The effect of PVP coatings on internalization and toxicological mechanisms of cerium oxide nanoparticles

Pulido-Reyes, G., Briffa S.M., Yudina, T., Leganés F., Puntes, V., Valsami-Jones E., Rosal R., & **Fernández-Piñas F.**

E-mail contact: francisca.pina@uam.es

Universidad de Alcalá

UAM UNIVERSIDAD AUTONOMA DE MADRID

FACULTAD DE CIENCIAS UNIVERSIDAD AUTÓNOMA DE MADRID

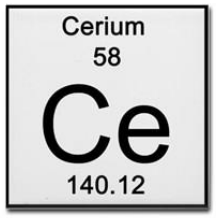
Departamento de BIOLOGÍA

ICN2 Institut Català de Nanociència i Nanotecnologia

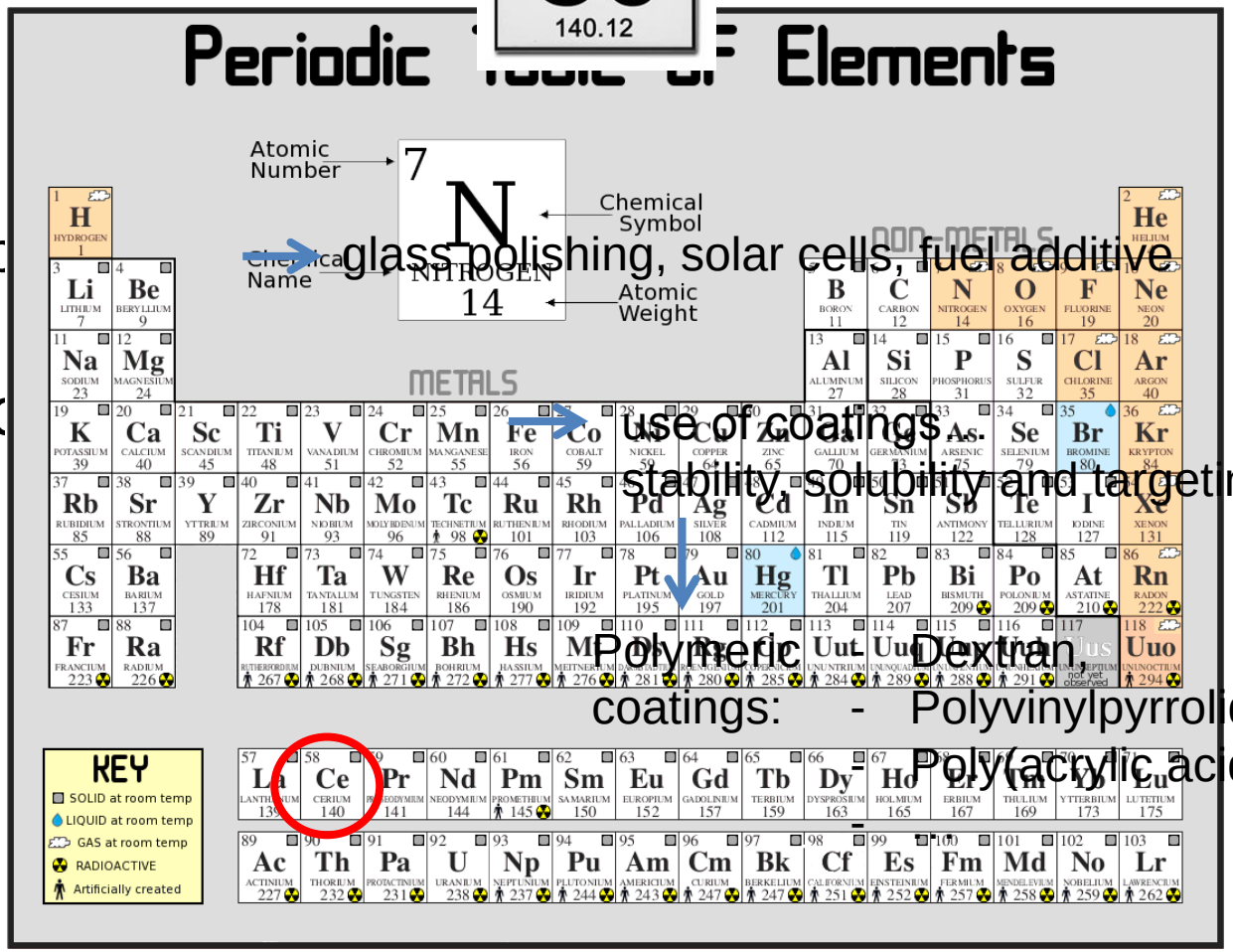
UNIVERSITY OF BIRMINGHAM

Introduction

- The element cerium



- CeO_2
  - app → glass polishing, solar cells, fuel additive
- Surfac → use of coatings → stability, solubility and targeting
  - Polymeric coatings: - Dextran,
    - Polyvinylpyrrolidone (PVP),
    - Poly(acrylic acid) (PAA),

