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Effects of Quantum Dots Adsorption on Algal Photosynthesis

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We report on the adsorption of CdSe/ZnS quantum dots and its effects on algal photosynthesis. A logarithmic trend was established for the adsorption process using UV−vis spectrophotometry, with Freundlich constants determined as $k = 0.588$ ppm^{1-n} and $n = 0.629$. Both our CO_2 depletion and O_2 production assays showed a significantly inhibited photosynthetic activity of the algae exposed to the quantum dots, suggesting the potential impact of nanoparticle adsorption on the photochemistry of plant species.

1. Introduction

Along with the rapid development of nanotechnology, a major technological advancement of the present time, there is a growing interest and a need to understand the biological and environmental consequences of engineered nanomaterials.[1−3] Obtaining such an understanding will benefit the biological, medicinal, and environmental applications of nanotechnology. Additionally, research in this area will guide the design and production of nanomaterials to minimize their potential adverse effects on human health and the environment.[2]

Quantum dots (QDs) are a major class of semiconducting nanocrystals which possess unique optical, electrical, and chemical properties.[4−6] Since their early development in the 1980s, QDs have been used extensively in such biological applications as cell labeling, fluorescence in situ hybridization, pathogen detection, ligand binding, genomic and proteomic detection, and high-throughput screening of biomolecules.[4−10] The toxicity of QDs has also been examined, and strategies—though far from optimal—have been developed to improve the biocompatibility of QDs through ligand exchange, hydrophobic interaction, and encapsulation.[6]

Like other classes of engineered nanoparticles (ENPs), QDs may eventually be discharged through industrial and research outlets and impact living organisms in the environment. Despite their broad use in many applications, fundamental research on the biological and environmental fates of ENPs is critically lacking.[11,12] Specifically, little is known about the interaction between ENPs and plant species,[11−15] the major component of ecosystems and the food chain. Among the literature available, mixtures of nano-SiO_2 and nano-TiO_2 were reported to increase nitrate reductase activity, enhance water and nutrient uptake, stimulate the antioxidant system, and hasten germination and growth of soybean (*Glycine max*).[16] Nano-TiO_2 at 0.25% increased seed germination, plant dry weight, chlorophyll production, and the RuBP activity and the rate of photosynthesis of spinach (*Spinacia oleracea*), while concentrations greater than 0.4% nano-TiO_2 were found to be detrimental to plant growth.[17] Chlorophyll-bound gold and silver nanoparticles were found to enhance the production of excited electrons in the photosynthetic complex.[18] Uncoated alumina nanoparticles at 2000 mg/L were reported to reduce the root growth of corn, carrot, cucumber, soybean, and cabbage seedlings, while alumina nanoparticles coated with phenanthrene had no effect on the root growth of vegetation.[19] Our recent study[20] demonstrated the uptake of fullerene C_{70} by rice plants. These fullerene particles were found to share the vascular systems with water and nutrients and were transmitted to the second generation of the rice plants through seeds harvested from the first generation.

Here we use *Chlamydomonas* sp., the single-celled green algae, as a model system for examining the interaction of QDs with plant species. Like most high plants and bacteria, algae possess a cell wall outside their cell membrane. However, unlike mammalian cells, algae do not show robust endocytosis when exposed to foreign materials and particles. Previous spectroscopic and electron microscopic results suggest that small QDs of less than 5 nm in diameter, when aided by light, can enter bacteria possibly by means of oxidative damage to the cell wall and the cell membrane.[21] Since functionalized QDs are typically larger than 5 nm, our current study focuses on addressing the effects of QDs adsorption on algae photosynthesis.

2. Experimental Section

2.1. Materials. Yellow fluorescent CdSe/ZnS core/shell quantum dots (Ex: <550 nm; Em: 570−585 nm) were purchased from NN-Laboratories, LLC. The QDs were rendered water-soluble by coating mercaptoundecanoic acid (MUA) ligands on the QD surfaces. The average hydrodynamic diameter of the QDs was determined by dynamic light scattering (Zetasizer S90, data not shown) as 11.69 nm. The dimensions of dried QDs, determined by transmission electron microscopy (TEM) (Figure 1a), were 5−9 nm. Fresh *Chlamydomonas* sp., the spherically shaped algal cells (Figure 1b), were harvested from the greenhouse at the Clemson University Facility.

