand proceeds. Actually. this red-shifted and broadened "trap emission" was also observed previously by several researchers under chemical environments,[11-13] but the origin of this peak has never been clearly identified until Kalyuzhny and Murray's work,[4] which claimed the chemical state responsible for the "trap emission" is associated with release of TOP-bound Se atoms at the surface of QDs. Formation of the TOPSe or similar type of TOPO species at the QD surface and following "trap emission" is very unique and will be very useful to monitor chemical state changes in QDs exposed to various biological and environmental systems. Since one of the main scenarios for nanoparticle (NP) toxicity is the accumulation of NPs inside the biological system followed by release of toxic ions/elements,[3] this unique spectral feature of "trap emission" (*e.g.*. enhanced intensity and unusually large red-shift of peak) could be a very sensitive probe (in the concentration range of sub nanomolar QD) for the progress of QD degradation accumulated within *D. magna*, as they experience loss of TOPO molecules at the interface, probably due to some kind of harsh biological environments (*e.g.*. high concentration of proteins. nucleic acids and other biomolecules) within *D. magna*. Finally, It is also important to mention that the "trap emission" was observed so far only in the case of *D. magna* with $^{CTAB/TOPO}$QD dosing. among several biological (*e.g.*. in the presence of microorganism, such as *E. coli*)[8] and chemical environments (*e.g.*. in the presence of various media, such as LB media used for microorganism, MHW for *D. magna* as well as UV exposure condition).[7] According to our interdisciplinary studies on QD toxicity on *D. magna*,[7] the EC_{50} of QDs for *D. magna* (neonate stage) are in the range of subnanomoles of [QD]. much higher than that of [Cd^{2+}] but still very low for most analytical tools to collect chemical information on this nanoparticles. Therefore, we think that the "trap emission" observed in *D. magna* will contribute significantly for better understanding of chemistry involved in the toxicity mechanisms of QD nanoparticles.

Supporting Information. Additional figure of autofluorescence spectra of *Daphnia magna* and its feeding materials are given as Figure S1 of supporting information and available at http://www.kcsnet.or.kr/bkcs

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