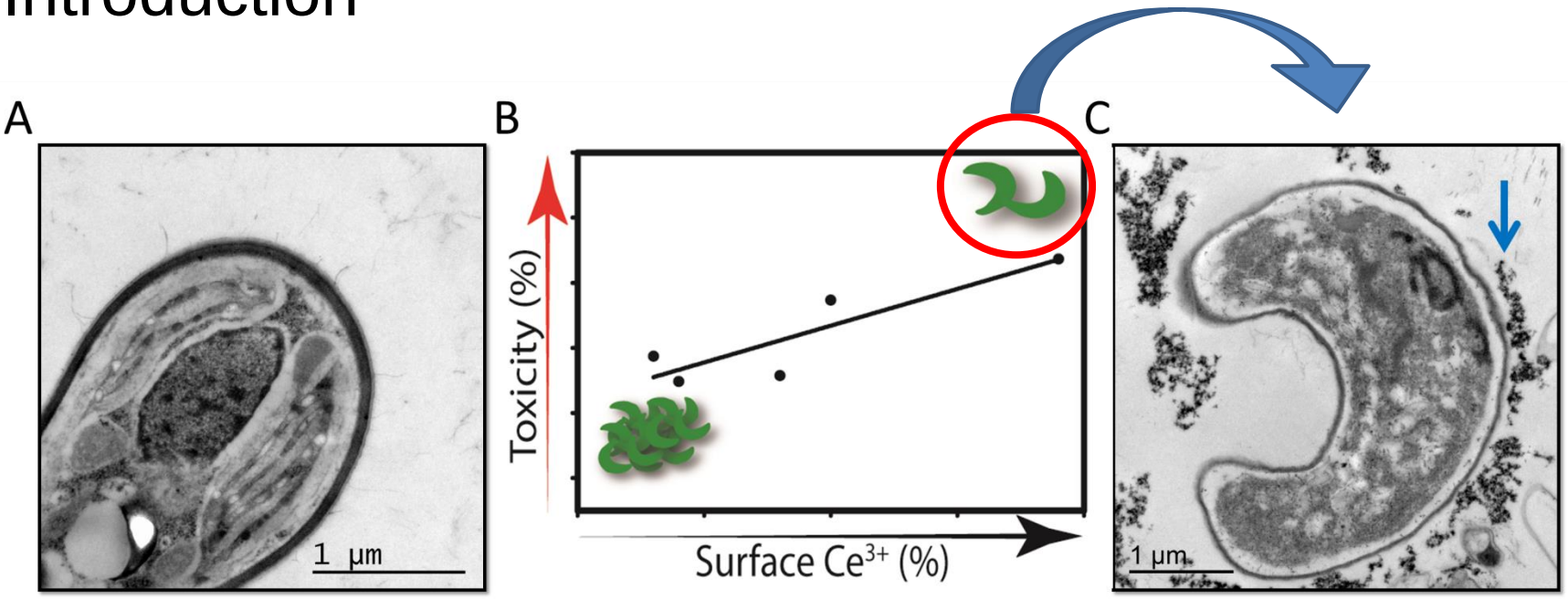


Introduction

Pro-oxidant or Antioxidant?

Cerium oxide NanoParticles (CeOxNP)

Introduction



www.nature.com/scientificreports

Article | OPEN

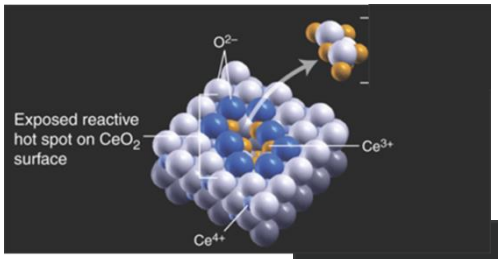
Untangling the biological effects of cerium oxide nanoparticles: the role of surface valence states

Gerardo Pulido-Reyes, Ismael Rodea-Palomares, Soumen Das, Tamil Selvan Sakthivel, Francisco Leganes, Roberto Rosal, Sudipta Seal & Francisca Fernández-Piñas

Scientific Reports **5**, Article number: 15613 (2015)
doi:10.1038/srep15613
Download Citation

Environmental sciences | Microbiology

The case of Cerium Oxide Nanoparticles

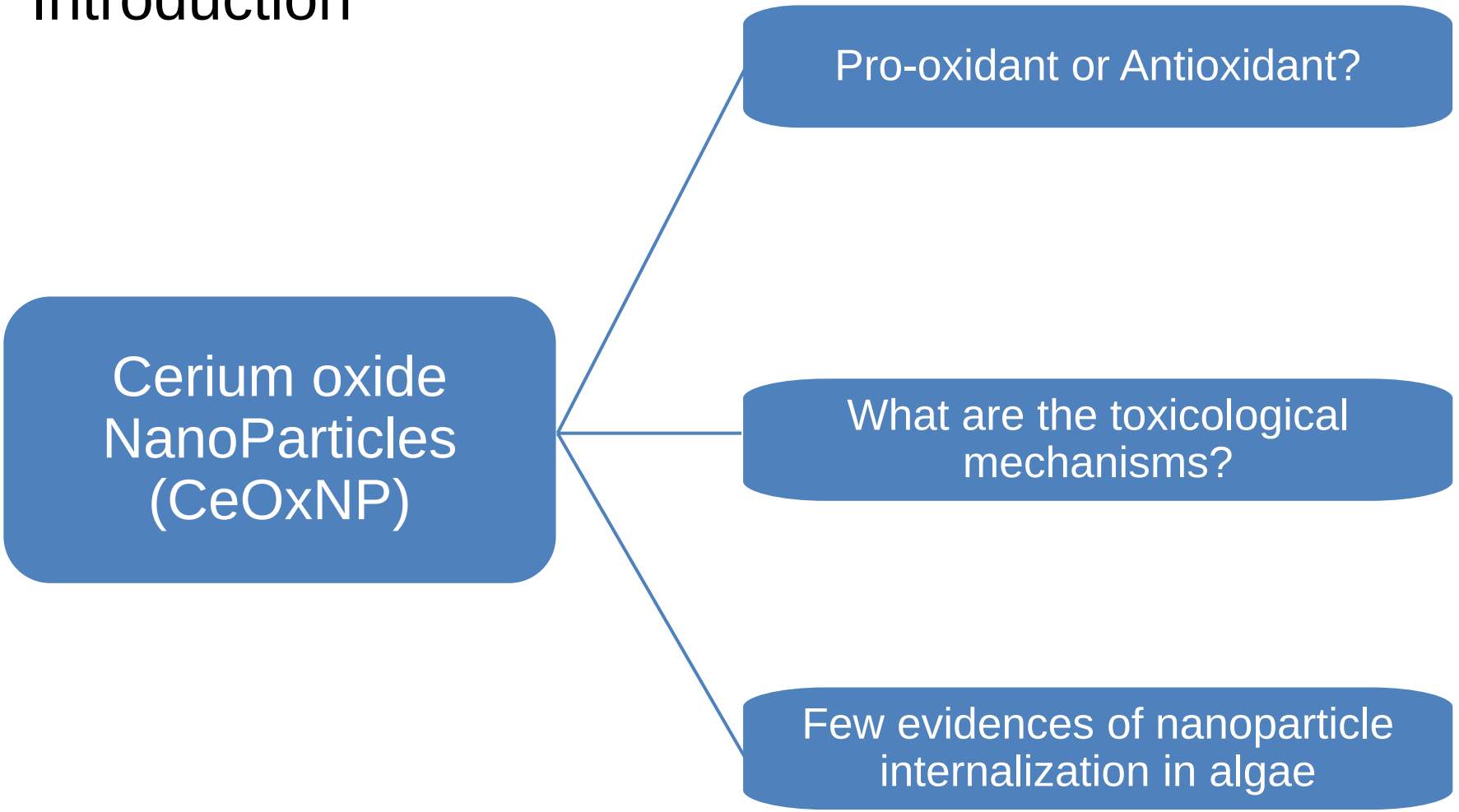


Campbell and Peden. Science. 309 (5735): 713-714

CATALYTIC ACTIVITY	Ce^{3+}	Ce^{4+}
Phosphatase	No	Yes
·NO scavenger	No	Yes
Catalase mimetic	No	Yes
SOD mimetic	Yes	No
Peroxynitrite scavenger	Yes	Yes
Hypochlorite scavenger	No	Yes

Dowding JM, Seal S, Self WT. *Drug Delivery and Translational Research*. 2013; 3 : 375-9.
Dowding JM, Das S, Kumar A, Dosani T, McCormack R, Gupta A, et al. *ACS Nano*. 2013 ; 7 : 4855-68.
Pulido-Reyes, G., Das, S., Leganés, F., Silva, S. O., Wu, S., Self, W., et al. *RSC Advances*. 2016 ; *6*(67), 62911-62915.