2.2. Incubation of Algae with QDs. Algal cells were concentrated by low-speed centrifugation (11 700 RCF/12 960 rpm) for 3 min. The concentrated algae were then incubated with QDs of 0.05−5 ppm at room temperature for 2 h. Four samples of each concentration were prepared to ensure experimental repeatability and to establish error bars.

2.3. Bright Field Imaging. QDs incubated algae were imaged under the bright field mode with use of a fluorescence

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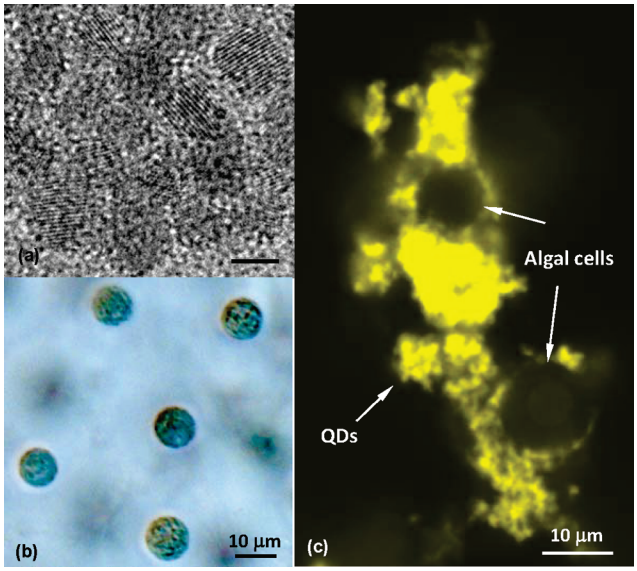

Figure 1. (a) Transmission electron microscopy image of CdSe/ZnS QDs, whose sizes are in the range of 5−9 nm. The QDs lattices are visible. Scale bar: 5 nm. (b) Bright field image of algal cells. (c) Confocal fluorescence image of QDs (yellow) adsorbed on algal cell (round) surfaces.

microscope (Imager A1, Zeiss). Approximately 10 μL of algae/QDs solution was flowed into a sample channel sandwiched between a glass substrate and a cover glass prior to imaging.

2.4. Confocal Fluorescence Imaging. Algal cells were incubated with QDs in an 8-well chamber glass overnight prior to confocal fluorescence imaging (LSM 510, Zeiss). Samples were excited with an argon ion laser at 488 nm and fluorescence images were captured with use of a BP 570−590 filter set and a 40× oil immersion objective.

2.5. Transmission Electron Microscopy. A small volume of QDs suspension (10 μL) was air-dried directly onto a TEM grid prior to imaging. The concentration of the QDs was 0.1 mg/mL. Imaging was conducted with a Hitachi H-9500 high-resolution transmission electron microscope, under a 100 kV accelerating voltage.

2.6. Quantification of QDs Adsorption. A UV−vis spectrophotometer (Biomate 3) was used to quantify the amount of QDs adsorbed on the algae. Absorbance was recorded at 545 nm before and after adding various concentrations of QDs into the algal growth medium, with the differences denoting total concentrations of the QDs. At this wavelength QDs showed a strong absorbance. After 2 h of incubation, 10 μL of NaOH was added in the algae/QDs solution before filtering through membranes with a pore size of 0.45 μm (Nalgene). The introduction of NaOH was to prevent the aggregation of negatively charged QDs in the weakly acidic environment of the algal growth medium (pH 6.45). After filtration, all algal cells were blocked by the membranes because of their large size (~10 μm, Figure 1b), while the absorbance of the filtrate indicated the amount of free (or unadsorbed) QDs. The amount of adsorbed QDs can be calculated by eq 1:

$$\mathrm{Abs_{adsorbed}} = (\mathrm{Abs_{QDs+algae}} - \mathrm{Abs_{algae}}) - \mathrm{Abs_{filtered}} \quad (1)$$

where $\mathrm{Abs_{filtered}}$ and $\mathrm{Abs_{QDs+algae}}$ denote the absorbance of the algae/QDs solution before and after filtration, and $\mathrm{Abs_{algae}}$ is the absorbance of the algae alone. An adsorption curve was established by varying the QDs concentration from 0.1 to 5 ppm (Figure 2).

