Oxidation of a ROS-indicator Dye by Inorganic Nanoparticles

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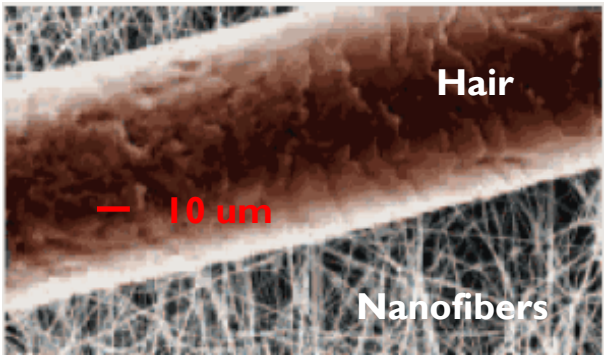
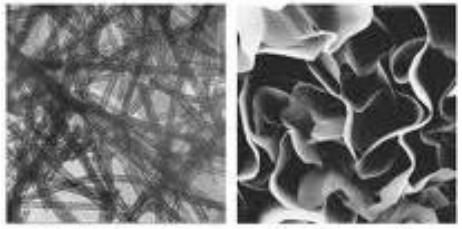
Nanoparticles

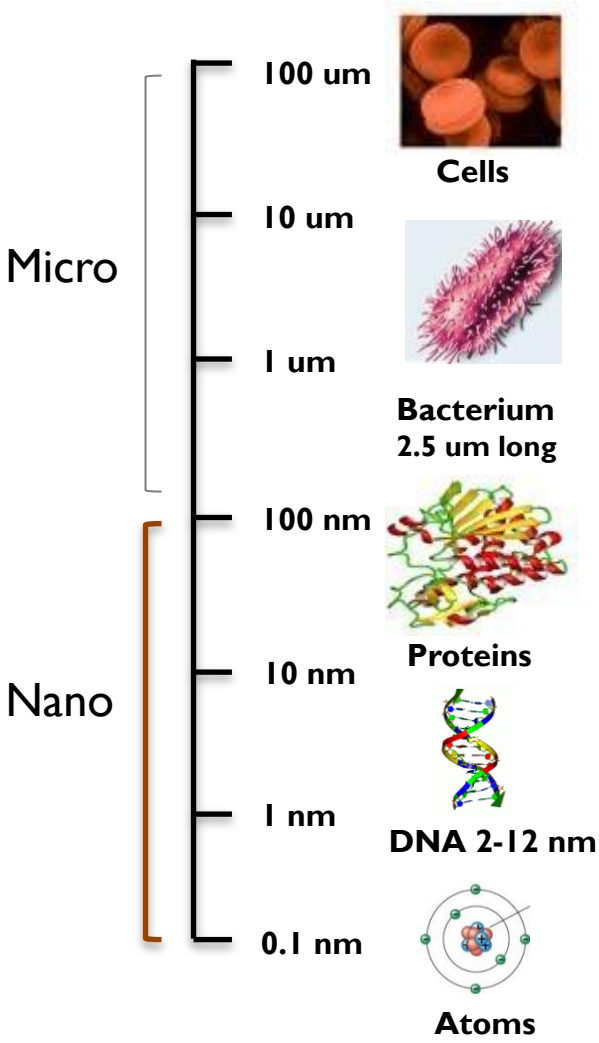
Nanoparticles (NPs):

Nano-sized materials (1-100 nm)



LARTA newsletter, June 24 ,2002





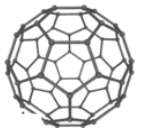
Nanoparticles in the Environment

Natural → Volcanic activity, erosion, dust, biological processes.

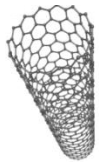
Incidental → Combustion, construction, mining.

Engineered →

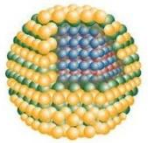
- Carbon-based
  (fullerene, carbon nanotubes)
- Inorganic
  (metal oxides & metals).
- Hybrid structures
  (Quantum dots, core shell structures).