Introduction



Preview

The effect of

- an uncoated CeOxNP
- polyvinylpyrrolidone (PVP)-coated CeOxNP

→ the green alga *Chlamydomonas reinhardtii*

With the aim of:

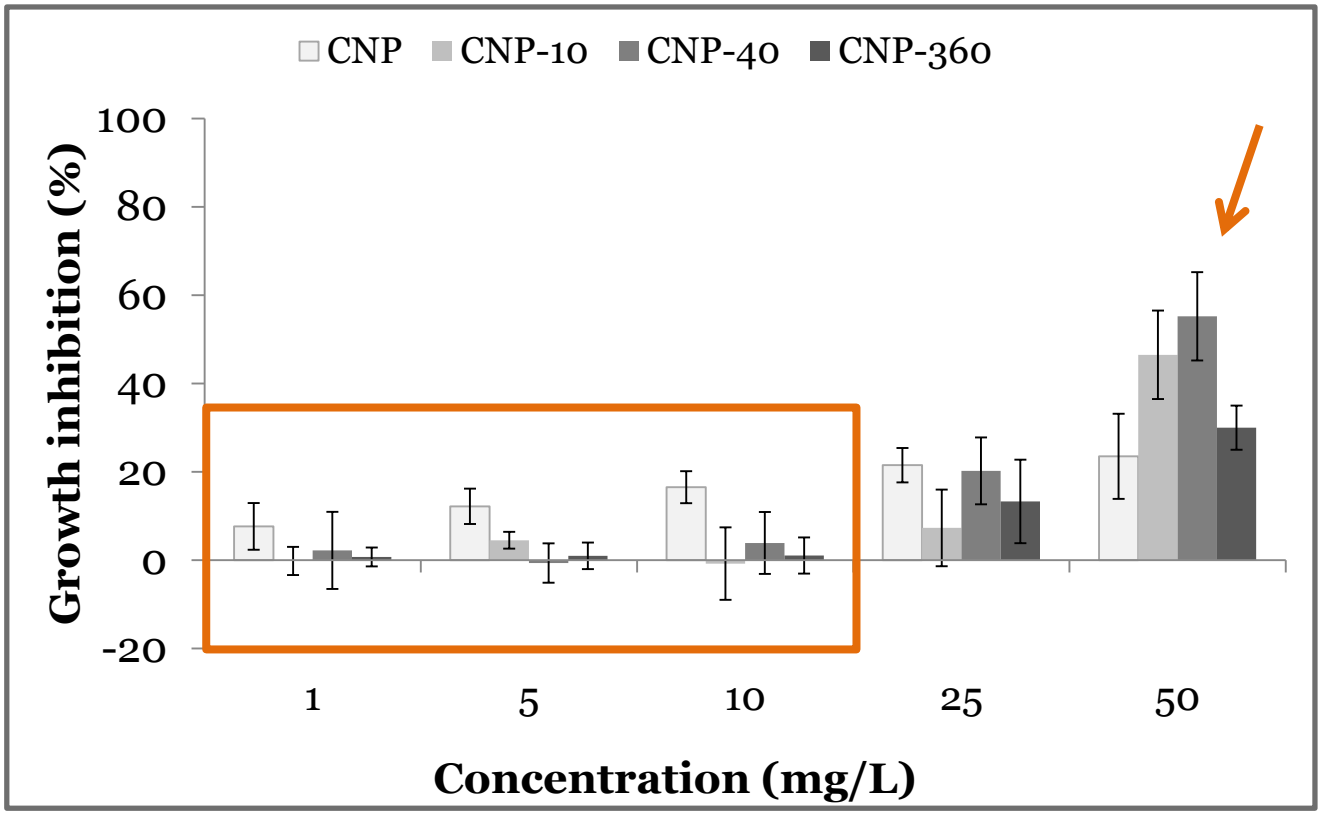
1. identifying their toxicological mechanisms and
2. studying the possibility of their cellular internalization.

