New Aspects of the Environmental Risks of Quantum Dots: Prophage Activation

Juan Xu[1,+], Huan He[1,+], Ying-Ying Wang[1], Ren Yan[1], Lian-Jiao Zhou[1], Feng-Lei Jiang[1,*], Thomas Maskow[2,*] Yi Liu[1,3,4,*]

[1] State Key Laboratory of Virology & Key Laboratory of Analytical Chemistry for Biology and Medicine (MOE) & Key Laboratory of Biomedical Polymer Materials (MOE), College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, P. R. China

[2] UFZ, Helmholtz Centre for Environmental Research, Department of Environmental Microbiology, Permoserstr. 15, 04318 Leipzig, Germany

[3] Key Laboratory of Coal Conversion and Carbon Materials of Hubei Province, College of Chemistry and Chemical Engineering, Wuhan University of Science and Technology, Wuhan 430081, P. R. China

[4] College of Chemistry and Material Sciences, Guangxi Teachers Education University, Nanning 530001, P. R. China

*Corresponding Author. Tel.: 86-27-68756667, 49-341-2351328. Fax: 86-27-68754067. 49-341-2351328 Email address: yiliuchem@whu.edu.cn, thomas.maskow@ufz.de, fljiang@whu.edu.cn.

[+] These authors contributed equally to this work.

Abstract

A few thousand tons of nanoparticles and quantum dots (QDs) are produced yearly worldwide, and a significant amount is released into ecosystems. This knowledge has stimulated numerous studies on the toxicological properties of these nanomaterials. However, an important ecotoxicological aspect has been largely overlooked: the activation of silent viruses in bacteria (so-called prophages). This is particularly important because, once the prophages are activated; phage replication using bacterial hosts is an autocatalytic process with a potentially exponential rate of bacteria killing under certain conditions. To shed light onto these underestimated processes, the interactions of differently functionalized CdTe QDs with *E. coli* containing prophages were investigated. We found that prophages can be activated with as little as a nanomolar concentration range of QDs. DNA damage due to oxidative stress induced by the CdTe QDs was revealed as the main reason for the prophage activation. The contribution of freely dissociated Cd^{2+} to prophage activation was on the order of 15 to 25%. Our pioneering work is intended to provide a first examination to better understand the role of nanoparticles in aquatic ecosystems.

Introduction

Nanoparticles and especially quantum dots (QDs) have numerous applications in various areas such as drug delivery, biosensors, biomedical imaging, solar energy generation and electronic devices due to their desirable optical and electrochemical properties[1]. The yearly production of nanomaterials is estimated to be 5000 t (TiO_2), 500 t (Ag) and 350 t (carbon nanotubes)[2]. Recent investigations have raised concerns over adverse effects on ecosystem health after the release of nanomaterials into soil and water[3] because QDs attack bacteria, fungi, algae and protozoa on different organismic levels even at very low concentrations[4]. Additionally, QDs can be enriched by surface adsorption, bio-accumulation and biomagnification in aquatic ecosystems[5]. Numerous studies have revealed the adverse effect of QDs on bacterial growth and biodegradation processes[6,7,8] and have defined the toxicity targets[9,10]. However, the potential influence of QDs on ecosystems through the activation of silent viruses inside of bacteria is mostly neglected. When temperate bacteriophages infect bacteria, they integrate their genome into the chromosome(s) of their host and replicate their DNA along with the bacterial chromosome without producing viruses[11]. This phenomenon is called lysogeny, and the integrated phage genome is called the prophage. Evidence has shown that lysogenic bacteria are common in water and soil environments,[12] and the proportion of bacterial strains containing prophages may be as high as 80% [13]. Environmental factors such as UV irradiation, chemical exposure and temperature alteration can activate the prophages[14]. A common feature of prophage activation is the damage to the bacterial DNA and a subsequent irreversible shift of lysogeny to lysis[15]. Numerous literatures have also pointed out that the DNA breaks are critical for the switch from the lysogenic cycle into the lytic cycle[16]. As a result, progeny phages capable of infecting other bacteria are spewing into the

environment. This could potentially cause a significant reduction in the abundance of host species and community composition, and finally result in the destruction of the micro-ecological balance[17]. Several conventional chemicals (organic and inorganic)[18,19,20] have been reported to activate prophages. In light of this evidence, and considering the ecological importance of prophages, it is surprising that the activation of prophages by nanomaterials and QDs has not already been reported. There is evidence suggesting that the uptake of QDs is accompanied by the release of heavy metal ions and the production of reactive oxygen species (ROS)[6, 7, 21]. ROS are known to damage DNA and thereby activate prophages. The ROS production rate can even be photochemically accelerated using nanocrystals and light[22,23]. Photochemically activated nanocrystalline TiO_2 is already reported to induce prophages[24]. Additionally, evidences have shown that heavy metal ions like Cd^{2+}, Cu^{2+} and Ag^{+} cause damage to the bacteria DNA.[25,26] It is important to clarify these findings to prove whether QDs alone (without light) can activate prophages and to determine the critical threshold concentrations. Furthermore, the possible mechanisms should be illuminated. Finally, the influence of the chemical structure of QDs on prophage activation has to be considered because the biological activities of QDs are closely related to their chemical structures[27]. We suggest *Escherichia coli* DSM4230 with λ prophage and CdTe with different surface ligands as suitable objects of investigation because there is already a large body of knowledge about both systems.

Materials and methods

Materials. $CdCl_2$ (99.99%), 3-mercaptopropionic acid (MPA, 99%), L-glutathione (GSH, reduced, 98%), N-acetyl-L-cysteine (NAC), DL-dithiothreitol (DTT), L-ascorbic acid (Vc), 2',7'-dichlorofluorescein diacetate (DCFH-DA) and tellurium powder (99.999%, approximately 200 mesh) were obtained from Sigma-Aldrich and

were used without further purification. All other reagents were of analytical grade. Ultrapure water with 18.2 MΩ cm^{-1} (Millipore Simplicity) was used in all aqueous solutions.

Preparation and quantification of CdTe QDs. CdTe QDs capped with MPA and GSH were synthesized according to Xiang et al.[27] Before using, freshly synthesized QDs were washed three times in 2-propanol. Afterwards, QDs were dispersed in ultrapure water, and the solutions were dialyzed for 4 h to remove impurities. The resultant QDs were stored at 4 °C in the dark for the subsequent experiments. QDs were quantified by UV/VIS spectroscopy using the extinction coefficients at 524 nm (MPA-CdTe QDs) and 504 nm (GSH-CdTe QDs)[28].

Bacterial strains and culture conditions. *Escherichia coli* DSM4230 (obtained from DSMZ Braunschweig, Germany) (without (λ-) and with prophages (λ+)) was used. The prophage was established in the λ+ strain as described by Xu et al[29]. In short: The *E. coli* DSM 4230 was activated by an overnight cultivation in LB medium to a final OD = 0.36 (corresponding to $2x10^8$ cfu/mL). 1 mL of this *E. coli* suspension was infected with the lambda phage (DSM 4499) (with a final titer: $5x10^7$ pfu/mL) corresponding to a MOI (multiplicity of infection) of 0.25. The mixture was incubated for 30 min at 37°C without shaking, serial diluted and plated on LB agar. After 2 days of incubation at 37°C and a further incubation at room temperature, single colonies inside the phage plaques were isolated. The isolates were purified several times, checked for lambda phage segregation and sensitivity, and used for further investigations. All growth experiments were conducted at 37 °C. Cultures were maintained on LB medium amended with maltose (2 g/L) and $MgSO_4 \cdot 7H_2O$ (0.12 g/L). All media were autoclaved at 121 °C fore final pH was 7.2 ± 0.2, and the buffer capacity was high enough to maintain the pH 20 minutes. The various amounts of

QDs were aseptically added to the medium. The final pH was 7.2 ± 0.2, and the buffer capacity was high enough to maintain the pH during growth.

