Internalization and toxicological mechanisms of uncoated and PVP-coated cerium oxide nanoparticles in the freshwater alga Chlamydomonas reinhardtii

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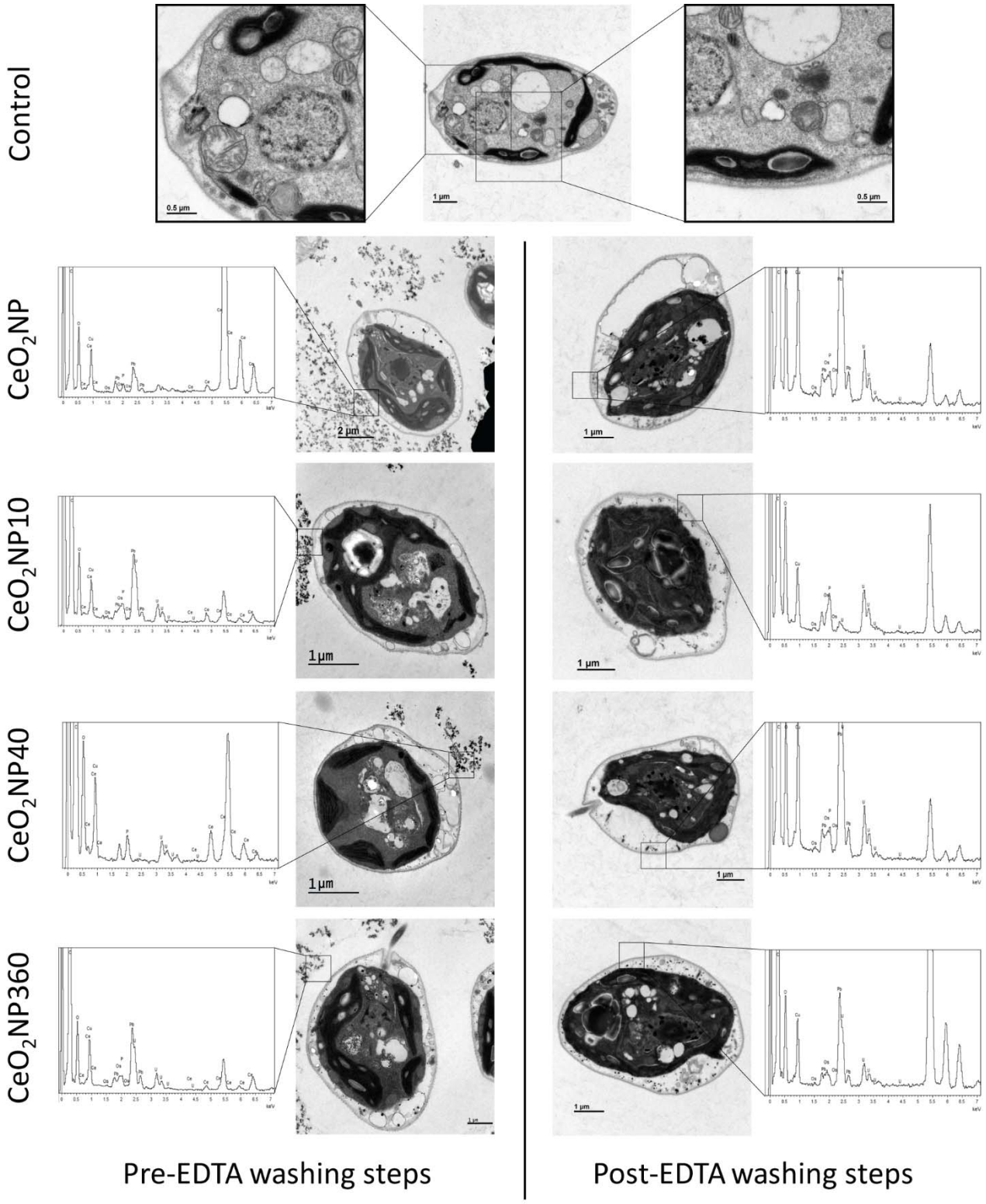
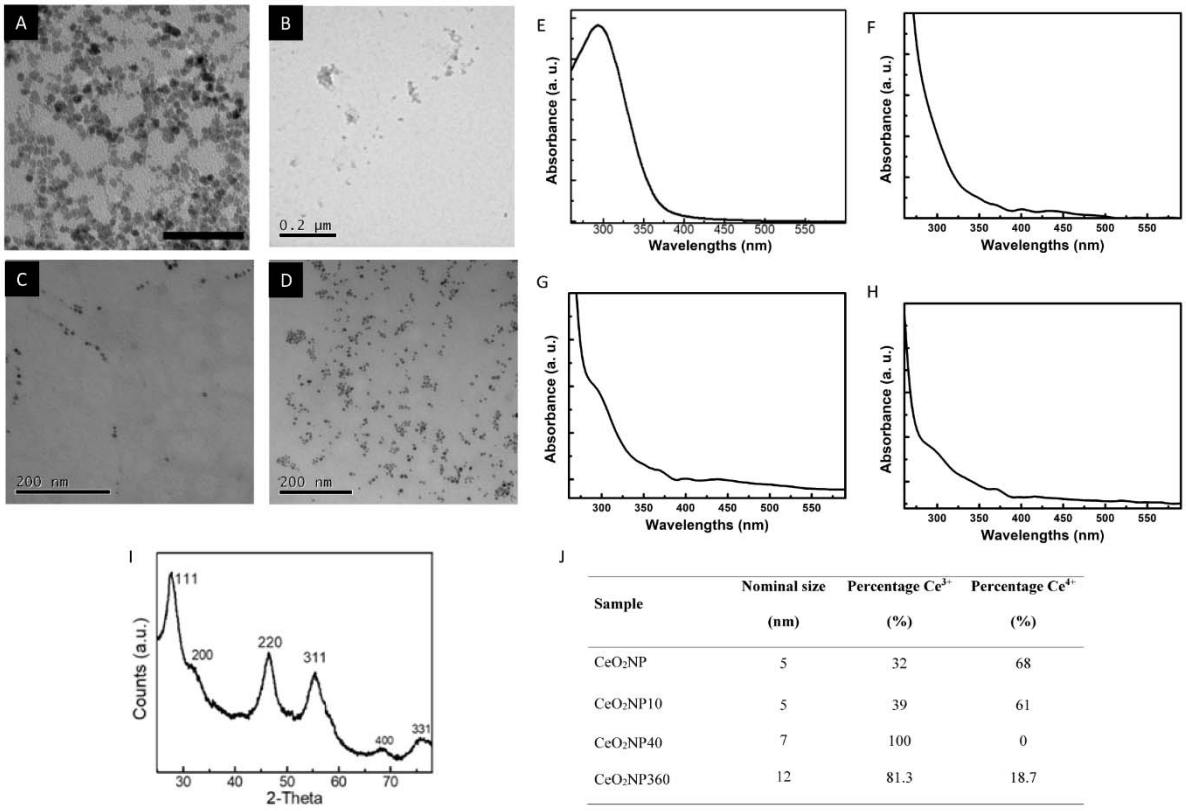