Physicochemical Properties of CeOxNP in algal OECD medium

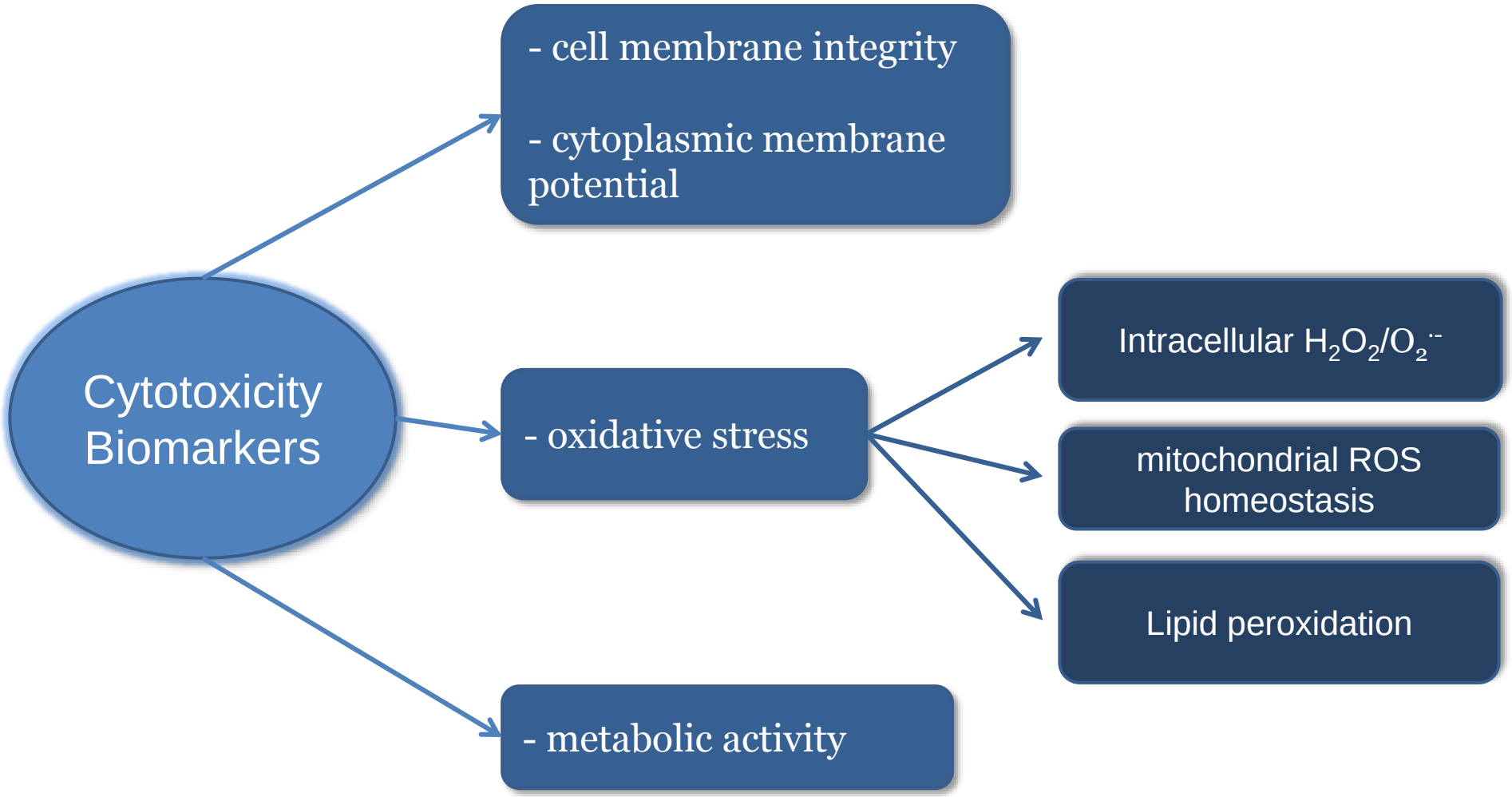
Sample Name	PVP chain length	ζ -Pot (mV)	DLS Diameter (nm)	PDI
CNP	-	-13.0 ± 1.1	10.25 ± 0.5	0.38
CNP-10	10 kDa	-8.11 ± 0.8	6.03 ± 0.1	0.18
CNP-40	40 kDa	-7.25 ± 0.4	8.20 ± 0.61	0.15
CNP-360	360 kDa	-9.23 ± 0.3	12.49 ± 0.1	0.28

- Ce^{3+} dissolution from the nanoparticles was very low (ICP-MS)
- Negative surface charge with similar values in the 7-13 mV range
- Slight increase in the effective diameter based on the PVP-chain length
- CNP-40 had the highest % of surface Ce^{3+}.

Effect of 72 h exposure to CeOxNP on growth of *C. reinhardtii*

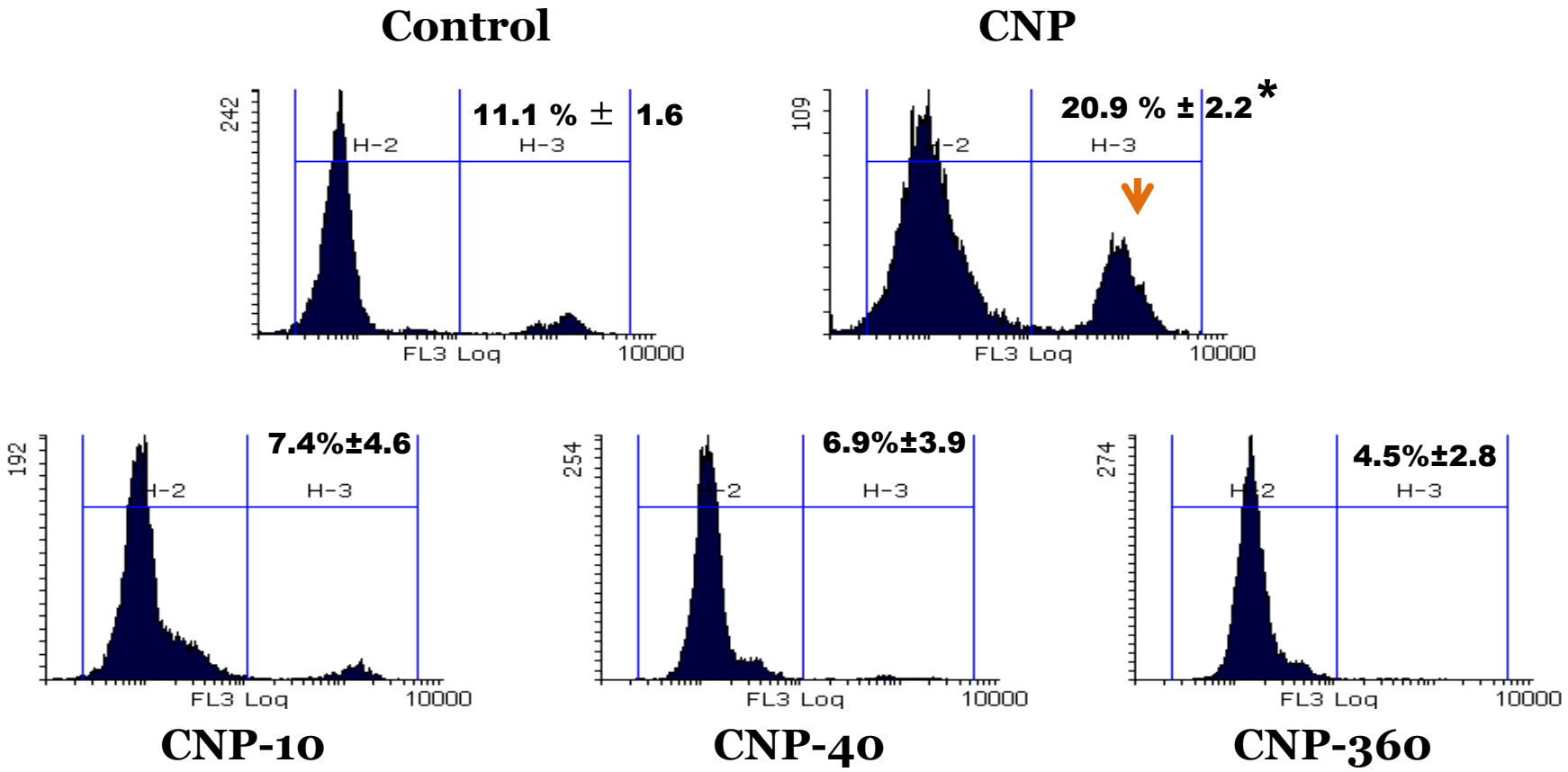


- CNP was more toxic at low concentrations than PVP-coated CNPs.
- CNP-10 and CNP-40 showed higher growth inhibition at 50 mg/L than CNP-360 and CNP.