Quantification of bacterial growth and phage propagation. Bacterial growth influenced by phage propagation was quantified off-line (as the optical density at 600 nm) and on-line (as the metabolic heat by microcalorimetry) in independent experiments. The optical densities were determined with a Hitachi U-2900 UV-Vis spectrophotometer, (Hitachi High-Tech, Tokyo, Japan). Growth was monitored on-line by a thermal activity monitor III (TAM III, TA Instruments, New Castle, USA). The ampoules and caps were autoclaved (30 min, 121 °C) before the experiment. Electric gain calibrations were regularly performed. Next, 1 mL of LB agar was put into the ampoules, and 10 µL of the bacterial suspension (1.9 mL of LB media, 0.09 mL of *E. coli* (λ-), 0.01 mL of *E. coli* (λ+); OD = 0.1 each in LB medium) with QDs was dropped on the solid agar. The ampoules were closed and made airtight before calorimetric monitoring.

Quantification of the phages. The concentrations of induced phages were quantified by the two-layer method[30]. In this technique, a phage particle causes a plaque in a bacterial layer. The results are provided in PFU (plaque forming units). The basic layer contained LB medium in 1.4% agar, and the cover layer contained 0.7% agar and the exponentially growing indicator bacteria (*E. coli* (λ-); OD = 0.3). Next, 1 mL of the sample for PFU quantification was mixed with 100 µL of chloroform. The samples were centrifuged with 4500 g for 20 minutes at 4 °C. The chloroform-free supernatants were stored in a refrigerator at 4 °C before the final PFU quantification, in which 10 µL serial dilutions of the cell-free supernatants were dropped on the double-layer plates. The plates were incubated overnight at 37 °C, and the plaques were counted on the following day as the PFU in each drop area.

Determination of the intracellular ROS level. *E. coli* (λ+) cultured overnight were harvested, washed twice with PBS and resuspended in tubes with PBS. Then, the microorganisms were cultivated in PBS in the presence of different concentrations of QDs. Finally, the cells were incubated with PBS buffer containing 2',7'-dichlorofluorescein diacetate (DCFH-DA, 5 μM) for 30 min in the dark at 37 °C. The ROS level was flow cytometrically analyzed (C6 flow cytometer, BD Biosciences, USA).

Quantification of superoxide dismutase activity and of lipid peroxides. *E. coli* (λ+) strains were cultivated under the same conditions as in the growth experiments. Afterwards, *E. coli* (λ+) was washed twice with PBS and disrupted using an ultrasonic cell disruption system. The activity of the superoxide dismutase (SOD) was assayed using a water-soluble tetrazolium salt (WST-8) kit (total superoxide dismutase assay kit with WST-8, Beyotime)[31]. The products of the lipid peroxidation (malondialdehyde, MDA) were quantified by the thiobarbituric reacting substances (TBARS) assay. The Lipid Peroxidation MDA Assay Kit (Beyotime, Nantong, China) was used as described by Liu et al[32].

Protective agents. To protect the bacteria against oxidative stress, N-acetyl-L-cysteine (NAC 2 mM), GSH (2 mM), dithiothreitol (DTT 1 mM), and vitamin C (Vc 1 mM) were applied.

Toxicity of Cd^{2+}. An attempt to reduce the toxicity of the heavy metal ion Cd^{2+} was made by masking the ion with ethylenediaminetetraacetic acid (EDTA, 1 mM). To demonstrate the success of masking, the concentration of free Cd^{2+} was analyzed with and without EDTA. For that purpose, the sample with and without EDTA was dialyzed in deionized water using a 200-Da membrane for 2 days. The solution outside of the dialysis tube was reduced in volume to 1mL. The concentrate was

mixed with concentrated nitric acid in an ampule and evaporated to dryness. Finally, 8mL of 3% nitric acid was used to wash the ampule, and the solution was prepared for the analysis using inductively coupled plasma atomic emission spectroscopy (ICP-AES).

Results

Synthesis and characterization of CdTe QDs. CdTe QDs with the two different surface ligands were prepared in aqueous medium as described by Xiang at al. [27]. Transmission electron microscopy (TEM) was used to measure the morphology and size distribution of the CdTe QDs. As depicted in Fig. 1, CdTe QDs on the ligand independently exhibit a spherical shape and do not agglomerate. The average diameter of the CdTe QDs coated with MPA and GSH were quantified using the microscopic data and were found to be 2.3 ± 0.5 nm and 2.2 ± 0.2 nm, respectively. The UV-visible absorption (red) and photoluminescence (green) spectra of the QDs at room temperature are shown in Fig. 1 B and D. MPA-CdTe QDs and GSH-CdTe QDs exhibit a well-resolved first electronic transition absorption maximum at 524 and 504 nm, indicating a narrow CdTe QD size distribution. The size distribution of the QDs determines the width of the peaks[21]. To characterize the potential electrostatic interactions of the QDs and the *E. coli* surface, the *zeta* potential of MPA-CdTe and GSH-CdTe QDs was quantified to be -22.3 mV and -24.0 mV, indicating a negatively charged surface for the QDs.

Activation of prophages by CdTe QDs. In our previous work, a method of microcalorimetry for monitoring of prophage activating chemicals was developed[33]. The method is based on the difference of the metabolic heat production rate between bacteria with prophages (λ+) and without prophages (λ-) under the influence of

prophage activating chemicals. Later research revealed (proved by simulations and experiments[34]) that a mixture of λ+ and λ- as a bioindicator is more sensitive against the chemical activation as a pure the λ+ strain alone[31].The optimum ratio of λ-/λ+ is 9/1. This is additionally confirmed by experiments shown in the supporting information (Fig. S1). Here, different mixtures of λ+ and λ- strains were exposed to the same concentration of a prophage-activating chemical (mitomycin C). To monitor the prophage-activating properties of QDs, *E. coli* (λ-) was mixed with *E. coli* (λ+) in at the optimum ratio of 9/1 (cell number). To be able to analyze the influence of MPA-CdTe QDs on prophage activation, growth curves from the described bacterial mixture and the *E. coli* (λ-) were monitored from the optical density at 600 nm[35] (Fig. 2). As shown in Fig. 2A, the inhibitive effects of the MPA-CdTe QDs on *E. coli* (λ-) at low concentrations (<1 nM) were negligible. Differently, the inhibition efficiency of MPA-CdTe QDs in the bacterial mix was much stronger (Fig. 2B). At a concentration of 10 nM, the growth of the *mixed culture* was completely suppressed. The reasons for the different behavior for the mixed culture were the activation, the subsequent production of phages, and the final infection of further bacteria, as shown from the formation of bacteriophage plaques (Fig. 2C). The amount of the phages depended on the concentration of the CdTe QDs (Fig. 2D). Notably, MPA-CdTe QDs already activate prophages at a concentration of 0.5 nM, which is not toxic to *E. coli* (λ-). The CdTe QDs are independent of the different ligand activated prophages. However, the MPA-CdTe QDs were more effective than GSH-CdTe QDs (see Fig. S1 in the supporting information (SI)). To exclude potential distortions by sampling and to prevent biases, the prophage-activating properties of the CdTe QDs were additionally monitored by microcalorimetry in real time (Fig. 3). Microcalorimetry has been proved to be a simple method to analyze the influence of chemicals on silent