Figure S1. TEM images and EDX spectra of *C. reinhardtii* cells before (left column) and after (right column) the application of EDTA washing steps.



Sample	Nominal size (nm)	Percentage Ce^{3+} (%)	Percentage Ce^{4+} (%)
CeO_2NP	5	32	68
CeO_2NP10	5	39	61
CeO_2NP40	7	100	0
CeO_2NP360	12	81.3	18.7

Figure S2. Characterization of the non-coated and coated CeO_2NPs used in this work. TEM image of CeO_2NP (A – scale bar: 50 nm), CeO_2NP10 (B), CeO_2NP40 (C) and CeO_2NP360 (D). UV-vis absorbance spectrum of CeO_2NP, CeO_2NP10, CeO_2NP40 and CeO_2NP360 is shown in E, F, G and H, respectively. XRD spectra of the CeO_2NP showing the characteristics peaks of CeO_2 crystals is shown in I (there was no good XRD data for PVP-coated nanoparticles due to the external presence of PVP as capping agent). Section J shows the nominal size and percentage of surface Ce^{3+}/Ce^{4+} of CeO_2NPs calculated by X-Ray photoelectron spectroscopy. TEM and UV-Vis spectra of PVP-coated CeO_2NPs are reproduced from Briffa *et al* (2017) with permission from the Royal Society of Chemistry. Additional characterization of these NPs can be also found in the cited reference.