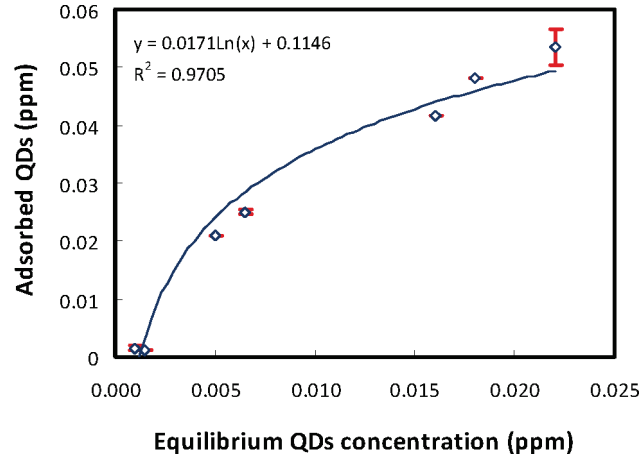

Figure 2. Adsorbed vs. equilibrium QDs concentration. The equilibrium QDs concentration is the adsorbed QDs subtracted from the initial QDs sample concentration. Also shown is a fitted logarithmic trendline.

2.7. Analysis of Algal Photosynthesis. The standard photosynthetic reaction is described by eq 2:

$$6CO_2 + 12H_2O + \text{photons} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O \quad (2)$$

To evaluate the effects of QDs absorption on algal bioactivities, we examined both oxygen production and carbon dioxide depletion of the algae, which were incubated with the QDs. A bicarbonate indicator solution (0.2 g of thymol blue, 0.1 g of cresol red, in 0.01 M $NaHCO_3$) was prepared to monitor the depletion of CO_2, the activities of which are depicted by eq 3:

$$HCO_3^- + H^+ = H_2O + CO_2 \quad (3)$$

The algae/QDs solution was mixed with the indicator solution and the samples were tightly sealed to prevent gas exchange. During photosynthesis, the algae consumed CO_2 over time, causing the pH value of the indicator solution to increase accordingly. A transition from acidic to basic condition was indicated by a color change from yellow to purple, accompanied by an increase of absorbance at 574 nm (TOC, right panel). The depletion rates of CO_2 were then calculated based on the increase of absorbance values for different sample concentrations. To measure oxygen production of the algae in the presence of the QDs, an Oxyg32 system (Hansatech Instruments) was used for a fixed amount of algal cells treated with various dosages of QDs. All measurements were made at room temperature, under identical lighting conditions.

2.8. Fluorescence of Algae in the Presence of Adsorbed QDs. A fluorescence spectrophotometer (Varian Cary Eclipse) was used to examine the effects of QDs fluorescence on the photo properties of algal cells. The excitation was set at 435 nm, a wavelength known to induce strong light absorption by the chlorophyll in algae.[22,23] Fluorescence spectra were recorded for algae, QDs (50 ppm), and algae/QDs solution (QDs: 50 ppm; incubation time: 2 h) within the wavelength range of 500−800 nm, at 1 nm/step.

3. Results and Discussion

3.1. Adsorption of QDs to Algal Cells. An adsorption curve is shown in Figure 2 for the amount of adsorbed vs. the amount