prophages by monitoring the growth processes in real time[36]. Moreover, the monitoring of the final concentration of free phages is a less suited indicator of prophage activation due to the very rapid adhesion of phages to bacteria, especially under the conditions used (in presence of maltose and magnesium). *E. coli* without prophages (λ-) and the *E. coli* mixtures (λ-:λ+ = 9:1) were grown in the presence of different concentrations of CdTe QDs on the surface of LB agar to ensure that the culture always had enough oxygen for respiratory metabolism[37] (Fig. 3). The on-line method confirms the results of the off-line measurements. It should be noted that the concentration for the QDs used in microcalorimetry were higher than those used in the off-line method. This was necessary because the cells were not completely surrounded by QDs except when in suspension in the agar experiment. The transport of QDs plays a role in the growth on solid surfaces. Similar to the off-line experiments, in the microcalorimetry experiments, the number of bacteriophages increased in a dose-dependent manner (Fig. 3, inserts).

Oxidative stress induced by CdTe QDs. It has been well established that QDs affect organisms mainly through the generation of ROS and the release of Cd^{2+}. [38] ROS are known to attack DNA.[39] For those reasons, the levels of ROS inside the bacterial cells were investigated flow cytometrically using the fluorescent probe DCFH-DA. DCFH-DA is taken up by cells and hydrolyzed by cellular esterase to yield DCFH, which is oxidized by ROS to the fluorescent stain DCF[40]. Thus, the fluorescence intensity reflects the ROS level within the cells. The increase in the fluorescence intensity after treatment with MPA-CdTe QDs or GSH-CdTe QDs indicates a significant increase in the cellular ROS level (Fig. 4), supporting the assumption that an oxidative attack on DNA occurs. Additionally, the tendency of MPA-CdTe QDs to be stronger than GSH-CdTe QDs is supported by the ROS measurement. The difference in the oxidative

damage treated by the two types of CdTe QDs could also be caused by the activities of antioxidative enzymes[41]. Thus, the activity of antioxidative enzymes may be an important indicator for evaluating the cellular oxidative stress[42]. In this work, the activity of superoxide dismutase (SOD), one of the most crucial enzymes in the ROS elimination system, was monitored. In the reaction catalyzed by SOD, two molecules of superoxide form hydrogen peroxide and molecular oxygen[43]. However, if the amount of oxygen free radical exceeds clearance capacity of the SOD, ROS will react with amino acids sulfur- (or selenium), which will decrease the activity of SOD[43, 44]. Polyunsaturated fatty acids will be attacked, because of their multiple double bonds, excellent targets for free radical attacks[45]. Both types of CdTe QDs reduced the SOD activities in a dose-dependent manner (Fig. 5). Again, the effect of MPA-CdTe QDs is much stronger than the effect of GSH-CdTe QDs. Oxidative damage to lipid molecules has also been regarded as an indicator of the occurrence of oxidative stress in cells[46]. Malondialdehyde[47] (MDA), a natural product of the lipid oxidation of organisms, was used to quantitatively determine the level of oxidative damage to lipids in lysogenic *E. coli*. Fig. 5C and 5D demonstrate a dose-dependent relationship between MDA and CdTe QD concentrations, suggesting the occurrence of significant oxidative damage of the lipids. Again, the lipid damage is greater for treatment with MPA-CdTe QDs than with GSH-CdTe QDs. To investigate whether oxidative stress is actually responsible for the activation of phages by CdTe QDs, a series of general antioxidants (N-acetyl-L-cysteine: NAC, L-glutathione: GSH, DL-dithiothreitol: DTT and L-ascorbic acid: VC) [48] were applied. The fluorescence intensity after the addition of the antioxidants (Fig. S4 in SI) demonstrates an effective elimination of the ROS produced by the CdTe QDs. Notably, the number of phages decreased by approximately 65% and 45% for the two CdTe QDs after the addition of antioxidants,

indicating effective suppression of the prophage induction activity (Fig. 6). Consequently, the survival rates for the bacteria increased.

Role of the CdTe QD dissociation. Cd^{2+}, a dissociation product of CdTe QDs, is a toxic compound and could be responsible for the observed prophage activation. Fig. 7 depicts the effects of Cd^{2+} and EDTA-masked Cd^{2+} on the growth and phage production. Interestingly, free Cd^{2+} ($\geq$ 3 μM) activated prophages, but it was almost nontoxic to *E. coli* (λ-). This indicates that the release of Cd^{2+} may also contribute to the prophage induction activity of CdTe QDs. To estimate the contribution of free Cd^{2+} under physiological conditions, mixtures of *E. coli* (λ+ and λ-) were simultaneously incubated with CdTe QDs and EDTA. EDTA (a popular chelating agent for Cd^{2+}) was chosen to mask the heavy metal ion. Indeed, the concentration of free Cd^{2+} was drastically reduced after incubation with EDTA as the Table 1 shows. The number of phages decreased (by between 15 and 25%), indicating the potential contribution of Cd^{2+}. Notably, the protective effect of EDTA for GSH-CdTe QDs is stronger than that of MPA-CdTe QDs. This might be attributed to the dosage of GSH-CdTe QDs being four times higher than the dosage of MPA-CdTe QDs which increased the concentration of free Cd^{2+}.

Obviously, EDTA is also able to form metal complexes with a component of the medium (i.e. Mg^{2+}), which could potentially affect the phage infection. To explore such potential side effects, EDTA-masking experiments in medium without Mg^{2+} were performed. The results shown in figure S4, demonstrates that the effect of Mg^{2+} reduction was not significant. Similar results for the EDTA effect were obtained for both the magnesium-containing and the magnesium-free medium, indicating that the protective effects of EDTA are not related to the binding of Mg^{2+}.

Discussion

The toxicity of nanomaterials and in particular of QDs has become a hot topic in recent years due to their accelerated development and growing applications in various fields. Here, a new aspect of ecotoxicology of such materials is illuminated. To the best of our knowledge, this is first report concerning the activation of silent viruses inside of bacteria (so-called prophages) by QDs. These viruses, once released, may infect and kill further bacteria and therefore disturb ecosystem functions.

For the investigation, spherical CdTe QDs were synthesized by the aqueous synthesis method using the MPA and GSH coating ligands. The sizes of the QDs were 2.3 ± 0.5 nm (MPA-CdTe QDs) and 2.2 ± 0.2 (GSH-CdTe QDs), which are in the typical size range to exhibit useful optical properties[49]. Because the direct measurement of the charge of the surface of QDs and bacteria is difficult[50], we used the zeta potential to characterize the electrostatic interactions between QDs and *E. coli*. The zeta potential of the QDs is negative (MPA-CdTe: -22.3 mV, GSH-CdTe QDs: -24.0 mV) as is the zeta potential of *E. coli* (between -34 and -48 mV)[51] in opposition of the electrostatic attraction. The activation of the prophages by the different CdTe QDs is demonstrated by a reduction in the growth rate (due to killing of the bacteria) as well as by an increase in the active phage numbers. This finding is confirmed by on-line measurements of the metabolic heat. The strength of the prophage activating properties of the QDs depends on the chemical structure, as demonstrated for the example of differently coated CdTe QDs (with MPA and GSH). The activation capability of MPA-CdTe QDs is stronger than that of GSH-CdTe QDs. This result was consistent with the previous finding that the biological impacts of QDs were closely related to their specific physicochemical properties including the size, surface charge, surface modification and core/shell materials[52,53].