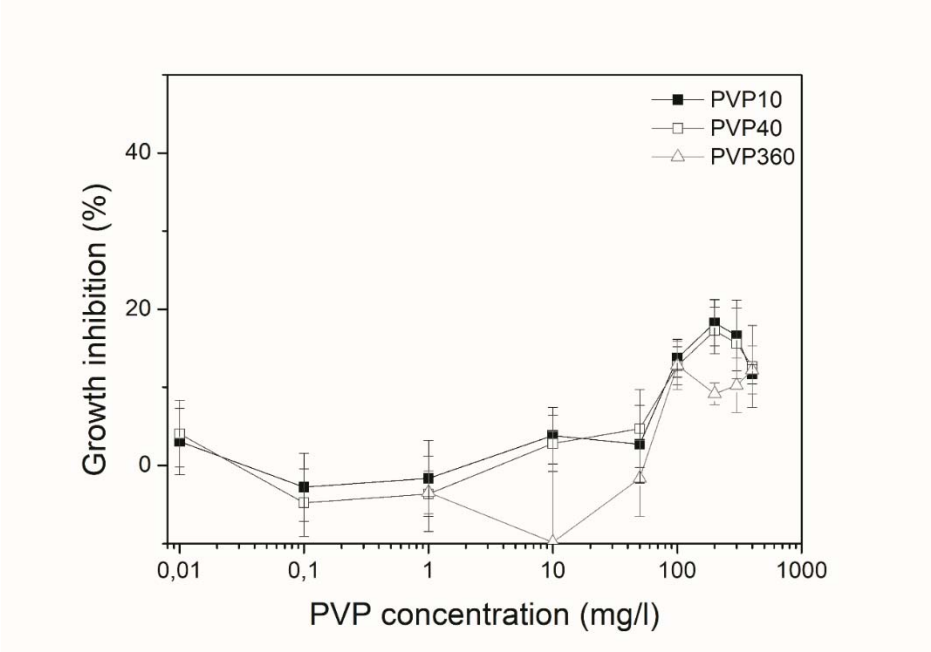


Figure S3. The biological effect of 72 h exposure to the three PVP used to synthesize the different CeO_2NPs on the growth of *C. reinhardtii.* Data are expressed as percentages of the value of untreated cells (mean ± standard deviation).

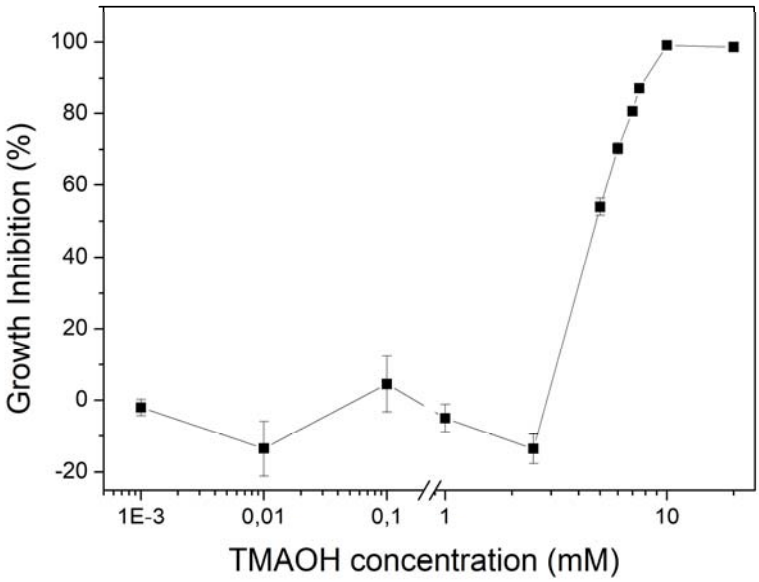


Figure S4. The biological effect of 72 h exposure to TMAOH used to stabilize the uncoated CeO_2NPs on the growth of *C. reinhardtii.* Data are expressed as percentages of the value of untreated cells (mean ± standard deviation).