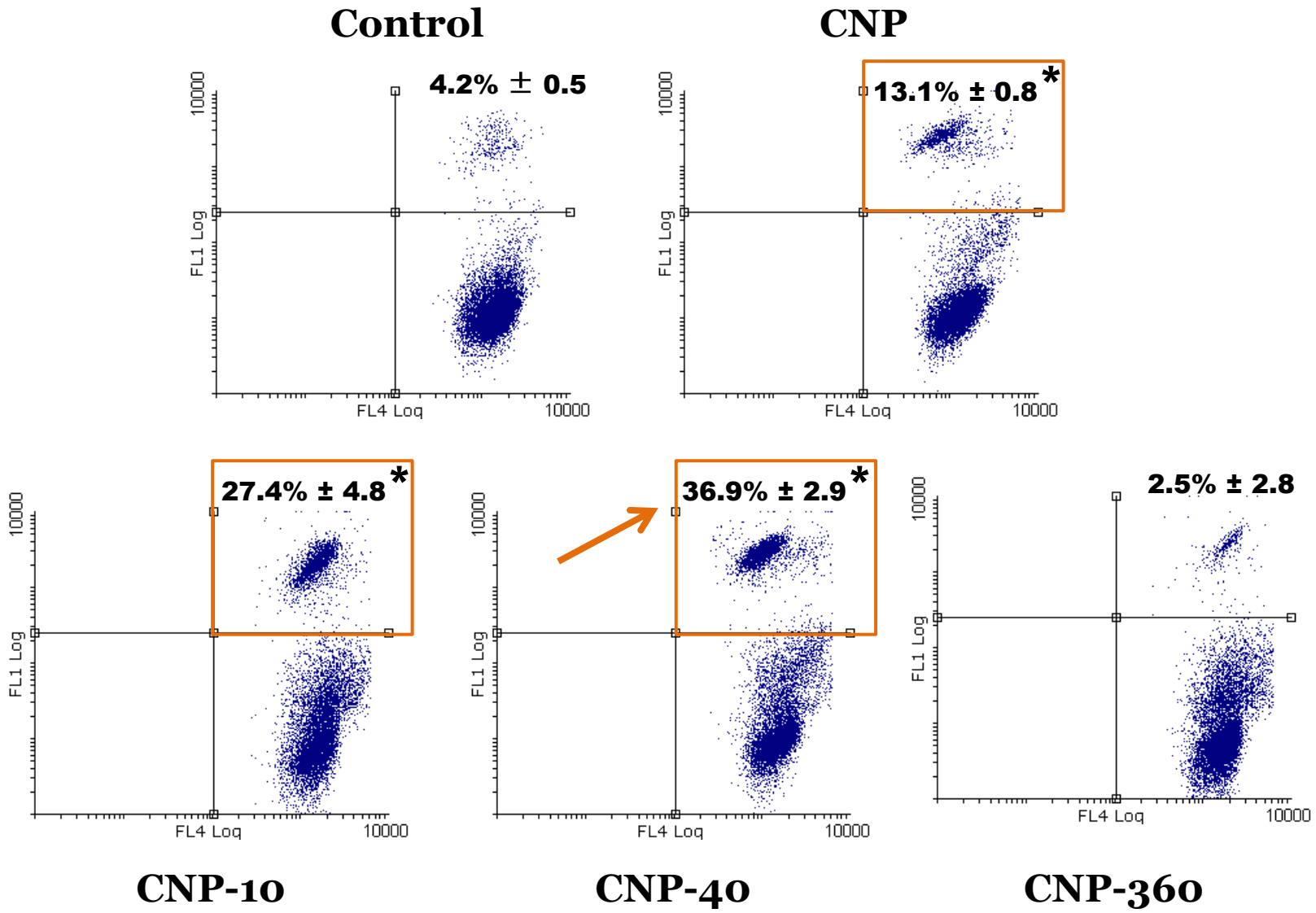

Cytotoxicity Biomarkers
- cell membrane integrity
- cytoplasmic membrane potential
- oxidative stress
Intracellular $H_2O_2/O_2^{\cdot-}$
mitochondrial ROS homeostasis
Lipid peroxidation
- metabolic activity

Cell membrane integrity with Propidium Iodide

- Only CNP caused a disruption of cell membrane integrity (PI-positive, non-viable cells) in agreement with the growth inhibition for this nanoparticle (10 mg/l).