It is widely accepted that changes in DNA (structural or fragmentation) are regarded as a common and essential event for the transition to the lytic cycle of phage replication. Treatments with DNA-damaging agents, leading to an SOS response within the bacteria, cause the activation of the RecA protein, inducing the expression of the phage genome. The observation of activated phages in CdTe QDs treated lysogenic bacteria indicated that chromosomal DNA of the bacteria has been damaged. The most likely reason for the DNA damage is oxidative stress, as demonstrated by the quantification of the ROS level and the protective effects of the ROS scavengers. It has been well established that increase in DNA damage is an inevitable consequence of raised ROS level[54]. This theory is further supported by the reduction in the superoxide dismutase activity in a dose-dependent manner. This is fatal because not only does the amount of ROS increase but also the ability of the cells to resist decreases. The final proof of the mechanism of CdTe QDs was the formation of oxidation products such as lipid peroxides. The oxidative stress has been reported for several other nanomaterials including metal nanoparticles[55], metal oxide nanoparticles[56], carbonaceous nanomaterials[57] and nanopolymers[58].

It has long been known that exposure of cells to Cd^{2+} induced DNA damage, e.g. DNA single- and double-strand breaks and DNA-protein crosslinks[25,26]. Thus, the phage-activating properties can be caused by the CdTe QDs themselves in addition to the dissociation product Cd^{2+}. The results of the growth experiments with Cd^{2+} show obvious prophage activation caused by the heavy metal ion. Experiments with CdTe QDs in the presence of a chelating agent allow the estimation of the contribution of dissociated Cd^{2+} (between 15 and 25% of the total effect).

In summary, our study suggests that more caution must be used with regard to the ecotoxicological properties of nanomaterials. The CdTe QD concentration needed

to activate prophages is far less than the concentration needed to inhibit bacterial growth. More importantly, during the test measurements, the bacteria were only exposed to the nanomaterial for 8 h. Long-time exposure may result in the continuous accumulation of QDs and may amplify the effect of prophage activation at very low concentrations. Moreover, in aquatic ecosystems, both metal-based NPs and their released metal ions can be taken up by aquatic organisms and further be bioaccumulated and biomagnified[2]. However, the technology exists to increase the stability and to make QDs more biocompatible through coating with protective polymers or several biomolecules[59]. However, over long times under local acid or alkaline conditions, QDs can lose their coating and become destabilized[60, 61]. Notably, recent findings suggested that the content of ROS induced by nanomaterials will increase several-fold after their exposure to light[6, 62], and thus their prophage induction effects could be amplified. Nanomaterials that were previously considered biocompatible or environmentally friendly may also be able to induce prophages due to the presence of light-activated redox species. After this initial study, more efforts should be taken to analyze the potential environmental risks of nanomaterials toward the activation of prophages and toward the disruption of ecosystem functions by killing beneficial bacteria in ecosystems.

Conclusions

In this work, spherical CdTe QDs with a size of circa 2 nm were synthesized by the aqueous synthesis method using the MPA and GSH coating ligand. Prophage induction activity of the CdTe QDs was investigated for the first time. The mechanism of the prophage induction activity of CdTe QDs was found to be the strand breakage of bacterial DNA. Oxidative stress induced by the QDs was found to be crucial for the prophage induction activity, while the contribution of Cd^{2+} release

was relative lower. Antioxidants provided a significant stronger protective effect on the prophage induction activity than the metal-ion chelator. This paper will deepen the understanding of effects of QDs on microbial environments, and provide new concerns for the potential environmental risks of quantum dots.

ACKNOWLEDGEMENTS

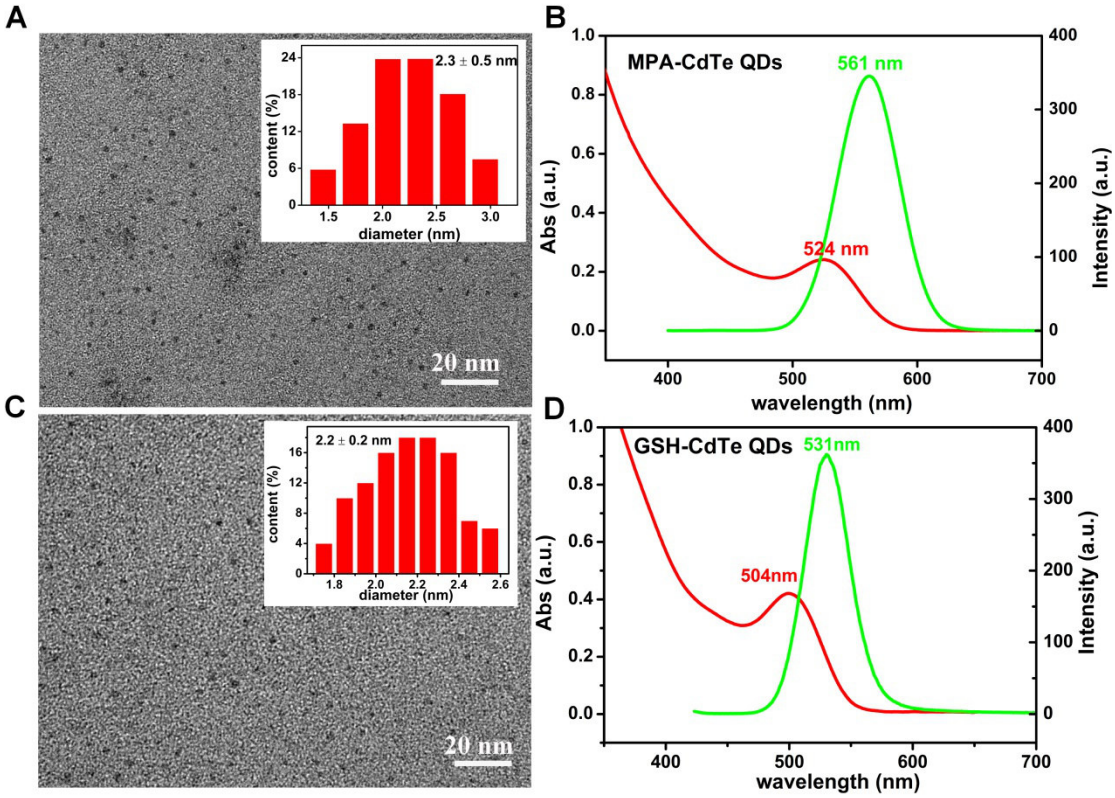
We gratefully acknowledge financial support from the National Natural Science Foundation of China (21673166, 21473125), Natural Science Foundation of Hubei Province (2014CFA003), Wuhan Yellow Crane Talents of Science and Technology Plan, and Bagui Scholar Program of Guangxi Province.

