Ecotoxicology (2020) 29:156–162
https://doi.org/10.1007/s10646-019-02158-3

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Quantum dots functionalized with gH625 attenuate QDs oxidative stress and lethality in *Caenorhabditis elegans*: a model system

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Accepted: 24 December 2019 / Published online: 11 January 2020

Abstract

Nanomaterials have revolutionized many scientific fields and are widely applied to address environmental problems and to develop novel health care strategies. However, their mechanism of action is still poorly understood. Several nanomaterials for medical applications are based on quantum dots (QDs). Despite their amazing physico-chemical properties, quantum dots display significant adverse effects. In the present study, the effects of QDs on the motor nervous system of nematodes *Caenorhabditis elegans* have been investigated as a non-mammalian alternative model. We also explored the possibility of modifying the toxicity of QDs by coating with a cell-penetrating peptide gH625 and thus we analysed the effects determined by QDs-gH625 complexes on the nematodes. With this work, we have demonstrated, by in vivo experiments, that the peptide gH625 is able to reduce the side effects of metallic nanoparticle making them more suitable for medical applications.

Keywords *Caenorhabditis elegans* · Quantum dots · Ecotoxicity · Drug delivery

Introduction

Development of nano-technological materials has produced significant advances in several scientific fields such as biomedical research. As a matter of fact, many nanomaterials, such as metallic nanoparticles, are engineered and used in numerous studies with diagnostic or therapeutic purposes (Wu et al. 2019; Pellico et al. 2019). The major advantages of using nanoparticles are the small size (less than 100 nm), the improved tissue diffusion, the lower sedimentation rates, the high effective surface areas, and the possibility to modify the surface and attach different cargos or moieties for a selective delivery (Martin-Serrano et al. 2019; Galstyan et al. 2019).

Despite these advantages, the use of nanoparticles also presents several drawbacks. Indeed, one of the main consequences of the increased use of nanoparticles is correlated to their environmental impact even after a short-term exposure (Khosravi-Katuli et al. 2018).

Quantum dots (QDs) are metal nanoparticles, which represent an increasingly important class of nanomaterials widely used in electronics, biomedical imaging, biosensors and drug delivery (Gao et al. 2005; Medintz et al. 2005; Jamieson et al. 2007). QDs are nanocrystal solids consisting of a semiconductor core often composed of cadmium-selenium (CdSe); previously reported in vivo and in vitro studies, showed that exposure to this nanoparticles could cause several toxic effects both to the environment and target organisms (McMahan et al. 2014; Ulusoy et al. 2016). Toxicity is associated with intracellular aggregation, accumulation into endo-lysosomal vesicles and release of cadmium ions (Cd^{2+}). The exposure to QDs and its ions produces reactive-oxygen species (ROS) which induce membrane lipid peroxidation as well as other significant cellular damages such as apoptosis and necrosis (Cho et al. 2007; Chen et al. 2012; Winnik and Maysinger 2013). Thus, it is highly important to develop strategies which are able to reduce toxicity of QDs in living organisms and surrounding environment for safe applications. Size, shape, dose, route of administration, and exposure time are key

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factors that affect the degree of toxicity. Over the years, these parameters have been analysed and modulated to find out the best conditions for a safe administration; moreover, many other strategies such as coating of the nanoparticles have been developed. Mostly, nanoparticles have been modified on their surface by conjugation of PEG or other different biomolecules (Li et al. 2019).

In our laboratory, the peptide gH625 was developed as a cell penetrating peptide (Galdiero et al. 2015; Falanga et al. 2015). It belongs to the glycoprotein H of *Herpes simplex virus type I*, and has proved exceptional vector properties (Galdiero et al. 2015; Falanga et al. 2015; Valiante et al. 2015) being essentially internalized by a non-endocytic pathway also at the level of the Blood Brain Barrier (BBB) both in vitro and in vivo (Valiante et al. 2015; Iachetta et al. 2019). gH625 is therefore a very good delivery vector and its coupling to nanoparticles can be exploited to escape the entrapment in endocytic vesicles (Carberry et al. 2012; Guarnieri et al. 2015) and reduce their toxicity (Galdiero et al. 2017). In previous in vivo studies on the aquatic biota, we have showed that the functionalization of QDs with gH625 can not only improve their delivery properties but also reduce their toxicity (Galdiero et al. 2015; 2017, 2012; Falanga et al. 2011; 2009).