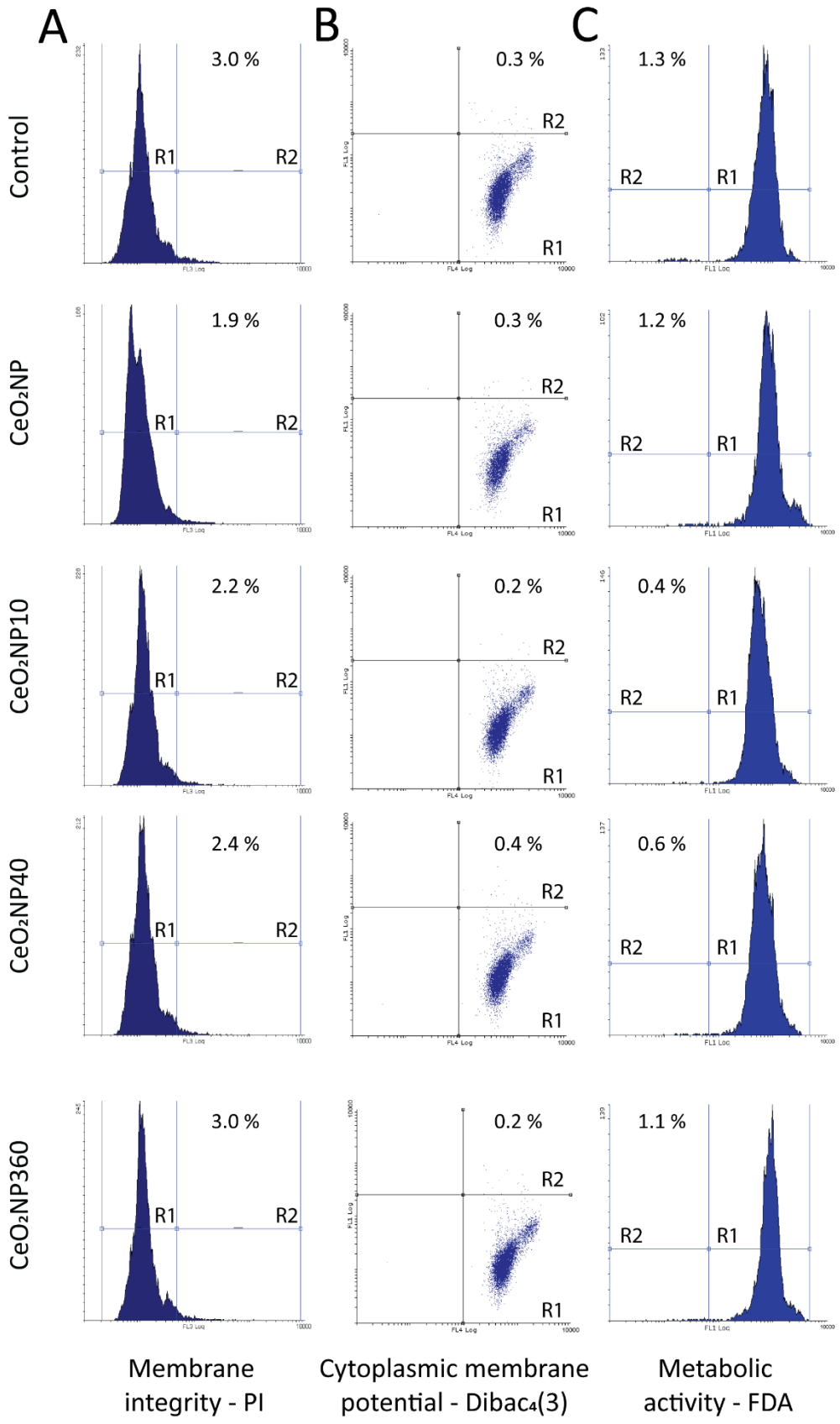


Figure S5. The biological effect of 0.1 mg/L of CeO_2NP, CeO_2NP10, CeO_2NP40 and CeO_2NP360 on cell membrane integrity (A), cytoplasmic membrane potential (B) and metabolic activity (C) of *C. reinhardtii*.