References

1. V. Biju, *Chemical Society reviews*, 2014, **43**, 744-764.
2. N. C. Mueller and B. Nowack, *Environmental Science & Technology*, 2008, **42**, 4447-4453.
3. G. V. Lowry, K. B. Gregory, S. C. Apte and J. R. Lead, *Journal*, 2012.
4. J. Lee, S. Mahendra and P. J. Alvarez, *ACS nano*, 2010, **4**, 3580-3590.
5. C. Peng, W. Zhang, H. Gao, Y. Li, X. Tong, K. Li, X. Zhu, Y. Wang and Y. Chen, *Nanomaterials*, 2017, **7**.
6. C. M. Courtney, S. M. Goodman, J. A. McDaniel, N. E. Madinger, A. Chatterjee and P. Nagpal, *Nature materials*, 2016, **15**, 529-534.
7. X. Han, L. Lai, F. Tian, F. L. Jiang, Q. Xiao, Y. Li, Q. Yu, D. Li, J. Wang and Q. Zhang, *Small*, 2012, **8**, 2680-2689.
8. M. Bottrill and M. Green, *Chemical communications*, 2011, **47**, 7039-7050.
9. J. Kloepfer, R. Mielke, M. Wong, K. Nealson, G. Stucky and J. Nadeau, *Applied and environmental microbiology*, 2003, **69**, 4205-4213.
10. L. Lai, S.-J. Li, J. Feng, P. Mei, Z.-H. Ren, Y.-L. Chang and Y. Liu, *Langmuir*, 2017, **33**, 2378-2386.
11. C. Howard-Varona, K. R. Hargreaves, S. T. Abedon and M. B. Sullivan, *The ISME Journal*, 2017, **11**, 1511-1520.
12. C. Canchaya, C. Proux, G. Fournous, A. Bruttin and H. Brüssow, *Microbiology and Molecular Biology Reviews*, 2003, **67**, 238-276.
13. J. Choi, S. M. Kotay and R. Goel, *Water research*, 2010, **44**, 4550-4558.
14. S. Bloch, B. Nejman-Faleńczyk, J. M. Łoś, S. Barańska, K. Łepek, A. Felczykowska, M. Łoś, G. Węgrzyn and A. Węgrzyn, *Archives of microbiology*, 2013, **195**, 693-703.
15. C. Janion, *International journal of biological sciences*, 2008, **4**, 338.
16. K. Norregaard, M. Andersson, K. Sneppen, P. E. Nielsen, S. Brown and L. B. Oddershede, *Bacteriophage*, 2014, **4**, e27517.
17. J. H. Paul, *The ISME journal*, 2008, **2**, 579.
18. L. McDaniel and J. Paul, *Applied and environmental microbiology*, 2005, **71**, 842-850.
19. T. Maskow, B. Kiesel, T. Schubert, Z. Yong, H. Harms and J. Yao, *Journal of Virological Methods*, 2010, **168**, 126-132.
20. T. C. Johnstone, S. M. Alexander, W. Lin and S. J. Lippard, *Journal of the American Chemical*

Society, 2013, **136**, 116-118.
21. R. Brayner, R. Ferrari-Iliou, N. Brivois, S. Djediat, M. F. Benedetti and F. Fiévet, *Nano Letters*, 2006, **6**, 866-870.
22. M. Aye, C. Di Giorgio, I. Berque-Bestel, A. Aime, B. P. Pichon, Y. Jammes, P. Barthelemy and M. De Meo, *Mutation research*, 2013, **750**, 129-138.
23. Y. Guo, C. Cheng, J. Wang, Z. Wang, X. Jin, K. Li, P. Kang and J. Gao, *Journal of hazardous materials*, 2011, **192**, 786-793.
24. E. V. Skorb, D. V. Andreeva, A. P. Raiski, N. A. Belyasova, H. Mohwald and D. V. Sviridov, *Photochem Photobiol Sci*, 2011, **10**, 1974-1978.
25. M. Filipic, T. Fatur and M. Vudrag, *Human & experimental toxicology*, 2006, **25**, 67-77.
26. A. Wang and D. E. Crowley, *Journal of bacteriology*, 2005, **187**, 3259-3266.
27. X. Xiang, C. Wu, B.-R. Zhang, T. Gao, J. Zhao, L. Ma, F.-L. Jiang and Y. Liu, *Chemosphere*, 2017.
28. W. W. Yu, L. Qu, W. Guo and X. Peng, *Chemistry of Materials*, 2003, **15**, 2854-2860.
29. D. Wacey, N. McLoughlin, O. R Green, J. Parnell, C. A. Stoakes and M. D. Brasier, *Int. J. Astrobiol.*, 2006, **5**, 333-342.
30. D. Lillehaug, *Journal of applied microbiology*, 1997, **83**, 85-90.
31. X. Li, L. Xu, W. Zhou, Q. Zhao and Y. Wang, *Environmental Pollution*, 2016, **210**, 48-56.
32. C. Liu, K. Gong, X. Mao and W. Li, *International journal of cancer*, 2011, **129**, 1519-1531.
33. J. Xu, B. Kiesel, R. Kallies, F. e. Jiang, Y. Liu and T. Maskow, *Microbial Biotechnology*.
34. J. Xu.
35. J.-C. Jin, X.-J. Wu, J. Xu, B.-B. Wang, F.-L. Jiang and Y. Liu, *Biomaterials science*, 2017, **5**, 247-257.
36. T. Maskow, R. Kemp, F. Buchholz, T. Schubert, B. Kiesel and H. Harms, *Microbial Biotechnology*, 2010, **3**, 269-284.
37. T. Maskow, F. M. Morais, L. F. M. Rosa, Y. G. Qian and F. Harnisch, *RSC Adv.*, 2014, **4**, 32730-32737.
38. D. Mo, L. Hu, G. Zeng, G. Chen, J. Wan, Z. Yu, Z. Huang, K. He, C. Zhang and M. Cheng, *Applied microbiology and biotechnology*, 2017, **101**, 2713-2733.
39. A. Pramanik, D. Laha, D. Bhattacharya, P. Pramanik and P. Karmakar, *Colloids and Surfaces B: Biointerfaces*, 2012, **96**, 50-55.
40. F. Caldefie-Chézet, S. Walrand, C. Moinard, A. Tridon, J. Chassagne and M.-P. Vasson, *Clinica Chimica Acta*, 2002, **319**, 9-17.
41. Z. Lu, C. M. Li, H. Bao, Y. Qiao, Y. Toh and X. Yang, *Langmuir*, 2008, **24**, 5445-5452.
42. A. D. de Azevedo Neto, J. T. Prisco, J. Enéas-Filho, C. E. B. de Abreu and E. Gomes-Filho, *Environmental and Experimental Botany*, 2006, **56**, 87-94.
43. J. Nordberg and E. S. J. Arnér, *Free Radic Biol Med*, 2001, **31**, 1287-1312.
44. T. Finkel and N. J. Holbrook, *Nature*, 2000, **408**, 239-247.
45. D. Steinberg, *Journal of Biological Chemistry*, 1997, **272**, 20963-20966.
46. F. M. El-Demerdash, M. I. Yousef and F. M. Radwan, *Food and Chemical Toxicology*, 2009, **47**, 249-254.
47. D. Del Rio, A. J. Stewart and N. Pellegrini, *Nutrition, metabolism and cardiovascular diseases*, 2005, **15**, 316-328.
48. X.-Y. Fan, X.-Y. Chen, Y.-J. Liu, H.-M. Zhong, F.-L. Jiang and Y. Liu, *Scientific reports*, 2016, **6**.
49. W. W. Yu, L. Qu, W. Guo and X. Peng, *Chemistry of Materials*, 2003, **15**, 2854-2860.
50. G. Karniadakis, A. Beskok and N. Aluru, *Springer*, 2005, New York.
51. K. A. Soni, A. K. Balasubramanian, A. Beskok and S. D. Pillai, *Current microbiology*, 2008, **56**, 93-97.
52. E. Oh, R. Liu, A. Nel, K. B. Gemill, M. Bilal, Y. Cohen and I. L. Medintz, *Nature nanotechnology*, 2016, **11**, 479-486.
53. R. Werlin, J. H. Priester, R. E. Mielke, S. Krämer, S. Jackson, P. K. Stoimenov, G. D. Stucky, G. N. Cherr, E. Orias and P. A. Holden, *Nature nanotechnology*, 2011, **6**, 65-71.
54. M. S. Cooke, M. D. Evans, M. Dizdaroglu and J. Lunec, *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 2003, **17**, 1195-1214.
55. O. Choi, K. K. Deng, N.-J. Kim, L. Ross, R. Y. Surampalli and Z. Hu, *Water research*, 2008, **42**, 3066-3074.
56. M. Auffan, J. Rose, J.-Y. Bottero, G. V. Lowry, J.-P. Jolivet and M. R. Wiesner, *Nature nanotechnology*, 2009, **4**, 634-641.