Literature is overloaded of examples of in vitro experiments and in vivo experiments with embryonic zebra fish, *Xenopus laevis*, and *Drosophila melanogaster* as models. However, there are few studies on toxicity in terrestrial organisms such as *Caenorhabditis elegans* that is a representative species in the nematode phylum with great ecological relevance.

Nematode *C. elegans* is a popular animal model that has been used to study the eco-toxicological impact of various nanomaterials such as silica, silver, titanium NPs (Wang et al. 2009; Roh et al. 2010; Ma et al. 2009). *C. elegans* has been used for toxicity assessment and eco-toxicological studies in vivo for heavy metals, organic compounds, drugs and engineered nanomaterials (ENMs) (Li et al. 2013).

It is a choice organism as animal model because of its properties such as small dimensions (about 1 mm), simple anatomy, short life cycle, short lifespan, easy handling, low cost and its genome is well documented. Furthermore, the worm is transparent, allowing monitoring in vivo and has a defense system highly homologous to mammalian innate immunity signaling pathways crucial for host defense such as p38 Mitogen-activated Protein Kinase (p38 MAPK), the Insulin Growth Factor (IIS) and Transforming Growth Factor-β (TGFβ) (Mallo et al. 2002; Troemel et al. 2006; Garsin et al. 2003). Altogether these advantages make *C. elegans* an ideal model organism for studies concerning drug discovery and delivery. Compared to other animal models, *C. elegans* gives a real-time toxicity information on nanomaterials so that researchers can obtain rapid relevant conclusions for their biosafety. Several authors have reported on QDs toxicity in *C. elegans* affecting growth, reproduction, neuronal development and function, lifespan (Qu et al. 2011; Hsu et al. 2012; Contreras et al. 2013; Zhao et al. 2015; Qu et al. 2011).

In this study, we investigated other biological effects induced in *C. elegans* by QDs and the possibility to reduce the biological risks by functionalization with gH625.

Materials and methods

Synthesis of gH625 and QD-gH625

The peptide gH625 was synthesized by a standard solid phase protocol using Fmoc chemistry, previously reported (de Alteriis et al. 2018). The crude peptide was precipitated with ethyl ether 4 times and freeze-dried. The purification was performed by preparative high-performance liquid chromatography (HPLC) on reversed-phase column with good yields (~70%).

Subsequently, the carboxylic group of the peptide was pre-activated in a buffered solution (PBS pH 7.4 for 30 min) of 1-ethyl-3(3-dimethylamino-propyl)-carbodiimide (EDC) and N-hydroxysuccinimide (NHS) with a molar ratio of peptide:EDC:NHS 4:4:1. The pre-activated peptide was added into a solution of QDs with amino groups on the surface (QDs525NC) in order to promote the reaction between the pre-activated carboxylic and amine groups. The mixture in the ratio of NH_2:COOH = 1:200 was allowed to react in MES buffer pH 5.8 for 3 h. The final solution obtained was 117 nM QDs and 500 μM gH625. QD-gH625 were previously characterized (Galdiero et al. 2017).

Synchronization of nematodes and toxicity assays

C. elegans strains N2 was cultured in Petri-dishes containing 15 ml of growth medium agar (NGM) seeded with *Escherichia coli* OP50 at 20 °C in our laboratory according to the ASTM E2172 – 01 Standard Method (2014) (Guarnieri et al. 2015; Oral et al. 2019). *C. elegans* N2 was used for initial testing and as a control in all assays.

To have synchronized *C. elegans*, gravid adult worms were lysed with a solution containing 10 g/L NaOH, 10.5 g/L NaOCl. The obtained eggs were washed by centrifugation in M9 Buffer (2.2 mM KH_2PO_4, 4.2 mM Na_2HPO_4, 85.6 mM NaCl, 1 mM $MgSO_4$) and allowed to stay overnight in NGM plates with OP50 food source. Standard methods were then used to analyze the reproductive schedule and lifetime fecundity (Hodgkin and Doniach 1997).

Toxicity tests in *C. elegans*, were performed using synchronized L1-larval nematodes injected into 24-well tissue culture plates. About 20-25 worms were transferred by

pipetting 5 µL into each well containing 0.5 mL test solutions at different concentrations (0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25 µM) and 0.5 ml K-medium (2.36 g KCl and 3.0 g NaCl in 1 L of distilled water) was used as negative control. All treatments were done in triplicate and without feeding the worms for 96 h at 20 °C. The toxicity and lifespan of worms were determined every day until the death. Worms were considered as dead when they no longer respond to gentle prodding with a platinum wire. The reproduction was assessed by counting the number of offspring after 96 h of incubation.