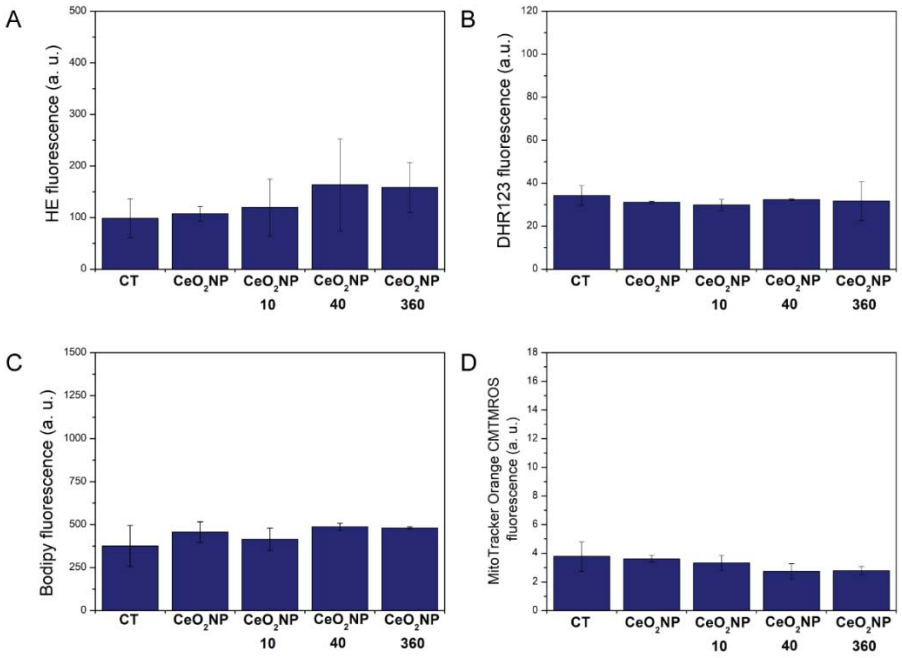


Figure S6. Effect of 0.1 mg/L of CeO_2NPs on intracellular superoxide anion and hydrogen peroxide levels of *C. reinhardtii* by FCM using the fluorochrome HE (A) and DHR123 (B), respectively. Alterations derived of oxidative stress in mitochondria and intracellular lipid peroxidation are also shown in (C) and (D), respectively.

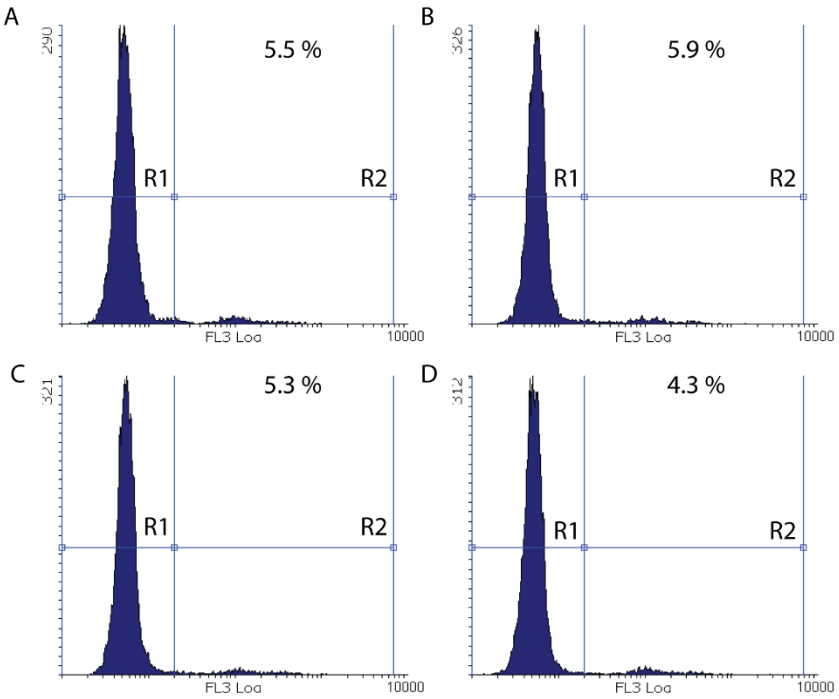


Figure S7. The effect of TMAOH (compound used to stabilize uncoated CeO_2NPs) at 0.1 mM (B), 1 mM (C) and 2.5 mM (D) on the cell membrane integrity of *C. reinhardtii*. A: control cells without TMAOH.

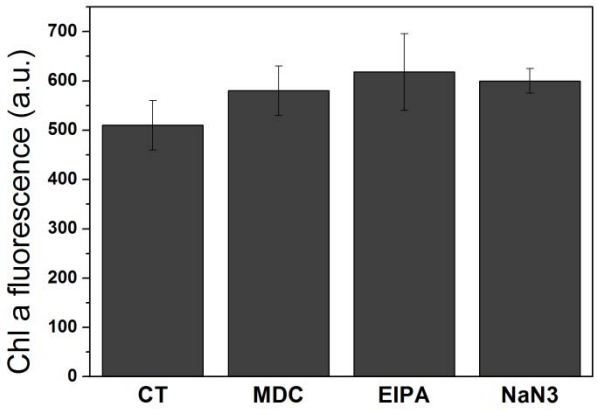


Figure S8. The effect of the different endocytic inhibitors towards chlorophyll *a* fluorescence of *C. reinhardtii*. CT: control. MDC: monodansylcadaverine. EIPA: 5-(N-ethyl-N-isopropyl)-amiloride. NaN_3: Sodium azide.