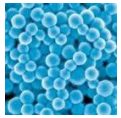
Fullerene



Carbon nanotube



Quantum dots



Metallic oxide

Properties & Applications

Unique properties of NPs

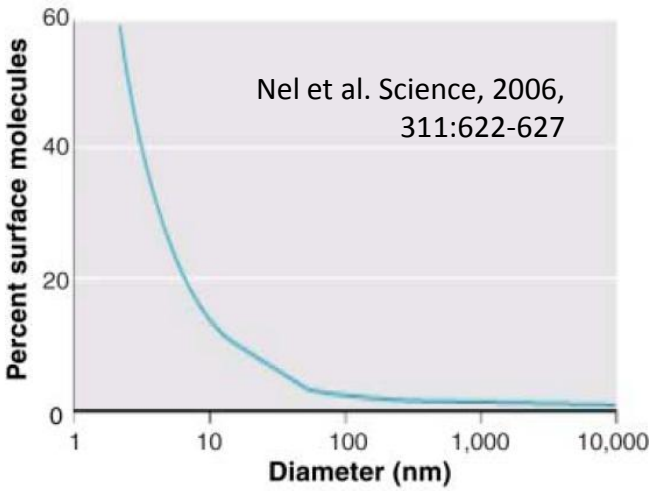
Purity

Small size

High surface area

Crystallinity

Shape



Nel et al. Science, 2006, 311:622-627

Nanotechnology applications

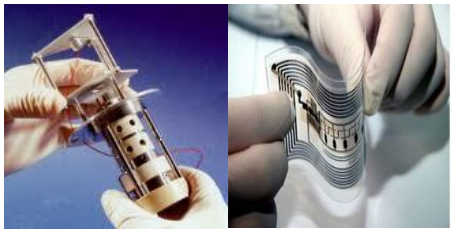
Pharmaceuticals

Cosmetics

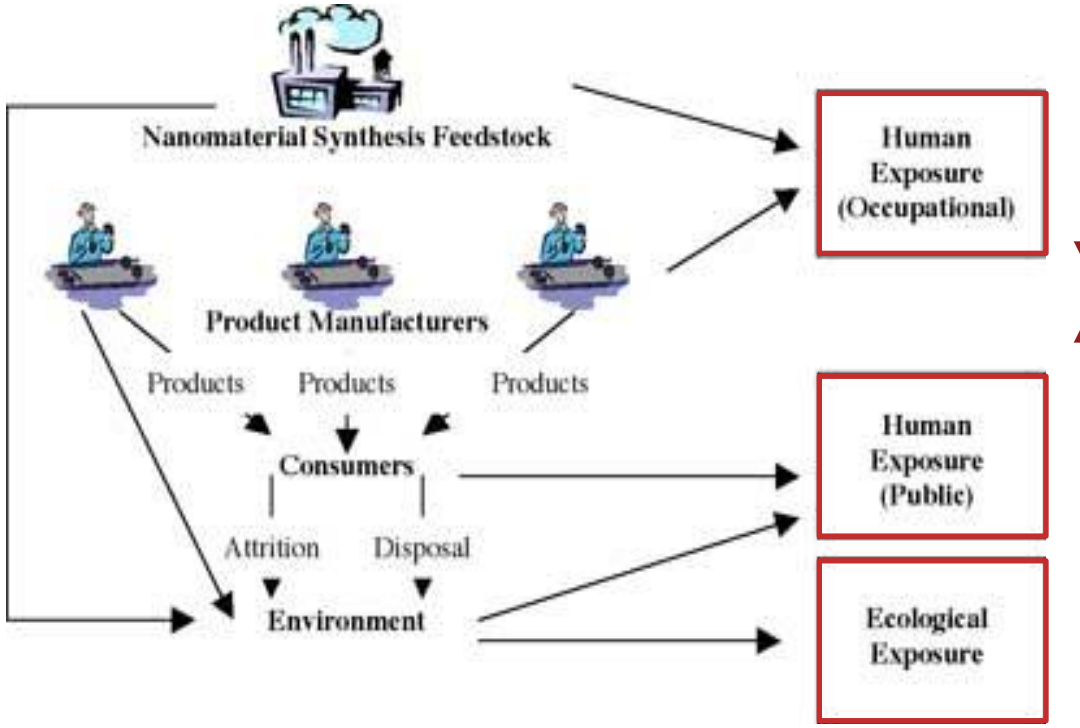
Materials science

Biomedicine

Electronics



Environmental Health & Safety Concerns