57. S. K. Manna, S. Sarkar, J. Barr, K. Wise, E. V. Barrera, O. Jejelowo, A. C. Rice-Ficht and G. T. Ramesh, *Nano letters*, 2005, **5**, 1676-1684.
58. P. C. Naha, M. Davoren, F. M. Lyng and H. J. Byrne, *Toxicology and applied pharmacology*, 2010, **246**, 91-99.
59. D. M. Aruguete, J. S. Guest, W. W. Yu, N. G. Love and M. F. Hochella, *Environmental Chemistry*, 2010, **7**, 28.
60. S. Mahendra, H. Zhu, V. L. Colvin and P. J. Alvarez, *Environmental Science & Technology*, 2008, **42**, 9424-9430.
61. D. R. Cooper, N. M. Dimitrijevic and J. L. Nadeau, *Nanoscale*, 2010, **2**, 114-121.
62. W. Zhang, Y. Li, J. Niu and Y. Chen, *Langmuir*, 2013, **29**, 4647-4651.



476

477 **Figure 1**. Characterization of MPA-CdTe and GSH-CdTe QDs. Transmission electron

478 microscopy (TEM) images of MPA-CdTe QDs (A) and of GSH-CdTe QDs

479 (C). Absorption spectra (red) and photoluminescence spectra (green) of

480 MPA-CdTe QDs (B) and of GSH-CdTe QDs (D).

481

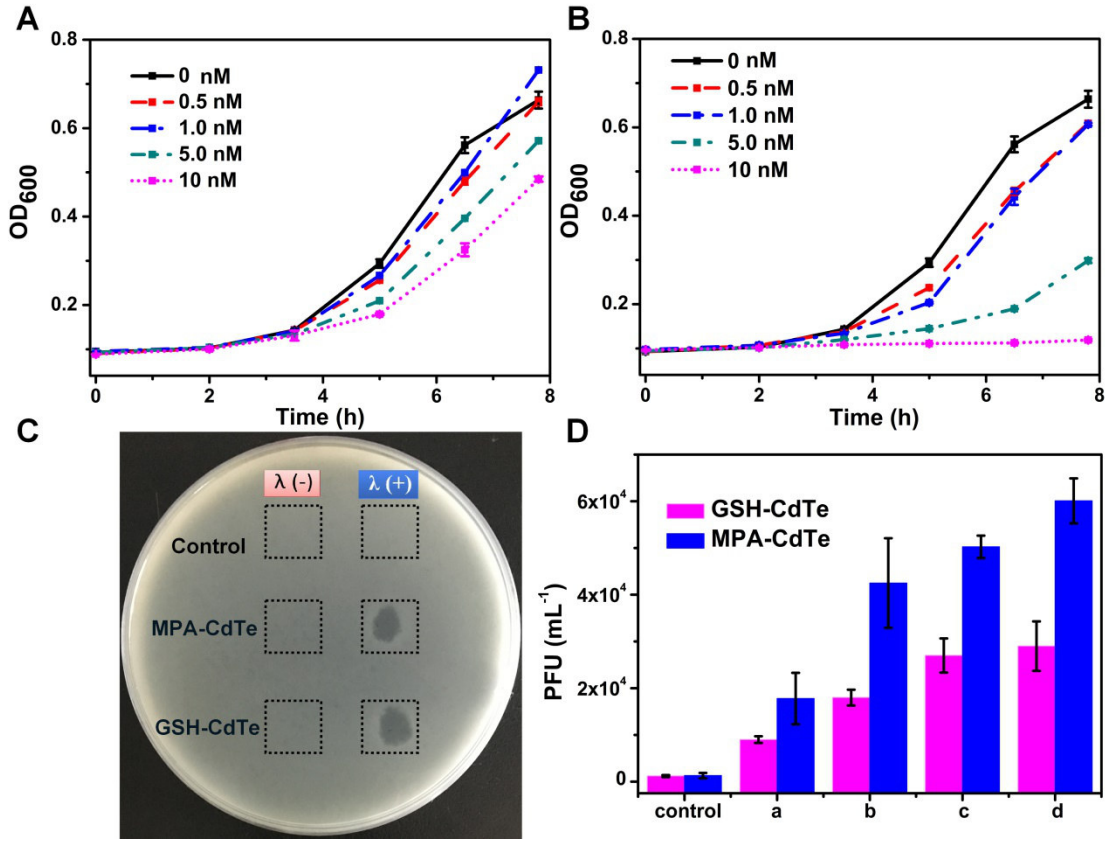


Figure 2. Prophage activation by CdTe QDs. Growth curves of *E. coli* (λ-) (A) and of mixtures of *E. coli* (λ-:λ+ = 9:1) (B) treated with MPA-CdTe QDs. Plaque formation on agar plate (C). Phage quantification (PFU) upon the interaction of mixtures of *E. coli* with CdTe QDs (D).