Oxidative stress assays: determination of ROS level and evaluation of CAT and SOD activity

The amount of reactive oxygen species (ROS), the enzymatic activity of superoxide dismutase (SOD) and catalase (CAT) were assayed according to protocols designed in our laboratory (Galdiero et al. 2017) and adapted for tests on nematodes. Alive nematodes were suspended in cold 50 mM PBS buffer pH 7.8, homogenized using a glass homogenizer for 6 min on ice; then the mixture was centrifuged (20,800 × *g*, 10 min) at 4 °C. The supernatant was transferred into 1.5 mL EP tube and used to evaluate ROS amount and enzyme activities. The total protein content was determined according to the Bradford method Bradford (1976). The fluorescence of H2DCFDA was recorded after 30 min at 37 °C using a fluorescence plate reader (Victor 3TM Multilabel Counter; Perkin Elmer, Shelton, CA, USA) with excitation wavelength set at 485 nm and emission wavelength at 530 nm. The intracellular reactive oxygen species levels (ROS) was expressed as relative fluorescent units (RFU). Superoxide dismutase (SOD) and Catalase (CAT) activities in worms were measured using commercial chemical assay kits purchased from Sigma-Aldrich according to the manufacturer's instructions.

Microscopy and imaging analysis

The uptake of QDs and QDs-gH625 by *C. elegans* was evaluated by a fluorescence microscope. In detail, nematodes were treated with 25 µM QDs and QDs-gH625 for 6 h then observed under microscope (Nikon Eclipse E 1000, equipped with a digital camera Nikon DXM1200F) and data were analyzed with the software Nikon ACT-1 (Acosta et al. 2018).

Data analysis

Results of bioassays are given as mean ± standard error and toxicity was expressed both as percentage of effect and median effective concentration (EC_{50}) if calculable. EC50 values and its 95% confidence intervals were calculated by linear regression. For single-dose tests, differences between samples and control group were determined by two-tailed Student's *t* test. For dose-response tests, after the verification of homoscedasticity (F test, $p < 0.05$) of data, the significance of differences between average values of different experimental treatments was assessed by the analysis of variance (ANOVA) with a 0.05 significance level. When ANOVA revealed significant differences among treatments, *post-hoc* analysis was carried on with Tukey's test ($p < 0.05$).

Results and discussion

In this study, the peptide gH625 was synthesized with the addition of the small sequence GGG at the C-terminus of the peptide which was used for the coupling to QDs. In particular, the peptide was coupled to QDs from its C-terminus in order to allow the N-terminus to mediate the internalization process as we previously reported Galdiero et al. 2010). The obtained complexes were analysed to determine their physico-chemical properties as previously reported (Galdiero et al. 2017).

In Fig. 1 we reported the mortality of nematodes at different concentrations of QD and QD-gH625 and clearly we observe a lower percentage of mortality when QDs are functionalized with gH625. Remarkably, at concentrations below 1.6 µM, it seems that the exposure to QD-gH625 does not affect nematode mortality, while a minimal lethal effect on *C. elegans* only occurs at higher concentrations; nonetheless, the percentage of mortality does not reach the value of 40% even at 25 µM. Nude QDs display a more toxic effect than QDs functionalized with the peptide; indeed, mortality occurs, although low, already at doses in the range 0.4–3.1 µM and increases above 60% at 25 µM.

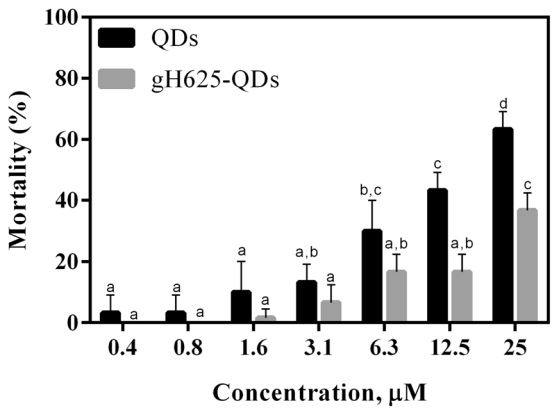

Fig. 1 QDs and gH625-QDs effects on *C. elegans* at 24 h. Mortality percentage of *C. elegans* exposed to different concentration of QDs and gH625-Qds, data with different letters (a–d) are significantly different (Tukey's, $p < 0.05$)

These results suggested a dose-dependent lethal effect of QDs compared with QDs-gH625.