Inhalation

Ingestion

Dermal

List of NPs for Testing

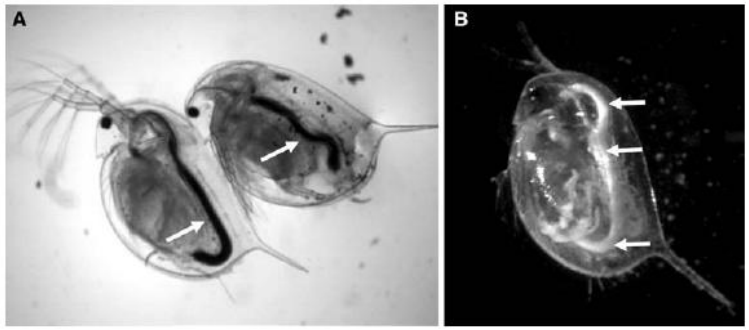
Organization for Economic Cooperation and Development (OECD)

Fullerenes
Carbon nanotubes
Silver
Iron
Carbon

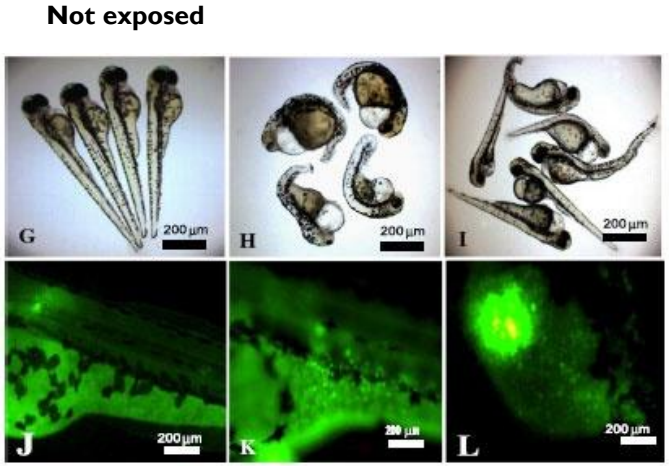
TiO_2
SiO_2
Al_2O_3
CeO_2
ZnO

Polystyrene
Dendrimers
Nanoclays

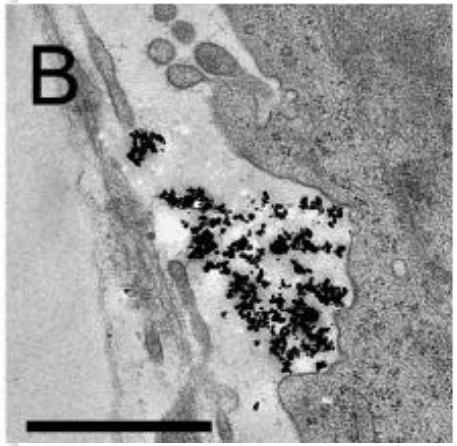
Toxicity of Nanoparticles

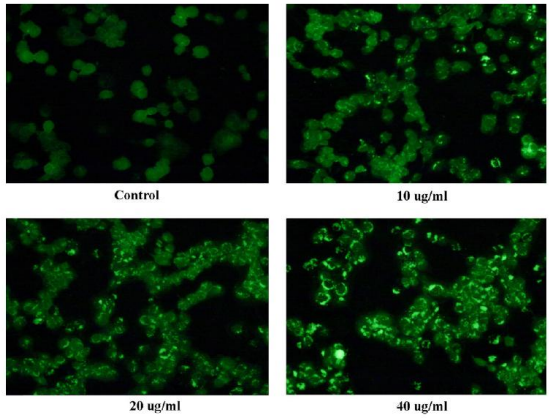


[1]*Daphnia magna*: A) C_{60} & B) TiO_2
NPs intake & translocation.