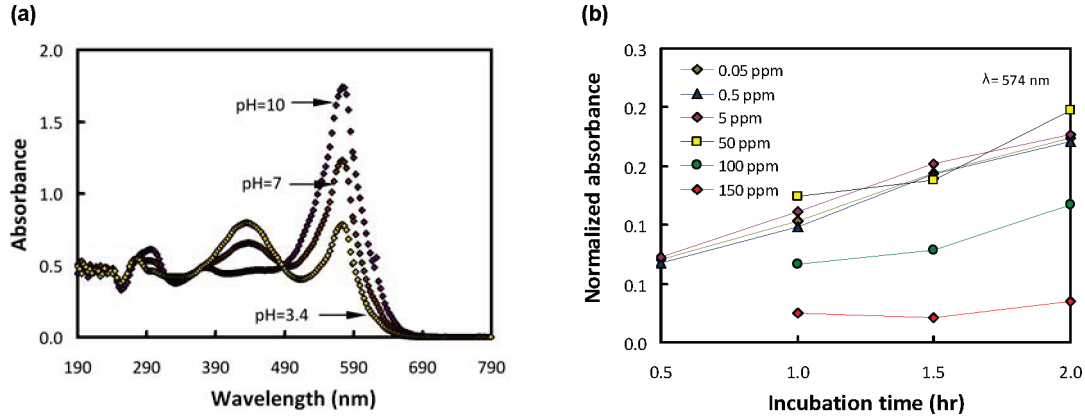

Figure 3. (a) Absorbance of bicarbonate indicator for acid, neutral, and basic solutions. The absorbance peaks occur at 574 nm, increasing with the pH values of the solutions. (b) Comparison of CO_2 depletion rates for QDs of various dosages, measured at 574 nm. Significantly reduced CO_2 depletions were found at and above 100 ppm of QDs.

of free QDs. This curve was derived from eq 1 as described in Section 2.6. Adsorption of QDs to algal cells increased with the dosage of the QDs. A logarithmic increase in the QD amount adsorbed was observed with increased equilibrium concentration of the QDs. The surface area of a typical algal cell (~10 μm in diameter) is at least 250 000 times larger than that of a QD (<20 nm in diameter), which allowed for a significant amount of the QDs to be adsorbed.

The QDs used in this study were surface-coated with mercaptoundecanoic acid (MUA), a ligand to elicit water solubility. When examined under bright field imaging, the algae exposed to the QDs appeared less mobile than the control algae. As stated in the literature,[24] the pore size of a plant cell wall is approximately 5−20 nm in diameter. The chemical composition of the porous algae cell wall consists of cellulose, polysaccharides, and glycoproteins, which afforded numerous QDs binding sites through nonspecific (electrostatic, hydrophobic, and hydrogen bonding) interactions. The binding affinity of QDs for algae can also be due to the interaction between the carboxylic groups (−COOH) of the MUA and the amine groups ($-NH_2$) of the algal cell wall. The overall interaction between algae and QDs can physically and/or chemically damage the algal cell wall and membrane to facilitate uptake of the QDs. However, no such uptake was observed in our electron microscopy imaging, possibly due to the size of the QDs, the aggregation of the QDs in the weakly acidic algal growth medium, as well as the thickness of the algal cell wall (~20 nm).[25]

To better understand the physical adsorption of QDs to algae, we used the Freundlich model[26] to fit the adsorption isotherms. The Freundlich model is a modification of the Langmuir adsorption scheme, and is appropriate for describing rough inhomogeneous adsorbent (i.e., algae) surfaces with multiple adsorption sites. Considering the adsorbate−adsorbate (i.e., QDs−QDs) interactions, the empirical Freundlich equation is expressed in eq 4[27]

$$q_{eq} = kC_{eq}^{\ n} \tag{4}$$

where k is a coefficient indicating the affinity of QDs for algae, n is a constant characteristic of the adsorption system and is related to the binding efficiency. An n value of less than 1 indicates a favorable adsorption, while an n value higher than 1 reflects a weak adsorption.[27] The parameters C_{eq} and q_{eq} represent the concentrations of nonadsorbed QDs and the QDs adsorbed on the algae at equilibrium, respectively.

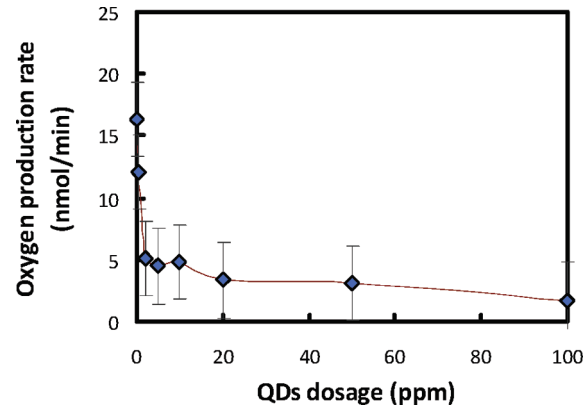

Figure 4. Oxygen evolution rate vs. dosage of QDs. Significant reduction of oxygen production was observed due to the introduction of QDs to algae solution.