The mortality data have been fitted in order to calculate the EC_{50} values. For QDs, the EC_{50} is 15.3 μM with the 95% confidence interval ranging from 12.8 to 18.8 μM while for QD-gH625 it has not been possible to determine the value as the mortality caused by QD-gH625 was not significantly different from the control (untreated *C. elegans)*. However, from our predictive calculation we estimate a probable EC_{50} value of 45.1 μM with the 95% confidence interval ranging from 33.8 and 70.4 μM. The peptide gH625 alone was also tested and showed to be not toxic for *C. elegans* (data not shown).

In general, several physiological parameters, apart from mortality, can be accounted as a measure of the toxic effect of a material; in particular, reduced fertility and survival should also be considered as a progression of toxicity. For this reason, we decided to analyse the effects on *C. elegans* after prolonged exposure (only at one concentration). In details, we observed the reproduction capacity and lifespan over 96 h. Prolonged exposure to QDs at the sublethal concentration of 6.3 μM significantly decreased lifespan of worms (Fig. 2a). These data are consistent with previous works, (Qu et al. 2011) which report that treatment with QDs induces toxicological effects in a shortened lifespan. On the other hand, we found that QD-gH625 do not affect lifespan of the worms at the concentration tested and the results are very similar to the control. Not only we observed a normal survival trend but also we noticed that nematodes exposed to QDs-gH625 have a normal reproductive capacity. Instead, when *C. elegans* is exposed to QDs, the reproductive capacity is reduced. Our results further confirmed that the functionalization of QDs with the peptide gH625 is effective in protecting worms and avoiding the toxic effect caused by nude QDs.

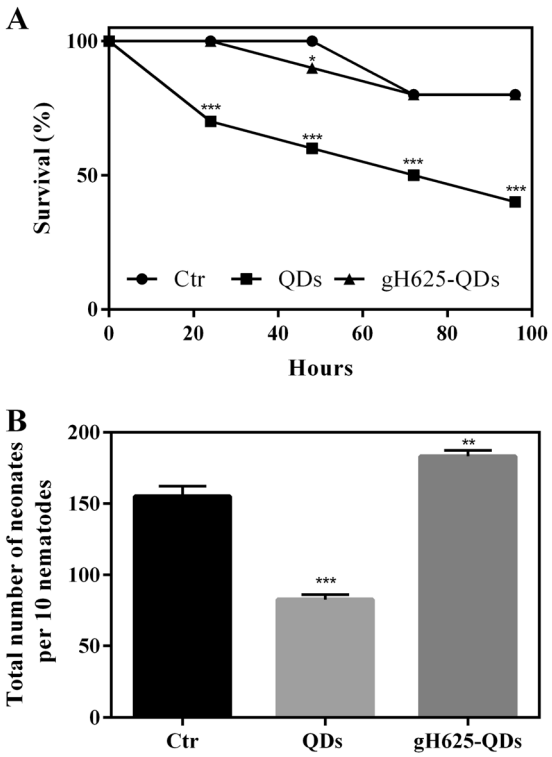

Fig. 2 Cumulative parent survival subjected to a chronic toxicity test for 96 h. Data are given as mean values ± SD (**a**). Total number of live offspring per 10 nematodes after 96 h of exposure to sublethal concentration of QDs and gH625-QDs (**b**). Asterisks indicate the difference compared to the control (Student *t* test): $*p < 0.05$; $**p < 0.01$; $***p < 0.001$

The relationship between nanotoxicity and oxidative stress is a mainstay and evidences suggest that oxidative damages may be caused in species ranging from *C. elegans* to Drosophila to humans (Finkel and Holbrook 2000; Hekimi and Guarente 2003). ROS (reactive oxygen species), such as superoxide, hydrogen peroxide, hydroxyl radical and others are produced as toxic byproducts of normal cellular metabolism and damage all cellular macromolecules, such as lipids, nucleic acids, carbohydrates and proteins. Level of ROS is surely an indicator of oxidative stress.