[2]Zebrafish embryos exposed to Ag
NPs deposition
Deformation & apoptosis

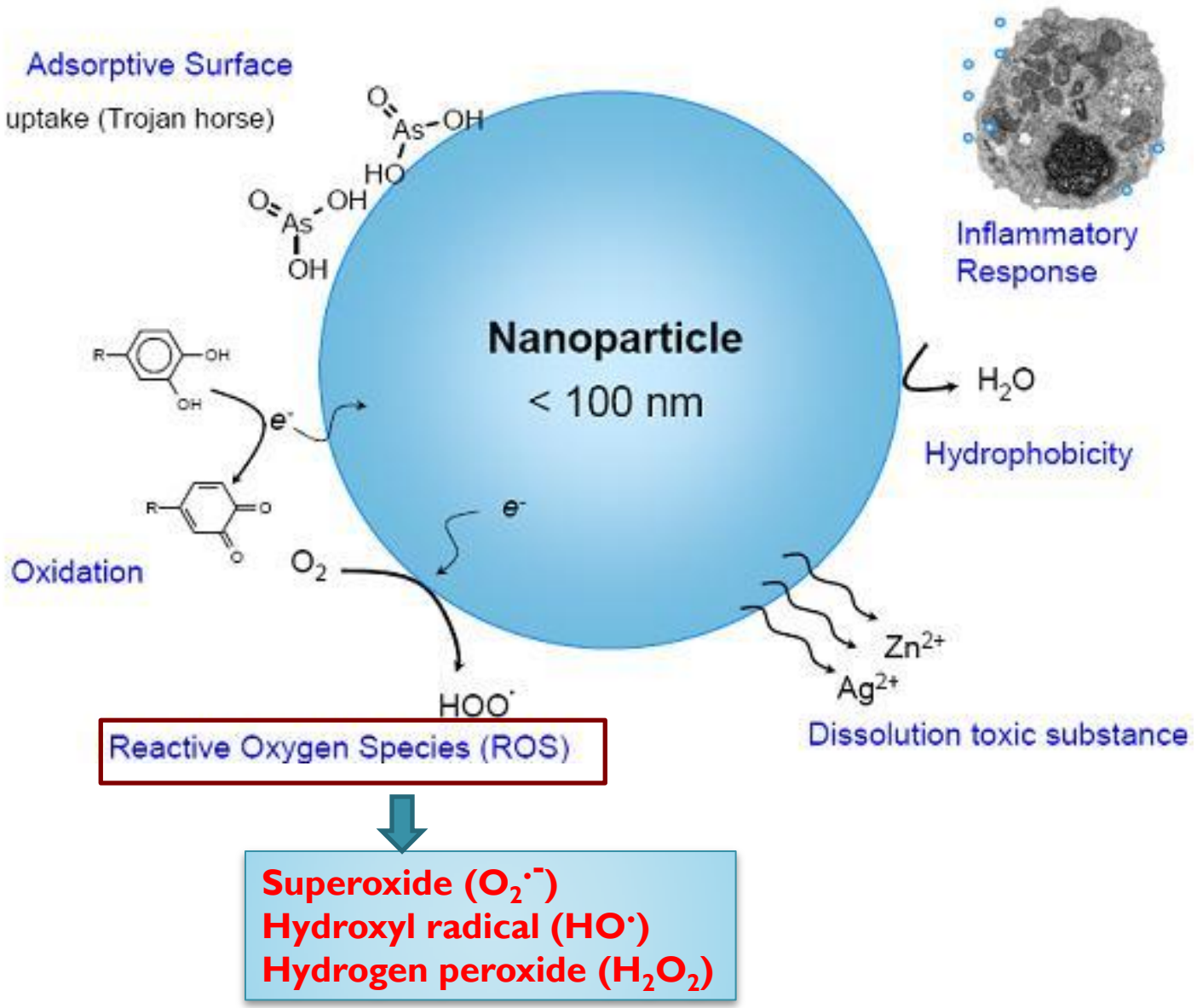




[3]Lung epithelial cells (BEAS-2B) exposed to CeO_2
Uptake
Oxidative stress