According to the Lambert−Beer law, absorbance is proportional to the concentration of the QDs. As such, the parameters of the Freundlich equilibrium model were fitted from a log−log plot of C_{eq} vs. q_{eq}. The plot slope represents the exponent $1/n$, and the value of k can be read from the intercept. For our absorbance data, the value of k was determined as 0.588 ppm^{1-n}, with its exponent n fitted at 0.629. This n value suggests a favorable binding of the QDs to the algae.

3.2. Adsorption Effect on Algal Photosynthesis. Algae consume CO_2 that is dissolved in aqueous solution for photosynthesis; they also produce O_2 as an end product. Thus, the rate of CO_2 depletion is an important parameter for indicating the photosynthetic activity of the algae. According to eq 3, an increase in the pH corresponds to a decrease in the concentration of CO_2 in the bicarbonate indicator solution. As shown in Figure 3a, the absorbance of the bicarbonate indicator at 574 nm increases with its pH. Therefore, the rate of the increasing absorbance can be treated as an indicator of the CO_2 depletion rate. As shown in Figure 3b, an increased dosage of QDs resulted in a significant decrease of CO_2 depletion rate at and above 100 ppm of QD dosage.

3.3. Reduced O_2 Production Rate. As shown in Figure 4, the oxygen production rate was significantly affected by the addition of different QD concentrations to a fixed concentration of algal solution. Above 5 ppm of QD dosage, the O_2 production rate decreased to nearly zero, indicating a significantly reduced photosynthetic activity. Both experiments proved that the introduction of QDs to algal growth media affected their photosynthetic activity. CO_2 depletion was significantly reduced

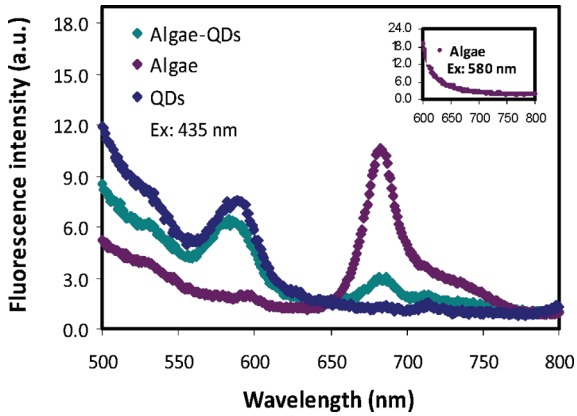

Figure 5. Fluorescence intensities of algae (purple), QDs (dark blue), and algae−QDs mixture (cyan), excited at 435 nm where the chlorophyll in algae absorbs light efficiently. The algae and the QDs show strong fluorescence peaks at 683 and 580 nm, respectively. By contrast, the mixture of algae−QDs displays a less prominent fluorescence peak than QDs at 580 nm, and a much less prominent peak than algae at 683 nm (cyan). The fluorescence emission of algae is negligible when excited at 580 nm (inset).

above 100 ppm of QDs dosage, while retarded O_2 production rate occurred in the low ppm range of QDs dosage. Previous studies with ENPs (ZnO, TiO_2, and CuO)[28−30] mainly focused on the growth inhibition of algae, perhaps due to the light shading effect, but neglected the photosynthetic function of the algae. As a primary producer in the food chain, the photosynthetic activity of algae is equally important as its reproduction behavior. Introducing QDs to algal growth media triggered the interactions between the algae and the QDs, which included both adsorption and possible translocation of the QDs within the algal cells. Adsorption of QDs is expected to hinder the CO_2 gas flow through algal cells needed for photosynthesis, block the pathways of nutrients uptake, and impede algal mobility via obstruction of their flagella movement. Furthermore, adsorption of QDs can damage algal cell walls to induce pore formation, which facilitated translocation of the QDs. Although not evident in our study, QDs post uptake may also bind to pyrenoids serving as centers for CO_2 fixation in algal chloroplasts, or generate reactive oxygen species (ROS) inside algal cells, both of which reduce algal bioactivity.