In this work, we evaluate ROS level in nematodes which have been exposed to QDs and QDs-gH625 both at all concentrations used in acute tests. We observe that after 24 h a significant enhancement of ROS production in nematodes treated with QDs. An enhancement of ROS level is also observed in nematodes treated with QDs-gH625 but this increase is less significant in comparison with the treatment with nude QDs (Fig. 3a). The exposure was prolonged to 48 h and again ROS was present in *C. elegans* at higher concentrations of QDs compared to QDs-gH625 (data not shown). These data clearly show the effectiveness of gH625 coating in reducing the toxic effect. This protective action has been observed with low dose treatments as well as at the highest doses tested; indeed at all concentrations we observed a ROS production higher than the control but ROS level is always lower with QDs-gH625 than QDs.

Among the most important defenses against oxygen radicals are the superoxide dismutase (SOD) and catalase (CAT) enzymes (Fridovich 1995). SOD generally exists in several forms in the cytoplasm of eukaryotic cell and in mitochondria and catalyzes the dismutation of the superoxide radical $O_2.-$ into either ordinary molecular oxygen O_2 and hydrogen peroxide H_2O_2. Instead, CAT is an enzyme involved in oxygen metabolism and catalyzes the decomposition of hydrogen peroxide H_2O_2 to water H_2O and oxygen O_2.

Usually, in the organisms SOD level increases in response to the increase of ROS level, and also in nematodes after the treatment with QDs or QDs-gH625 SOD is

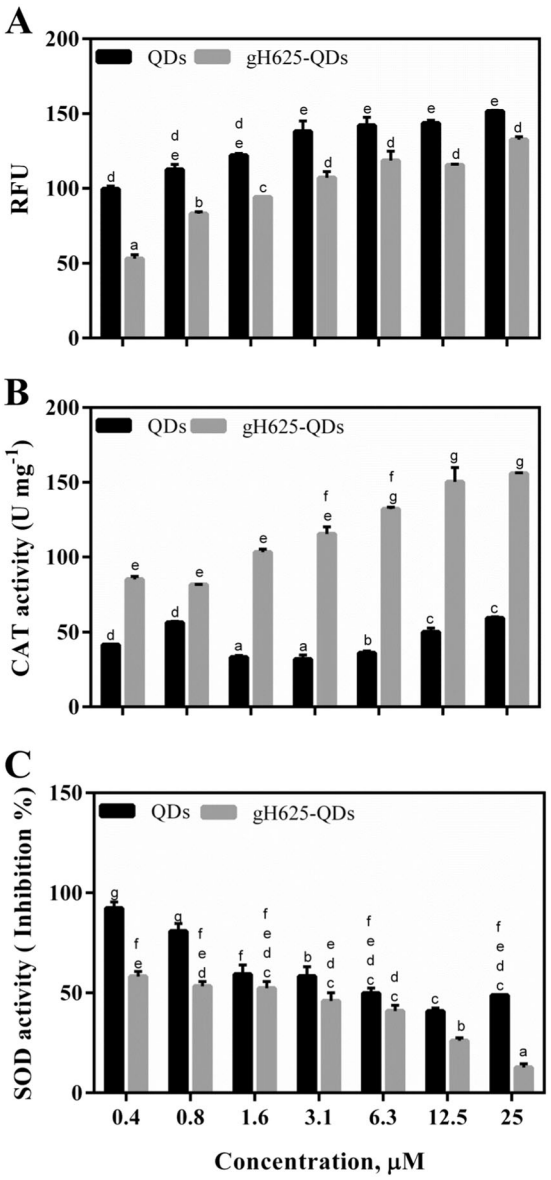

Fig. 3 ROS production by dichlorofluorescein fluorescence. Data are given as fluorescence values (mean ± SD) after normalization on control group (**a**). CAT activity is expressed as units mg^{-1} of protein mean ± SD (**b**). SOD activity is given as percentage of SOD inhibition compared to the control mean ± SD (**c**). Letters (a– i) indicate significant differences between treatments (Tukey's, $p < 0.05$)

activated. The experiment to evaluate SOD activity is reported in Fig. 3c as percentage of inhibition of the enzymatic activity. We observed that SOD activity is slightly dependent from the particle concentration; in particular, the enzymatic activity is more inhibited at high concentrations of both QDs-gH625 and QDs as expected and is less inhibited with QDs-gH625 than nude QDs, confirming the importance of the coating and the effectiveness of gH625 in reducing the toxic effect of the metal particles.