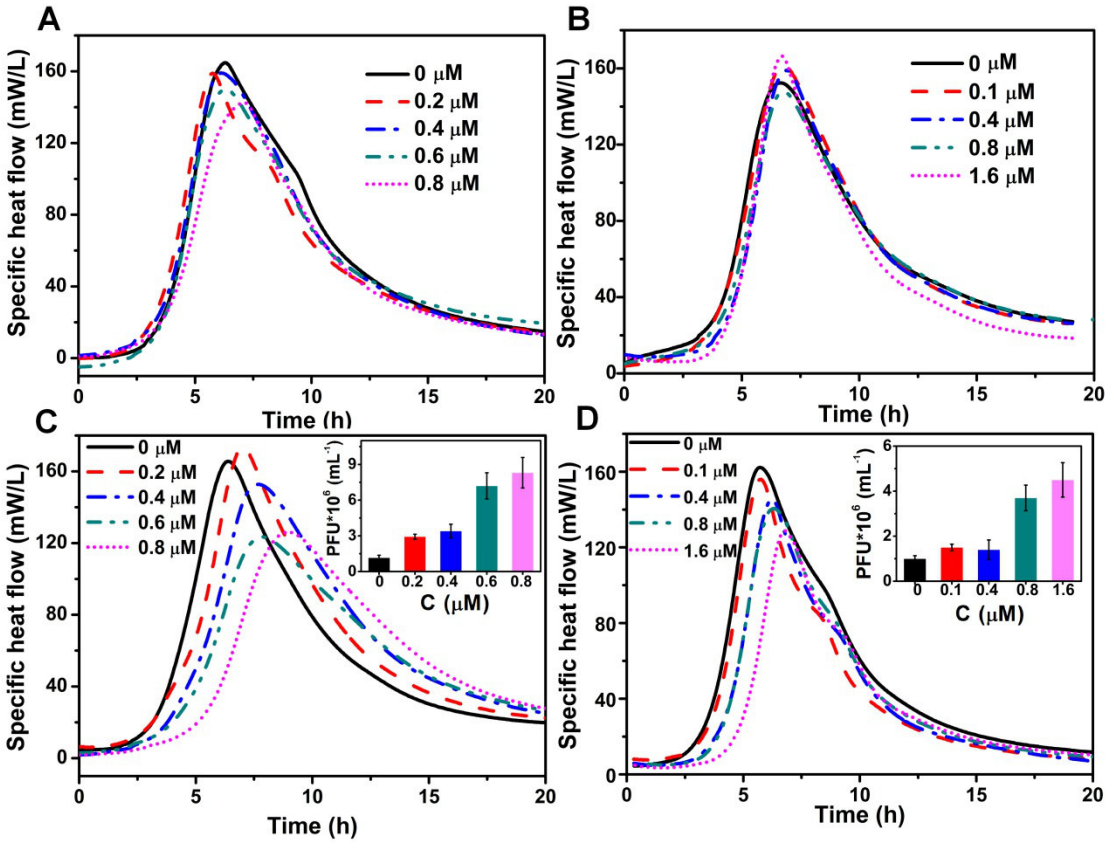


Figure 3. Dependency of the metabolic heat production rate of *E. coli* on the dosage of MPA-CdTe and GSH-CdTe QDs. *E. coli* (λ-) treated with MPA-CdTe QDs (A) and with GSH-CdTe QDs (B). Mixture of *E. coli* (λ-:λ+ = 9:1) treated with MPA-CdTe QDs (C) and with GSH-CdTe QDs (D). The inserted bar diagram shows the phage propagation.

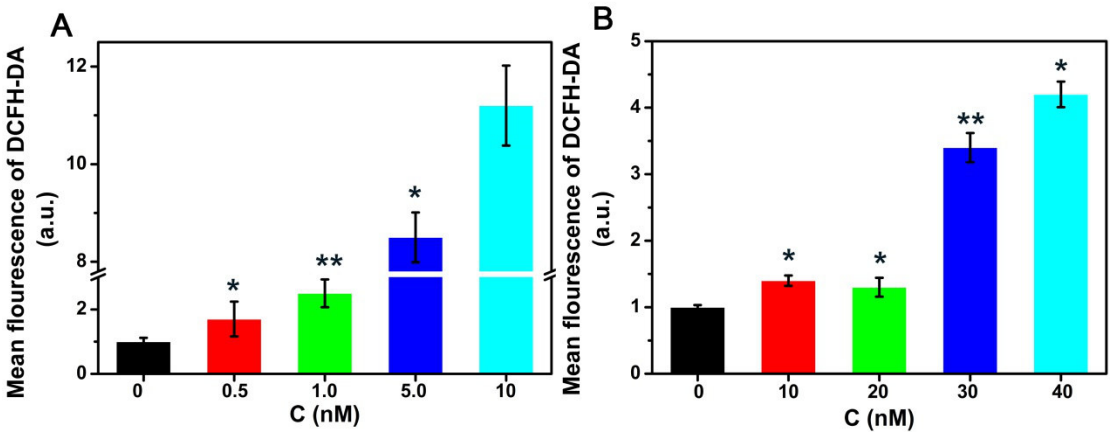
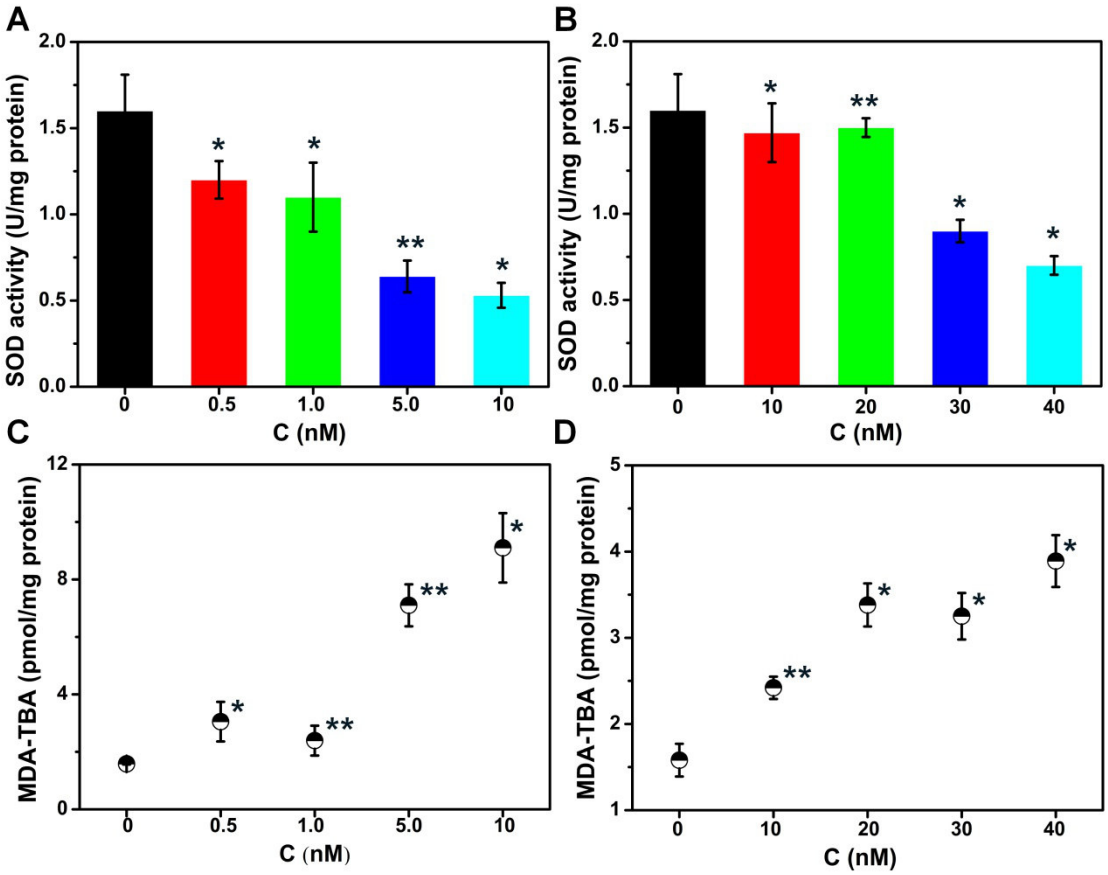


Figure 4. Dosage dependence of the production of reactive oxygen species (ROS) for MPA-CdTe QDs (A) and GSH-CdTe QDs (B), *: $P < 0.05$, **: $P < 0.01$.