3.4. Effects of QDs Fluorescence on Algae. As shown in Figure 5, when excited by light at 435 nm, QDs emitted a strong fluorescence peak at 580 nm (blue) while algae emitted a prominent fluorescence peak at 683 nm (purple). The algae/QDs solution, by contrast, displayed two fluorescence peaks both at 580 and 683 nm (cyan). Specifically, the fluorescence peak at 580 nm for the algae/QDs solution was reduced by 20% as compared to that for free QDs, while the peak at 683 nm for the algae/QDs solution was reduced remarkably by 73% as compared to that for the algae. The reduction of QDs fluorescence at 580 nm could be due to the inefficient light absorption by the QDs when adsorbed into multilayers on the algal cell surfaces, or the fluorescence resonance energy transfer (FRET)[31−33] from the QDs (donor) to the algae (acceptor). However, we exclude FRET as a major cause for the reduced fluorescence intensities based on these following observations: (a) lack of anticorrelation between the QDs fluorescence peak at 580 nm and the algal fluorescence at 683 nm, as indicated by the cyan curve, and (b) lack of fluorescence emission (resonance) when algae were directly excited by light at 580 nm (Figure 5 inset). Since intracellular chloroplasts were separated from the extracellular QDs by far more than a Förster distance,[31] fluorescence energy transfer between the adsorbed QDs and the algae was deemed unfeasible. The subdued fluorescence peak at 683 nm for the algae/QDs solution (Figure 5) is attributed to light emission from free or less coated algae, which could be directly excited by light at 435 nm.

4. Conclusions

The large-scale production of ENPs is fueled by the promises and applications of these nanocrystals in bioimaging, sensing, and nanomedicine. Along with these technological developments, understanding the impact of ENPs on biological and ecological systems is becoming increasingly relevant for the exploration of new research schemes and for protection of the environment from the potentially adverse effects of nanomaterials. In this study, we found that water-soluble CdSe/ZnS QDs, a major class of ENPs, have a high affinity for the *Chlamydomonas* sp. algae. The adsorption of the QDs to the algal cell surfaces is from a combined result of nonspecific interactions, as well as possible reactions between the amine groups of the polysaccharides or glycoproteins in the algal cell wall and the carboxyl groups of the MUA ligands coated on the QDs. The porous structure of the algal cell wall also afforded ample binding sites for the QDs. We have shown that the amount of QDs adsorbed onto algae logarithmically depends upon the equilibrium concentration of the QDs. No clear evidence of QDs internalization by algae was found from our TEM study, although QD adsorption on the algae surface was apparent from our bright field and confocal fluorescence imaging. This lack of internationalization may be explained as a result of the thick algal cell wall, the size and mutual aggregation of the QDs, and a lack of endocytosis capability of the algae. Consistently, our fluorometry study indicates that FRET is unfeasible between the adsorbed QDs and the algal cells. Although heat conduction from the light-absorbing QDs could impact on the biochemical activities of the algae, Fourier's law predicts no significant effect of such process on the photochemistry of the current study, which was conducted under equilibrium conditions at controlled room temperature. The adsorption of QDs has been found to hinder the photosynthetic activity of algae, as indicated by both reduced CO_2 depletion for QDs over 100 ppm and declined O_2 production for small dosages of QDs. These physiochemical phenomena were likely caused by the adsorbed QDs which impeded gas flow and nutrients uptake for the algae, although no apparent algal cell death was observed in our current study. Since algae are primary food sources for aquatic organisms in natural ecosystems, further studies to decipher the mechanisms and long-term effects of the interactions between algae and various types of ENPs are deemed necessary.

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References and Notes

(1) Nel, A.; Xia, T.; Madler, L.; Li, N. *Science* **2006**, *311*, 622.
(2) Maynard, A. D.; Aitken, R. J.; Butz, T.; Colvin, V.; Donaldson, K.; Oberdörster, G.; Philbert, M. A.; Ryan, J.; Seaton, A.; Stone, V.; Tinkle, S. S.; Tran, L.; Walker, N. J.; Warheit, D. B. *Nature* **2006**, *444*, 267.