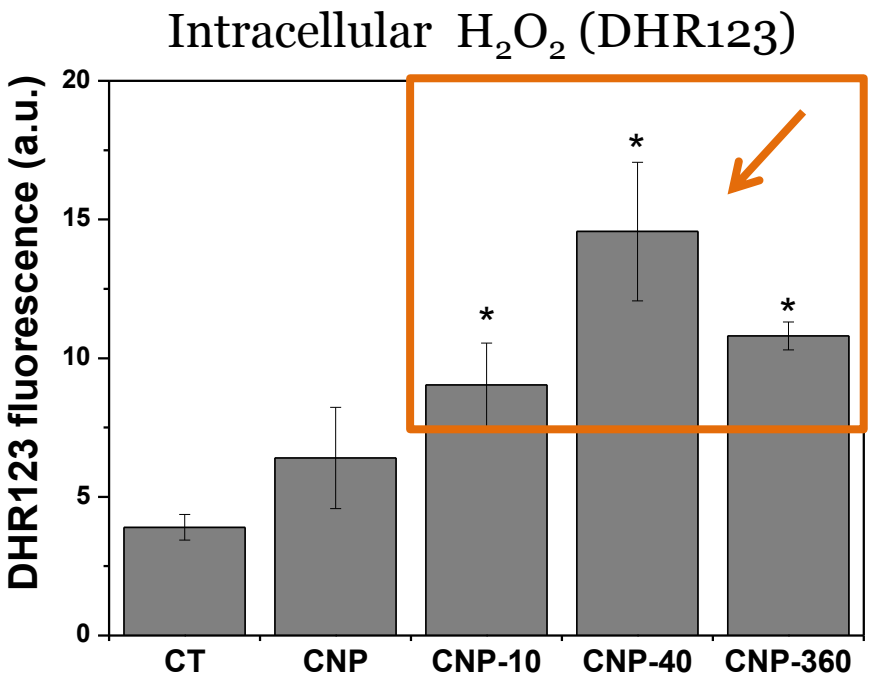


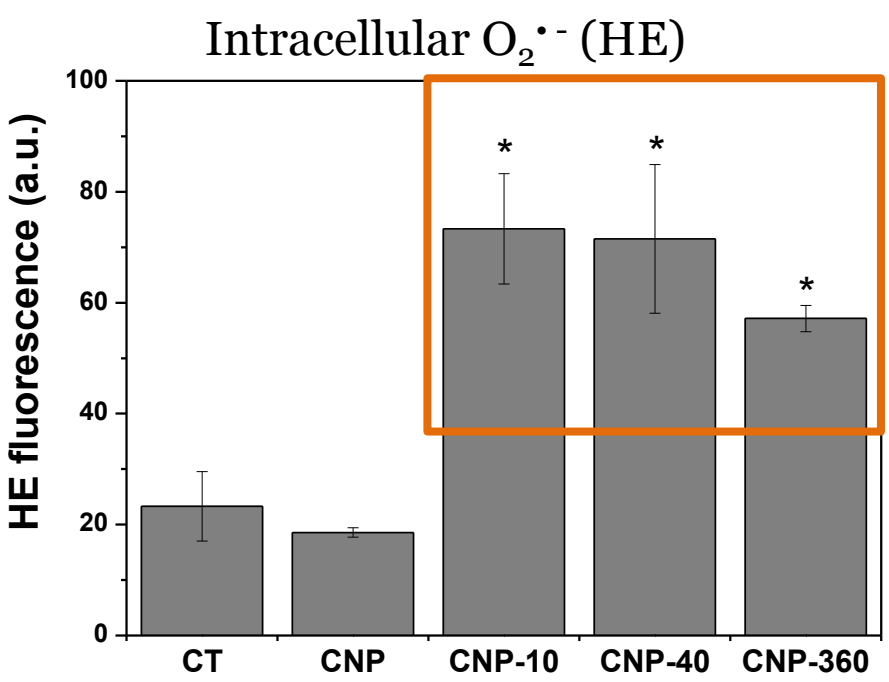
Cytoplasmic membrane potential: depolarization



Oxidative stress (I)

- CNP-10, CNP-40 and CNP-360 caused a significant increase in the intracellular level of both oxidative species.

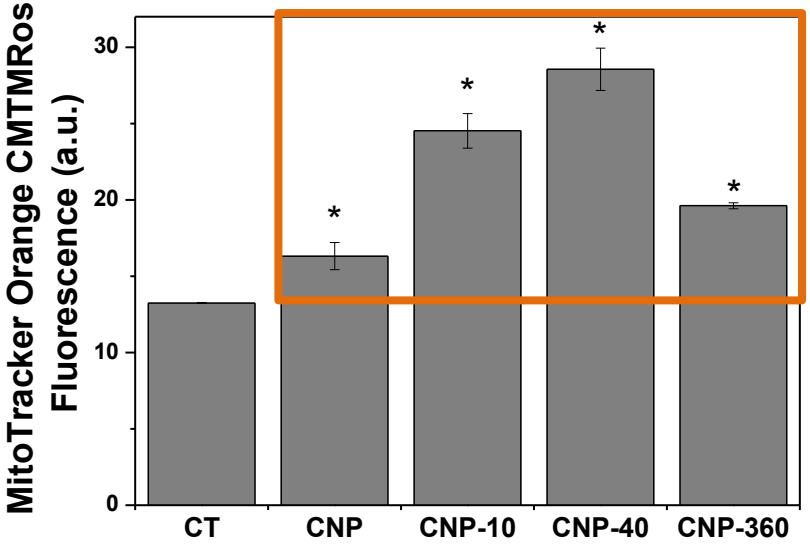


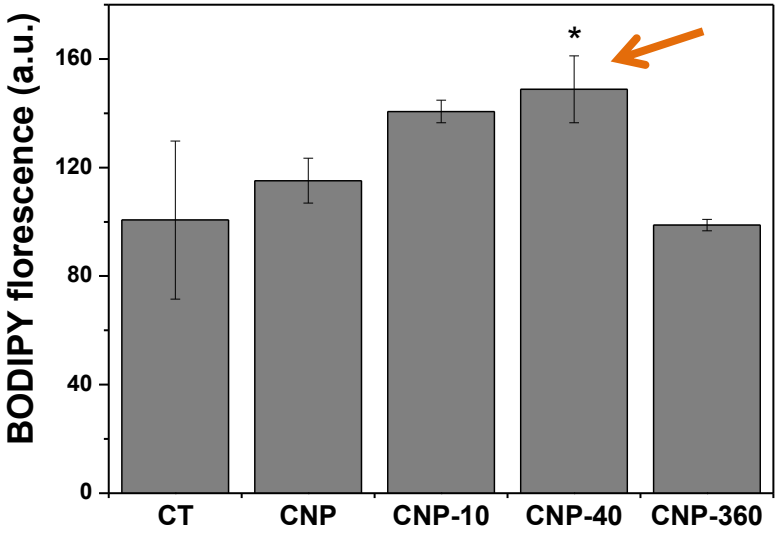


Oxidative stress (II)