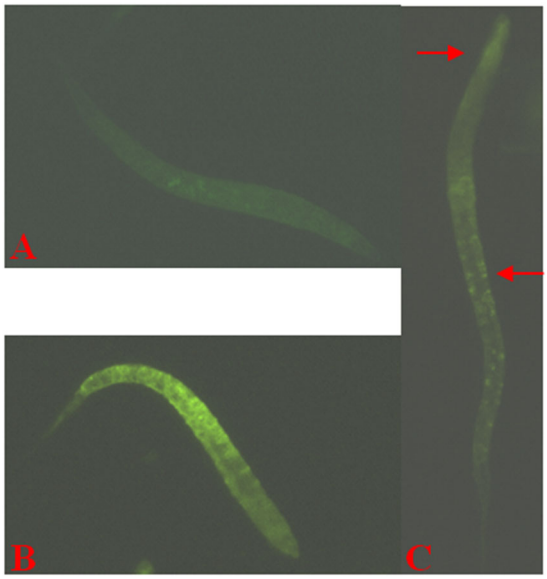

Fig. 4 Fluorescence image of *C. elegans* after 6 h of exposure to QDs and gH625-QDs (25 µM): **a** control, **b** QDs, **c** gH625-QDs

Moreover, we evaluated catalase activity as well (Fig. 3b). CAT displays a constant activity over the tested concentration in nematodes exposed to QDs while its activity is more intense with QDs-gH625. The higher CAT activity in presence of QDs-gH625 is reasonable as it is a natural consequence of the increase of ROS level; however, we observed a constant and less intense activity of this enzyme with QDs. The nude QDs may be able to inhibit the activity of this enzyme being another reason of toxicity.

C. elegans are good laboratory model also for their transparent body which allows a direct visualization of live worms without any specific pre-treatment. Fluorescence images were obtained (Fig. 4) after the administration of QDs or QDs-gH625 to visualize distribution of the nanoparticles in the body of our model. As shown in Fig. 4b, QDs could accumulate in several organs, both primary and secondary organs. The random accumulation of QDs in the body of small animals was also demonstrated in other scientific articles. The distribution changes when QDs-gH625 are administrated (Fig. 4c). The presence of QDs-gH625 in *C. elegans* is not homogeneous, rather the particles are significantly located in neurologic organs showing a predilection for these organs or, in other words, gH625 allows a greater internalization and also favors uptake by the brain. This was also demonstrated in previous works (Galdiero et al. 2017; Falanga et al. 2018) where gH625 was shown to change the internalization mechanism and distribution of the cargo. These results may be very significant for the design and development of appropriate molecules for drug delivery.

Conclusion

We have focused on quantum dots and their application as nanomaterials for healthcare. Well-known are the amazing physico-chemical properties of this nanoparticles as well as their human and environmental toxicity. In this study, we have explored the possibility of coating QDs with a cell-penetrating peptide gH625 and investigated the effects caused on the motor nervous system in *C. elegans* used as non-mammalian model.

In particular, we have previously identified gH625, a viral peptide with challenging delivery properties. Here, we explored the possibility to use this peptide for the coating of metallic nanoparticles. Through different biological assays, we have shown that the coating with our cell-penetrating peptide is a promising strategy to make quantum dots more suitable for medical treatments and to modify their mechanism of internalization; indeed, gH625 is also able to reduce the side effects, as well as the tropism, of metallic nanoparticles. Furthermore, as there are similarities between nematodes and higher vertebrates at all levels, we think it may be possible to translate our results collected on *C. elegans* to more complex living beings.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

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References

Acosta C et al. (2018) Toxicological assessment of mesoporous silica particles in the nematode *Caenorhabditis elegans*. Environ Res 166:61–70. https://doi.org/10.1016/j.envres.2018.05.018

de Alteriis E et al. (2018) Polymicrobial antibiofilm activity of the membranotropic peptide gH625 and its analogue. Microb Pathog 125:189–195. https://doi.org/10.1016/j.micpath.2018.09.027

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Carberry TP et al. (2012) Dendrimer functionalization with a membrane-interacting domain of herpes simplex virus type 1: towards intracellular delivery. Chemistry 18:13678–13685. https://doi.org/10.1002/chem.201202358

Chen N et al. (2012) The cytotoxicity of cadmium-based quantum dots. Biomaterials 33:1238–1244. https://doi.org/10.1016/j.biomaterials.2011.10.070

Cho SJ et al. (2007) Long-term exposure to CdTe quantum dots causes functional impairments in live cells. Langmuir 23:1974–1980. https://doi.org/10.1021/la060093j

Contreras EQ et al. (2013) Toxicity of quantum dots and cadmium salt to *Caenorhabditis elegans* after multigenerational exposure. Environ Sci Technol 47:1148–1154. https://doi.org/10.1021/es3036785