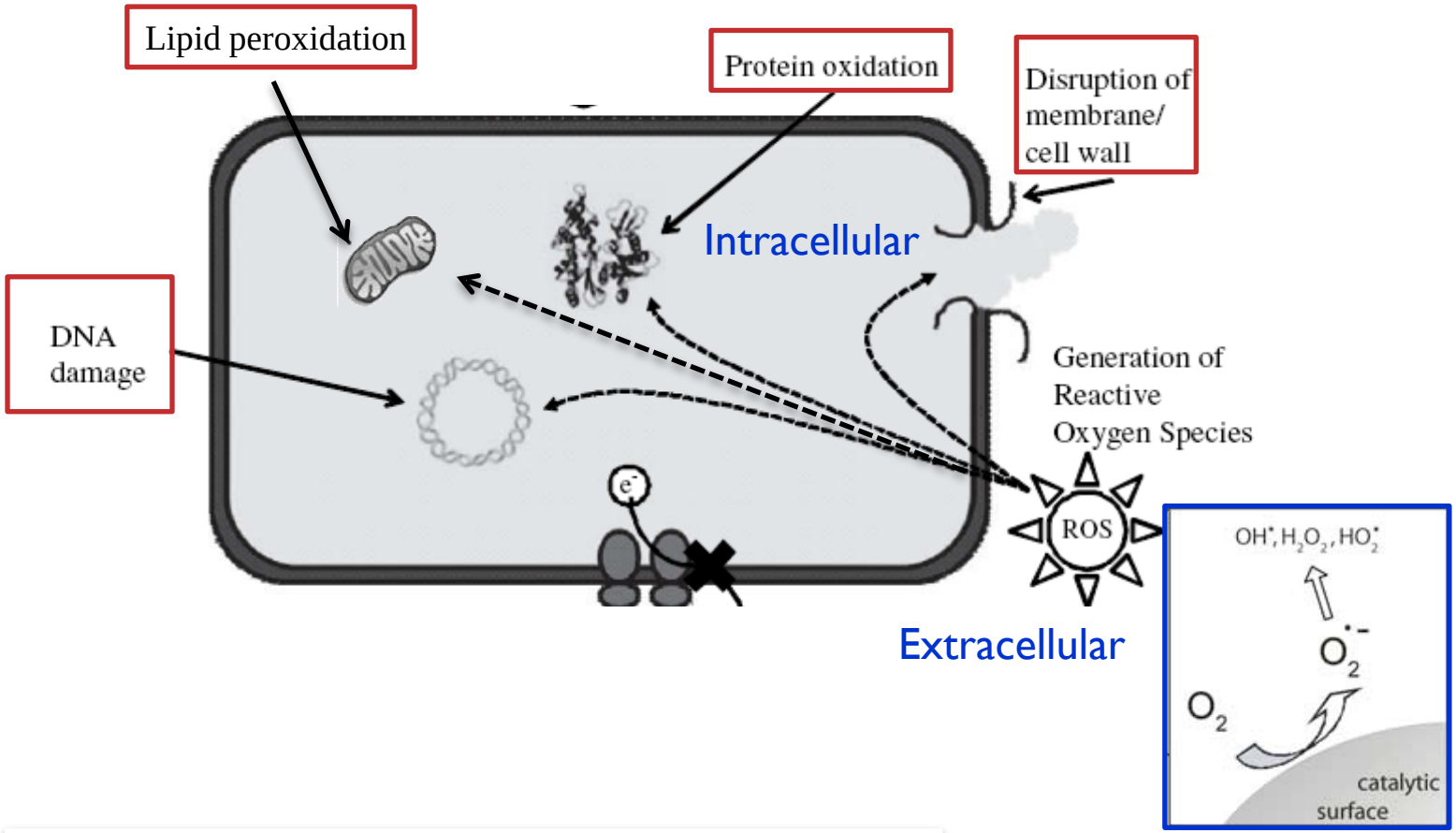
[1]Baun *et al.* 2008. Ecotoxicol.17:387-395; [2]Asharani *et al.* 2008. Nanotechnol. 19:255102; [3]Park et al. 2008. Toxicol. 245:90-100.