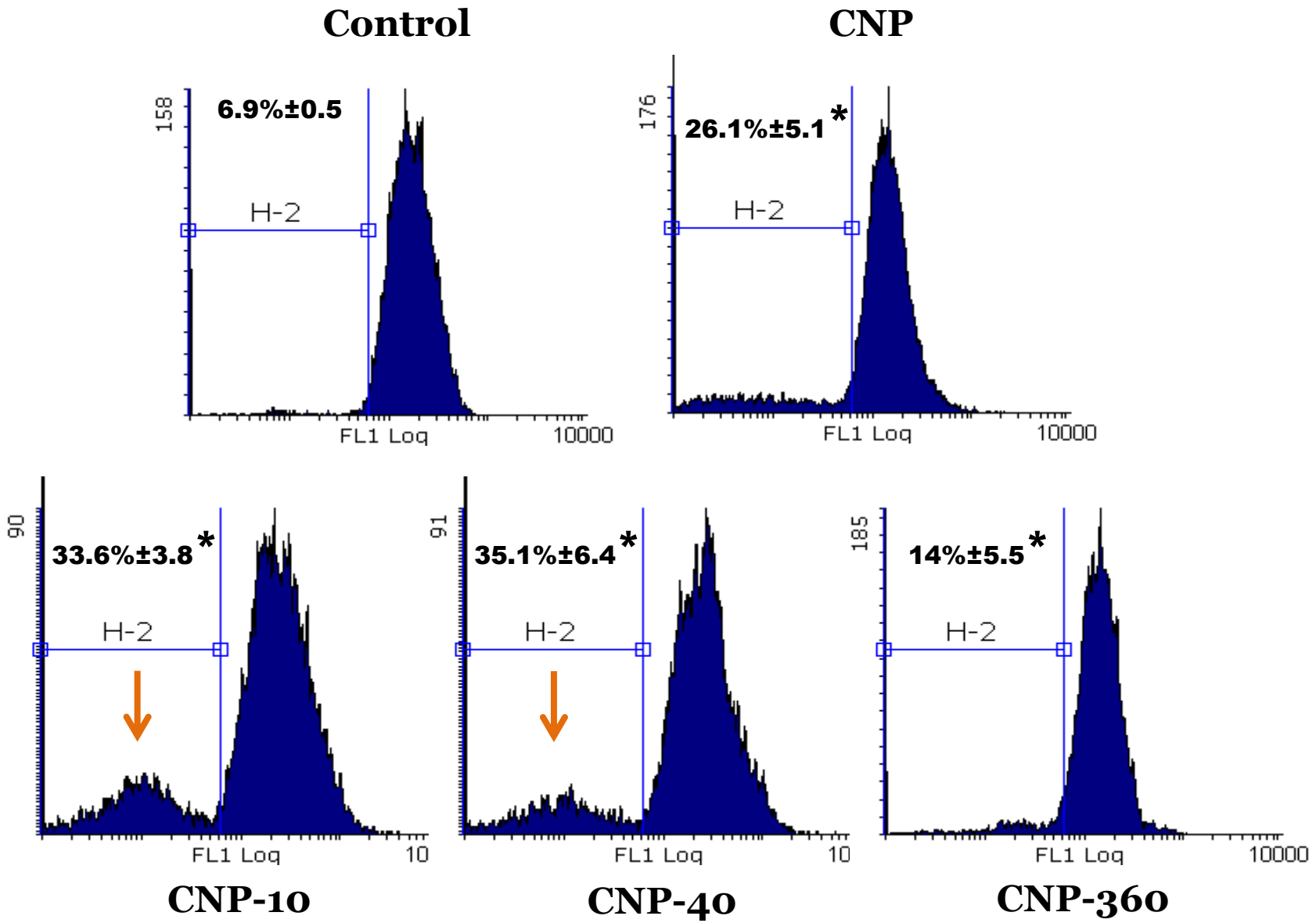
- Mitocondria were affected in all treated cells.

- Only CNP-40 produced an increase in cellular lipid peroxidation



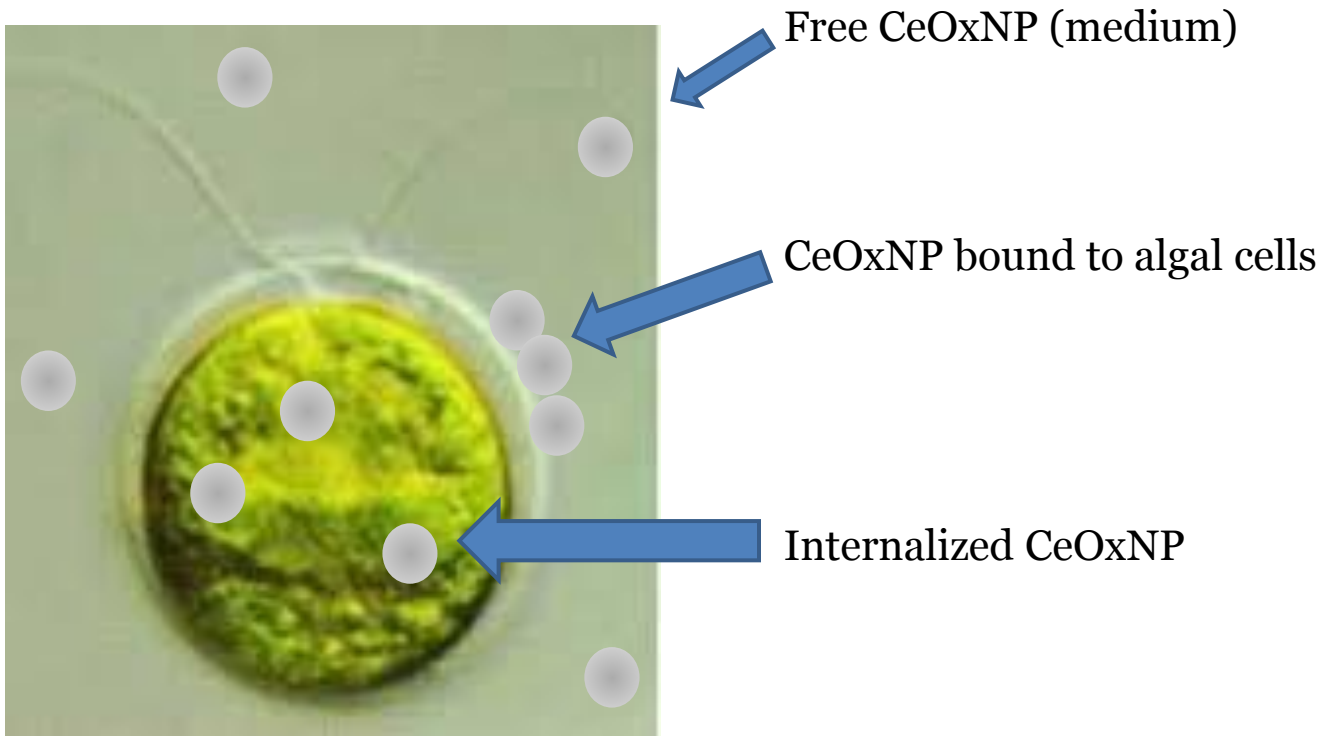


Metabolic activity: unspecific esterase activity



Internalization studies (I)

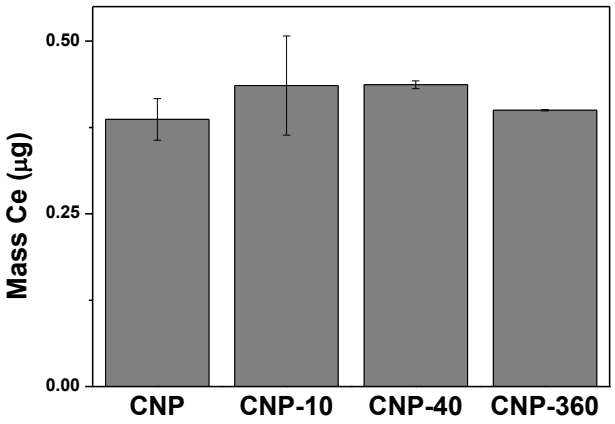
- We have analyzed the Ce content in three main compartments by ICP-MS.