Falanga A et al. (2011) A peptide derived from herpes simplex virus type 1 glycoprotein H: membrane translocation and applications to the delivery of quantum dots. Nanomedicine 7:934. https://doi.org/10.1016/j.nano.2011.04.009

Falanga A et al. (2018) Enhanced uptake of gH625 by blood brain barrier compared to liver in vivo: characterization of the mechanism by an in vitro model and implications for delivery. Sci Rep 8:13836–13836. https://doi.org/10.1038/s41598-018-32095-w

Falanga A, Cantisani M, Pedone C, Galdiero S (2009) Membrane fusion and fission: enveloped viruses. Protein Pept Lett 16:751–759

Falanga A, Galdiero M, Galdiero S (2015) Membranotropic cell penetrating peptides: the outstanding journey. Int J Mol Sci 16:25323–25337. https://doi.org/10.3390/ijms161025323

Finkel T, Holbrook NJ (2000) Oxidants, oxidative stress and the biology of ageing. Nature 408:239. https://doi.org/10.1038/35041687

Fridovich I (1995) Superoxide radical and superoxide dismutases. Annu Rev Biochem 64:97–112. https://doi.org/10.1146/annurev.bi.64.070195.000525

Galdiero E et al. (2017) Daphnia magna and xenopus laevis as in vivo models to probe toxicity and uptake of quantum dots functionalized with gH625. Int J Nanomed 12:2717–2731. https://doi.org/10.2147/ijn.s127226

Galdiero S et al. (2010) The presence of a single N-terminal histidine residue enhances the fusogenic properties of a membranotropic peptide derived from herpes simplex virus type 1 glycoprotein H. J Biol Chem 285:17123–17136. https://doi.org/10.1074/jbc.M110.114819

Galdiero S et al. (2012) Structure and orientation of the gH625-644 membrane interacting region of herpes simplex virus type 1 in a membrane mimetic system. Biochemistry 51:3121–3128. https://doi.org/10.1021/bi201589m

Galdiero S, Falanga A, Morelli G, Galdiero M (2015) gH625: a milestone in understanding the many roles of membranotropic peptides. Biochim Biophys Acta 1848:16–25. https://doi.org/10.1016/j.bbamem.2014.10.006

Galstyan A et al. (2019) Blood-brain barrier permeable nano immunoconjugates induce local immune responses for glioma therapy. Nat Commun 10:3850. https://doi.org/10.1038/s41467-019-11719-3

Gao X et al. (2005) In vivo molecular and cellular imaging with quantum dots. Curr Opin Biotechnol 16:63–72. https://doi.org/10.1016/j.copbio.2004.11.003

Garsin DA et al. (2003) Long-lived C. elegans daf-2 mutants are resistant to bacterial pathogens. Science 300:1921. https://doi.org/10.1126/science.1080147

Guarnieri, D et al. (2015) Surface decoration with gH625-membranotropic peptides as a method to escape the endo-lysosomal compartment and reduce nanoparticle toxicity. Nanotechnology 26. https://doi.org/10.1088/0957-4484/26/41/415101

Hekimi S, Guarente L (2003) Genetics and the specificity of the aging process. Science 299:1351. https://doi.org/10.1126/science.1082358

Hodgkin J, Doniach T (1997) Natural variation and copulatory plug formation in *Caenorhabditis elegans*. Genetics 146:149–164

Hsu P-CL, O'Callaghan M, Al-Salim N, Hurst MRH (2012) Quantum dot nanoparticles affect the reproductive system of *Caenorhabditis elegans*. Environ Toxicol Chem 31:2366–2374. https://doi.org/10.1002/etc.1967

Iachetta G et al. (2019) gH625-liposomes as tool for pituitary adenylate cyclase-activating polypeptide brain delivery. Sci Rep. 9:9183–9183. https://doi.org/10.1038/s41598-019-45137-8

Jamieson T et al. (2007) Biological applications of quantum dots. Biomaterials 28:4717–4732. https://doi.org/10.1016/j.biomaterials.2007.07.014

Khosravi-Katuli K et al. (2018) Effects of ZnO nanoparticles in the Caspian roach (Rutilus rutilus caspicus). Sci Total Environ 626:30–41. https://doi.org/10.1016/j.scitotenv.2018.01.085