Toxicity Mechanisms



Toxicity by ROS

Oxidative damage



In vitro ROS testing might predict NPs toxicity

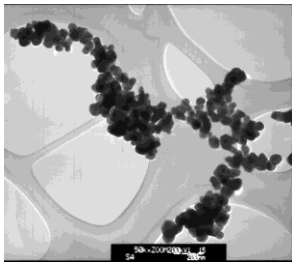
Adapted from Li et al. 2008, Water Res, 42:4591

Objectives

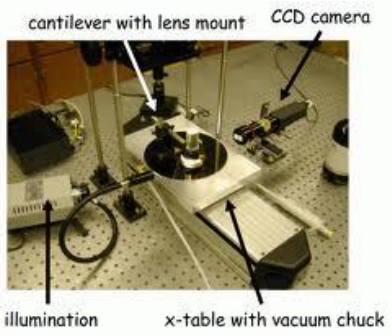
- Determine if the chemical reaction of NPs with dissolved oxygen together with biological molecules can cause increased formation of ROS.
- Determine if NPs can directly from ROS with dissolved oxygen or directly oxidize ROS-indicator dye in the absence of dissolved oxygen.
- Confirm and identify the ROS species formed by the NPs studied.
- Determine if the NPs studied can cause oxidation of the protein BSA.

Materials

- Hafnium oxide (HfO_2) → ***Immersion Lithography***

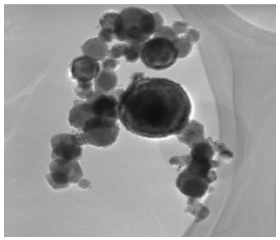


HfO_2

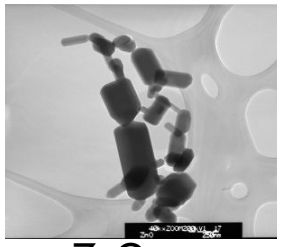


- Cerium oxide (CeO_2)
  Silicon oxide (SiO_2)
  Aluminum oxide (Al_2O_3) → ***Chemical Mechanical Planarization (CMP)***

- Others:
  Fe_2O_3, ZrO_2, ZnO, Mn_2O_3,etc.



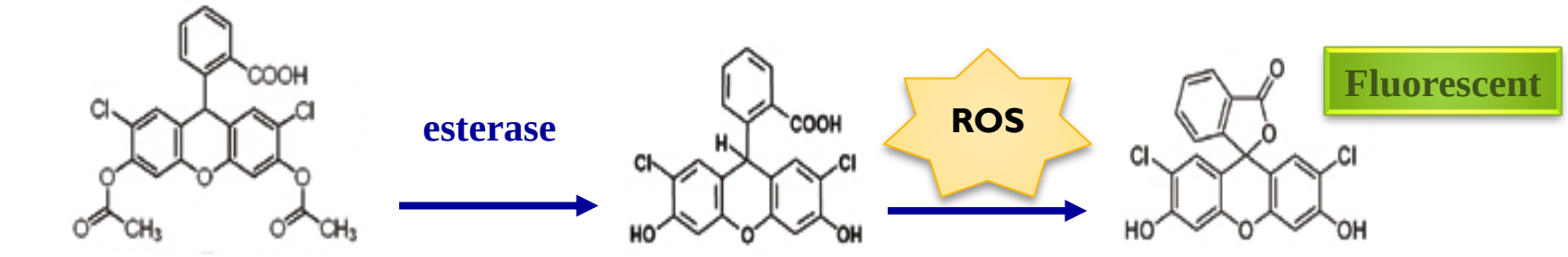
Mn_2O_3



ZnO

ROS Indicator-Dye Reaction

Based on fluorescence of ROS-sensitive dye



2′,7′-dichlorodihydrofluorescein diacetate
(DCFH-DA)