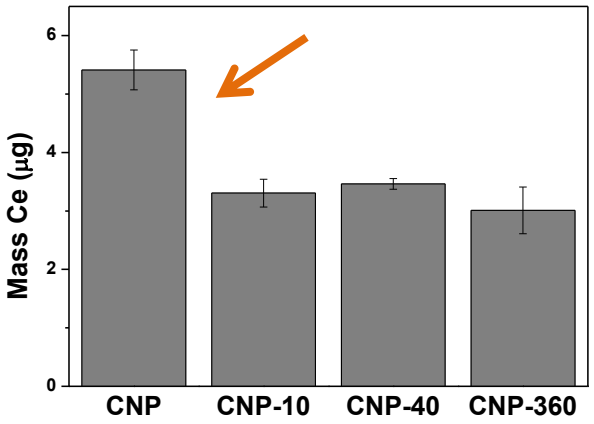
Internalization studies (II) 12h

- There were no differences in the amount of free CeOxNP between nanoparticles
- CNP was attached to the cell wall in a higher amount than PVP coated nanoparticles
- The PVP-coated nanoparticles were internalized to a larger extent than CNP.

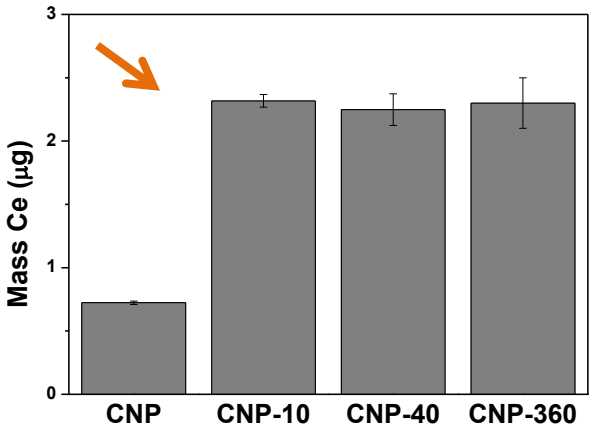
Free CeOxNP (medium)



CeOxNP bound to algal cells



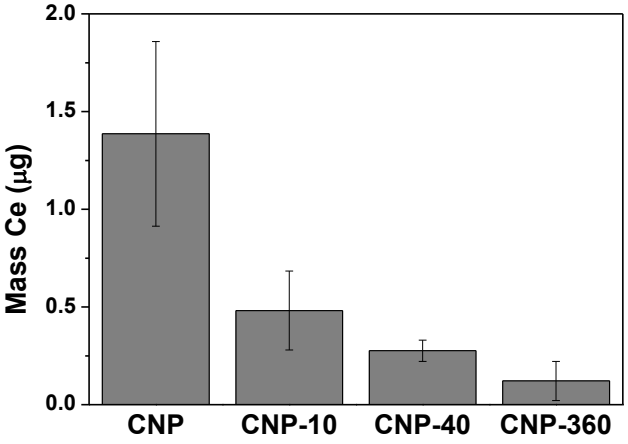
Internalized CeOxNP



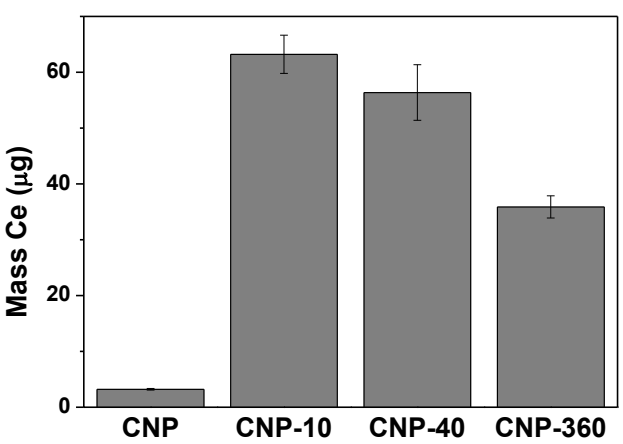
Internalization studies (I) – 48 h

- CNP was found more prominently in suspension than the other nanoparticles
- PVP coated nanoparticles were attached to the cell wall in a higher amount than CNP
- The uncoated nanoparticle, CNP, was internalized to a larger extent than the PVP coated nanoparticles

Free CeOxNP (in medium)

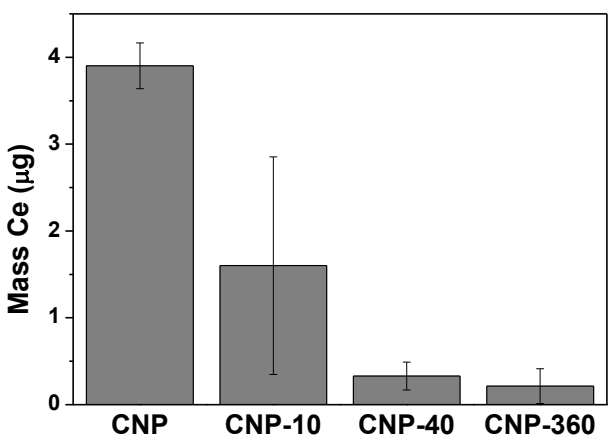


CeOxNP bound to algal cells



A

Internalized CeOxNP



A

Conclusions

1. The main toxicological mechanism of the nanoparticle without coating (CNP) was through damage in cytoplasmic membrane.

2. Regarding PVP coated nanoparticles, the mechanisms of toxicity relied on intracellular ROS formation without directly damaging the cell membrane, however different degrees of cell damage were found depending on PVP chain length.

3. CNP-40 was the most toxic nanoparticle according to the employed cytotoxicity biomarkers.

4. Cerium internalization depended on the time of exposure: after 12 h, internalization was found mostly for PVP-coated nanoparticles while after 48 h of exposure it was found for CNP and CNP with the smallest coating (CNP-10). This study is still under progress.

5. This type of research may be useful for the synthesis of safer-by-design nanomaterials.

Thank you for your attention!

GOBIERNO DE ESPAÑA
MINISTERIO DE EDUCACIÓN, CULTURA Y DEPORTE

NanoSpain Conf 2017
March 07-10, 2017 – San Sebastian (Spain)

THE LEADING N&N EVENT IN SPAIN

NanoMILE

Universidad de Alcalá

UAM UNIVERSIDAD AUTONOMA DE MADRID

FACULTAD DE CIENCIAS
UNIVERSIDAD AUTÓNOMA DE MADRID

Departamento de BIOLOGÍA

ICN2
Institut Català de Nanociència i Nanotecnologia

UNIVERSITY OF BIRMINGHAM