502

503 **Figure 5.** Superoxide dismutase (SOD) activity in relation to the dosage of MPA-

504 CdTe QDs (A) or GSH-CdTe QDs (B). Effects of MPA-CdTe QDs (C) and

505 GSH-CdTe QDs (D) on lipoperoxidation. (n=3), *： $P < 0.05$, **:$P < 0.01$.

506

507

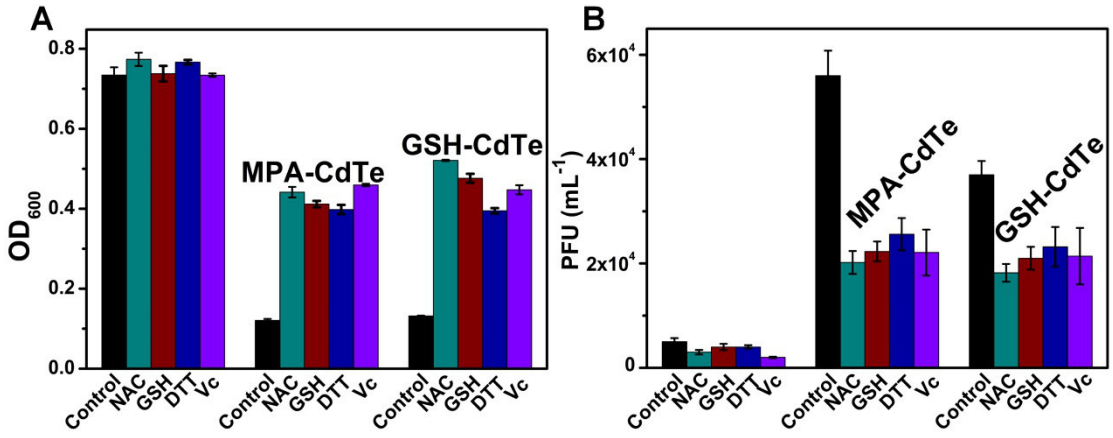


Figure 6. Treatment of antioxidants suppresses the lysogenic activity of MPA-CdTe and GSH-CdTe QDs. The abbreviations stand for NAC - acetyl-L-cysteine, GSH - L-glutathione, DTT - DL-dithiothreitol, Vc - L-ascorbic acid. (A) Optical density of the suspension at 600 nm. (B) PFU measurements.

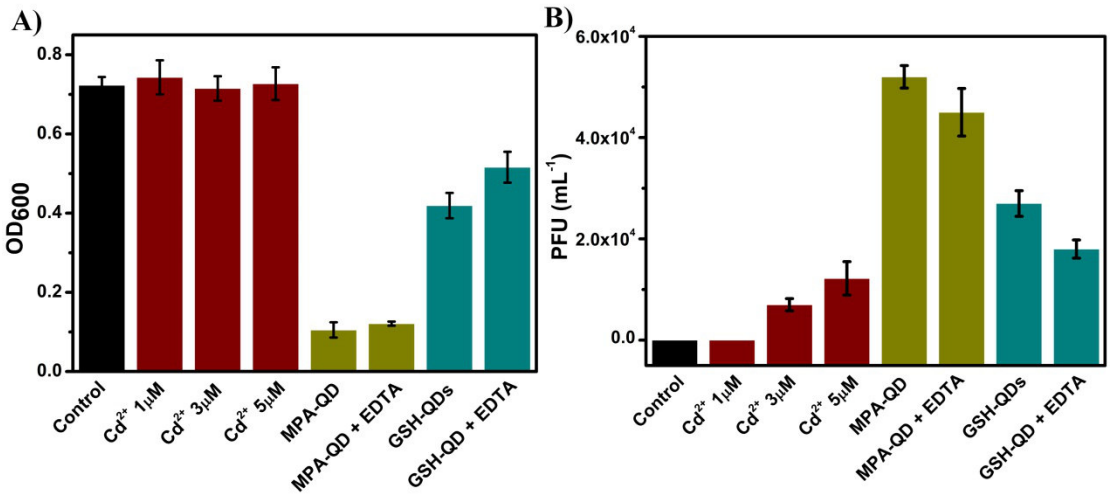


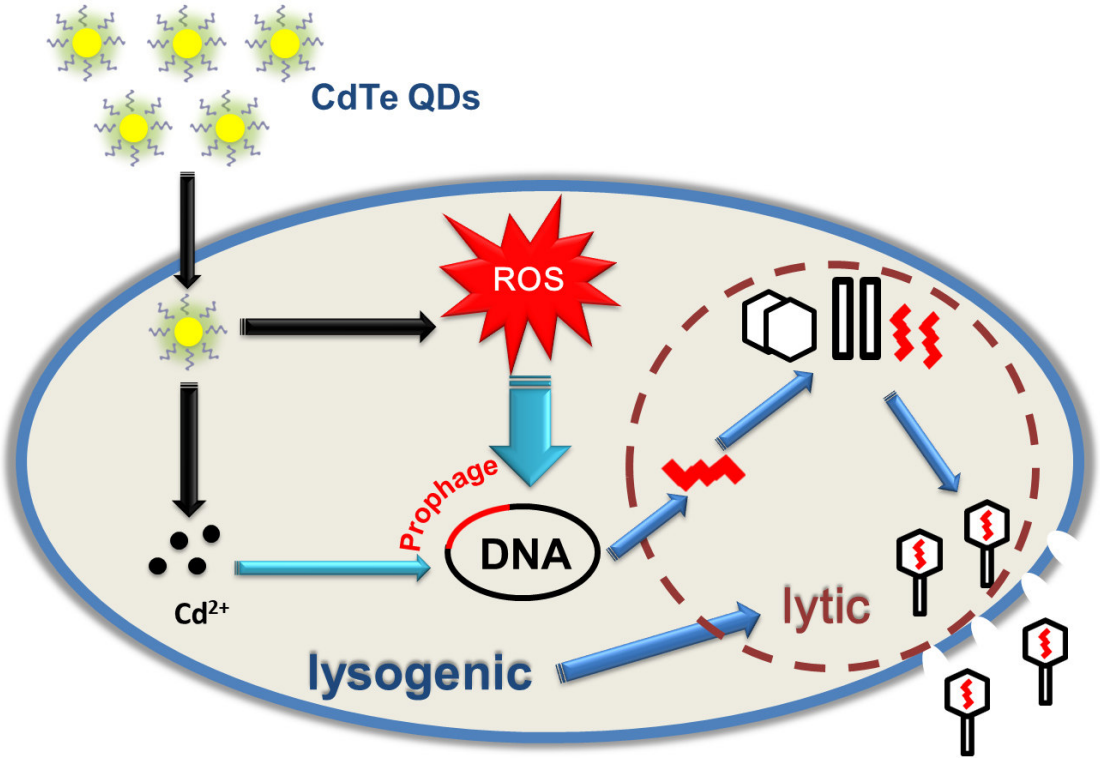
Figure 7. Protective effects of the complexation of Cd^{2+} by EDTA against prophage activation. Optical density of the bacterial suspension at 600 nm (A). PFU measurements (B) (MPA-CdTe QDs, 10 nM; GSH-CdTe QDs, 40 nM; EDTA, 1 mM).

521 **Table 1.** The influence of EDTA masking on the concentration of free Cd2+.
522

Concentration of Cd^{2+}	Without EDTA (μM)	With EDTA (μM)
MPA-CdTe QDs	2.12±0.073	0.245±0.045
GSH-CdTe QDs	5.75±0.066	0.212±0.037

523

524 **TOC**



525

526 **CdTe QDs induced lysogenic bacteria into the lytic cycle by**

527 **damaging the DNA.**

528