Li D et al. (2019) Cross-linked poly(ethylene glycol) shells for nanoparticles: enhanced stealth effect and colloidal stability. Langmuir 35:8799–8805. https://doi.org/10.1021/acs.langmuir.9b01325

Li Y et al. (2013) High concentration of vitamin E decreases thermosensation and thermotaxis learning and the underlying mechanisms in the nematode *Caenorhabditis elegans*. PLoS One 8:e71180. https://doi.org/10.1371/journal.pone.0071180

Ma H, Bertsch PM, Glenn TC, Kabengi NJ, Williams PL (2009) Toxicity of manufactured zinc oxide nanoparticles in the nematode *Caenorhabditis elegans*. Environ Toxicol Chem 28:1324–1330. https://doi.org/10.1897/08-262.1

Mallo GV et al. (2002) Inducible antibacterial defense system in C. elegans. Curr Biol 12:1209–1214. https://doi.org/10.1016/S0960-9822(02)00928-4

Martin-Serrano, A, Gomez, R, Ortega, P & de la Mata, FJ (2019) Nanosystems as vehicles for the delivery of antimicrobial peptides (AMPs). Pharmaceutics 11. https://doi.org/10.3390/pharmaceutics11090448

McMahan RS, Lee V, Parks WC, Kavanagh TJ, Eaton DL (2014) In vitro approaches to assessing the toxicity of quantum dots. Methods Mol Biol 1199:155–163. https://doi.org/10.1007/978-1-4939-1280-3_12

Medintz IL, Uyeda HT, Goldman ER, Mattoussi H (2005) Quantum dot bioconjugates for imaging, labelling and sensing. Nat Mater 4:435. https://doi.org/10.1038/nmat1390

Oral R, Pagano G, Siciliano A, Toscanesi M, Gravina M, Di Nunzio A, Palumbo A, Thomas PJ, Tommasi F, Burić P, Lyons DM, Guida M, Trifuoggi M (2019) Soil pollution and toxicity in an area affected by emissions from a bauxite processing plant and a power plant in Gardanne (southern France). Ecotoxicol Environ Saf 170:55–61. https://doi.org/10.1016/j.ecoenv.2018.11.122

Pellico, J et al. (2019) Unambiguous detection of atherosclerosis using bioorthogonal nanomaterials. Nanomedicine https://doi.org/10.1016/j.nano.2018.12.015

Qu Y et al. (2011) Full assessment of fate and physiological behavior of quantum dots utilizing *Caenorhabditis elegans* as a model organism. Nano Lett 11:3174–3183. https://doi.org/10.1021/nl201391e

Roh J-Y, Park Y-K, Park K, Choi J (2010) Ecotoxicological investigation of CeO2 and TiO2 nanoparticles on the soil nematode *Caenorhabditis elegans* using gene expression, growth, fertility, and survival as endpoints. Environ Toxicol Pharmacol 29:167–172. https://doi.org/10.1016/j.etap.2009.12.003

Troemel ER et al. (2006) p38 MAPK regulates expression of immune response genes and contributes to longevity in C. elegans. PLOS Genet 2:e183. https://doi.org/10.1371/journal.pgen.0020183

Ulusoy M et al. (2016) Evaluation of CdTe/CdS/ZnS core/shell/shell quantum dot toxicity on three-dimensional spheroid cultures. Toxicol Res 5:126–135. https://doi.org/10.1039/c5tx00236b

Valiante S et al. (2015) Peptide gH625 enters into neuron and astrocyte cell lines and crosses the blood-brain barrier in rats. Int J Nanomed 10:1885–1898. https://doi.org/10.2147/ijn.s77734

Wang H, Wick RL, Xing B (2009) Toxicity of nanoparticulate and bulk ZnO, Al2O3 and TiO2 to the nematode *Caenorhabditis elegans*. Environ Pollut 157:1171–1177. https://doi.org/10.1016/j.envpol.2008.11.004

Winnik FM, Maysinger D (2013) Quantum dot cytotoxicity and ways to reduce it. Acc Chem Res 46:672–680. https://doi.org/10.1021/ar3000585

Wu, L et al. (2019) Enzyme-responsive multifunctional peptide coating of gold nanorods improves tumor targeting and photothermal therapy efficacy. Acta Biomater. https://doi.org/10.1016/j.actbio.2019.01.026

Zhao Y et al. (2015) Quantum dots exposure alters both development and function of D-type GABAergic motor neurons in nematode *Caenorhabditis elegans*. Toxicol Res 4:399–408. https://doi.org/10.1039/c4tx00207e