(3) Ke, P. C.; Qiao, R. *J. Phys.: Condens. Matter* **2007**, *19*, 373101.
(4) Alivisatos, P. *Nat. Biotechnol.* **2004**, *22*, 47.
(5) Yu, W. W.; Chang, E.; Drezek, R.; Colvin, V. L. *Biochem. Biophys. Res. Commun.* **2006**, *348*, 781.
(6) Klostrance, J. M.; Chan, W. C. W. *Adv. Mater.* **2006**, *18*, 1953.
(7) Xing, Y.; Wang, M. D.; Nie, S. M. *Nat. Protoc.* **2007**, *2*, 1152.
(8) Mortensen, L. J.; Oberdörster, G.; Pentland, A. P.; DeLouise, L. A. *Nano Lett.* **2008**, *8*, 2779.
(9) Smith, B. R.; Cheng, Z.; De, A.; Koh, A. L.; Sinclair, R.; Gambhir, S. S. *Nano Lett.* **2008**, *8*, 2599.
(10) Xie, H.; Li, Y. F.; Kagawa, H. K.; Trent, J. D.; Mudalige, K.; Cotlet, M.; Swanson, B. I. *Small* **2009**, *5*, 1036.
(11) Navarro, E.; Baun, A.; Behra, R.; Hartmann, N. B.; Filser, J.; Miao, A.-J.; Quigg, A.; Santschi, P. H.; Sigg, L. *Ecotoxicology* **2008**, *17*, 372.
(12) Hannah, W.; Thompson, P. B. *J. Environ. Monit.* **2008**, *10*, 291.
(13) Zhu, H.; Han, J.; Xiao, J. Q.; Jin, Y. *J. Environ. Monit.* **2008**, *10*, 713.
(14) Etxeberrie, E.; Gonzalez, P.; Baroja-Fernandez, E.; Romero, J. O. *Plant Signaling Behavior* **2006**, *1*, 196.
(15) Liu, Q.; Chen, B.; Wang, Q.; Shi, X.; Xiao, Z.; Lin, J.; Fang, X. *Nano Lett.* **2009**, *9*, 1007.
(16) Lu, C. M.; Zhang, C. Y.; Wen, J. Q.; Wu, G. R.; Tao, M. X. *Soybean Sci.* **2002**, *21*, 168.
(17) Zheng, L.; Hong, F.; Lu, S.; Liu, C. *Biol. Trace Elem. Res.* **2005**, *104*, 83.
(18) Govorov, A. O.; Carmeli, I. *Nano Lett.* **2007**, *7*, 620.
(19) Yang, L.; Watts, D. *Toxicol. Lett.* **2005**, *158*, 122.
(20) Lin, S.; Reppert, J.; Hu, Q.; Hudson, J. S.; Reid, M. L.; Ratnikova, T. A.; Rao, A. M.; Luo, H.; Ke, P. C. *Small* **2009**, *5*, 1128.
(21) Kloepfer, J. A.; Mielke, R. E.; Nadeau, J. L. *Appl. Environ. Microbiol.* **2005**, *71*, 2548.
(22) Frense, D.; Müller, A.; Beckmann, D. *Sens. Actuators, B* **1998**, *51*, 256.
(23) Morrison, S. S.; Mullineaux, C. W.; Ashby, M. K. *BMC Microbiol.* **2005**, *5*, 47.
(24) Smith, H. *The Molecular Biology of Plant Cells*; University of California Press: Berkeley, CA, 1978.
(25) Roberts, K.; Hills, G. J. *J. Cell Sci.* **1976**, *21*, 59.
(26) Weber, W. J., Jr. *Adsorption Technology: A Step-by-Step Approach to Process Evaluation and Application*; Marcel Dekker: New York, 1985.
(27) Ribeiro, M. H. L.; Lourenco, P. A. S.; Monteiro, J. P.; Ferreira-Dias, S. *Eur. Food. Res. Technol.* **2001**, *213*, 132.
(28) Hund-Rinke, K.; Simon, M. *Environ. Sci. Pollut. Res.* **2006**, *13*, 225.
(29) Aruoja, V.; Dubourguier, H.-C.; Kasemets, K.; Kahru, A. *Sci. Total Environ.* **2009**, *407*, 1461.
(30) Fujiwara, K.; Suematsu, H.; Kiyomiya, E.; Aoki, M.; Sato, M.; Moritoki, N. *J. Environ. Sci. Health, Part A* **2008**, *43*, 1167.
(31) Stryer, L.; Haugland, R. P. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *58*, 719.
(32) Weiss, S. *Science* **1999**, *283*, 1676.
(33) Lin, S.; Keskar, G.; Wu, Y.; Wang, X.; Mount, A. S.; Klaine, S. J.; Moore, J. M.; Rao, A. M.; Ke, P. C. *Appl. Phys. Lett.* **2006**, *89*, 143118.

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