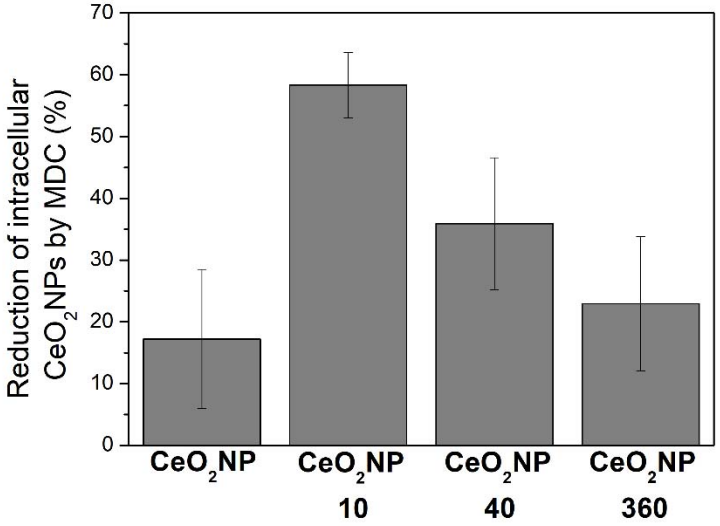


Figure S9. The level of reduction of intracellular CeO_2NPs after the treatment with the MDC inhibitor. Data are expressed as percentage of reduction in comparison with CNPs samples without inhibitor (mean ± standard deviation).

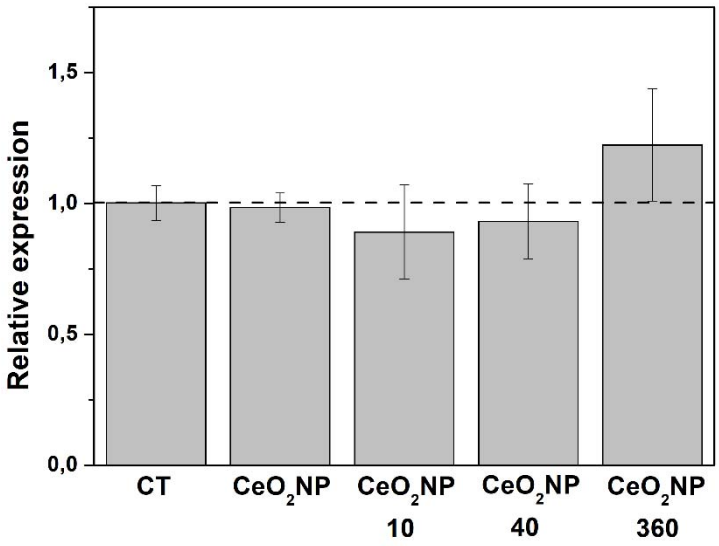


Figure S10. Effect of CeO_2NPs on expression of *CHC1* gene after 4 h of exposure. Data are represented as relative expression of the genes with respect to the unexposed control. Control values were set to 1 for easy comparison.

Table S1: Fluorochromes used to analyze several physiological parameters of *C. reinhardtii* by flow cytometry.

Fluorochrome	Acronym	Applications	Stock concentration (mg mL^{-1})	Final concentration (µg mL^{-1})	Incubation time (min)
Dihydrorhodamine123	**DHR 123**	Intracellular levels of hydrogen peroxide	2	10	40
Hydroethidine	**HE**	Intracellular levels of superoxide anion	3.154	5	30
Propidium iodide	**IP**	Membrane integrity	1	5	10
Fluorescein Diacetate	**FDA**	Unspecific esterase activity	5	2.5	15
bis-(1,3-dibutylbarbituric acid) trimethine oxonol	**$DiBAC_4(3)$**	Cytoplasmic membrane potential	0.5	2.5	10
5-Butyl-4,4-Difluoro-4-Bora-3a,4a-Diaza-s-Indacene-3-Nonanoic Acid	**BODIPY- C4-C9**	Lipid peroxidation	101.08	0.01	10
MitoTracker® Orange CM-H2TMRos	**Mitotracker**	Mitochondrial ROS homeostasis	0.05	2.5	60

References

Briffa, S. M., Lynch, I., Trouillet, V., Bruns, M., Hapiuk, D., Liu, J., ... & Valsami-Jones, E. (2017). Development of scalable and versatile nanomaterial libraries for nanosafety studies: polyvinylpyrrolidone (PVP) capped metal oxide nanoparticles. *RSC Advances*, *7*(7), 3894-3906.