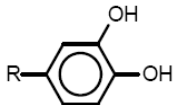
2′,7′-dichlorodihydrofluorescein
(DCFH)

2′,7′-dichlorofluorescin
(DCF)

ROS Assay

NPs

Sonicated
pH 7.2

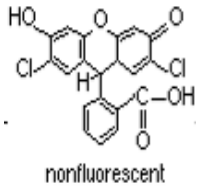


L-dopa or catechol

+

NPs

+



nonfluorescent

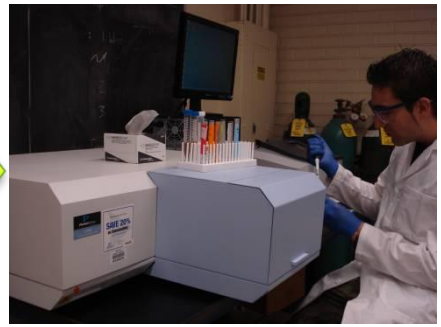
ROS-dye

Aerobic

Anaerobic

Head space flushed with N_2 gas

Fluorescence Measurements

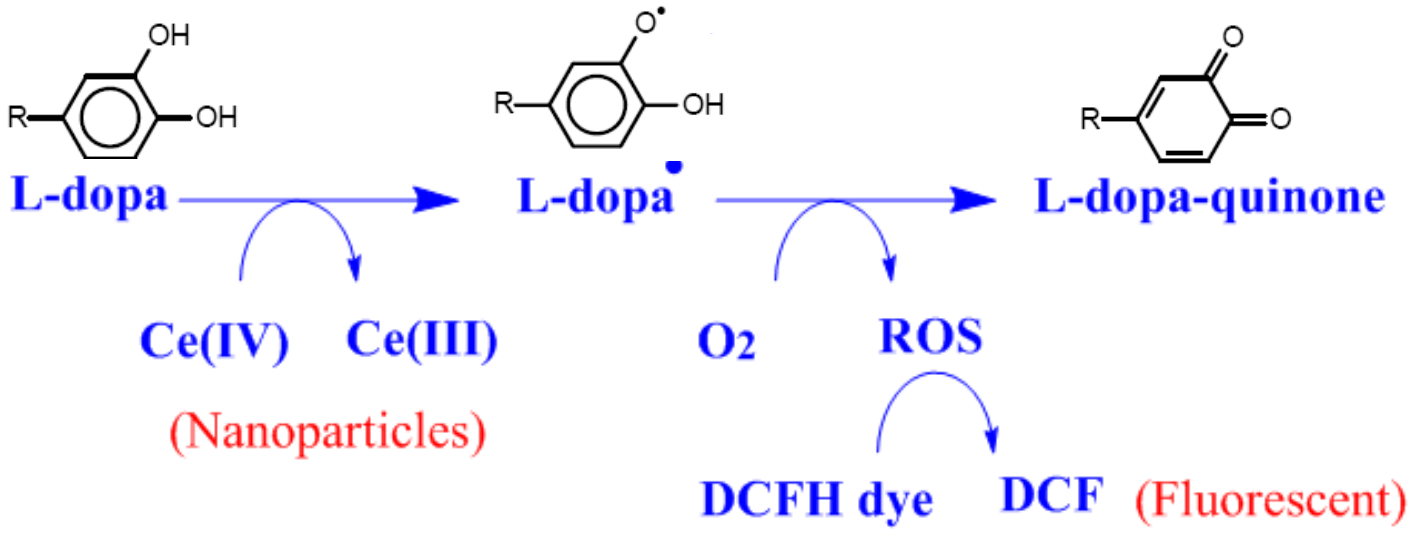


Controls:
NPs alone
Dye alone
L-dopa alone

Incubated in phosphate buffer pH 7.4 at 37 °C, Protected from the light.

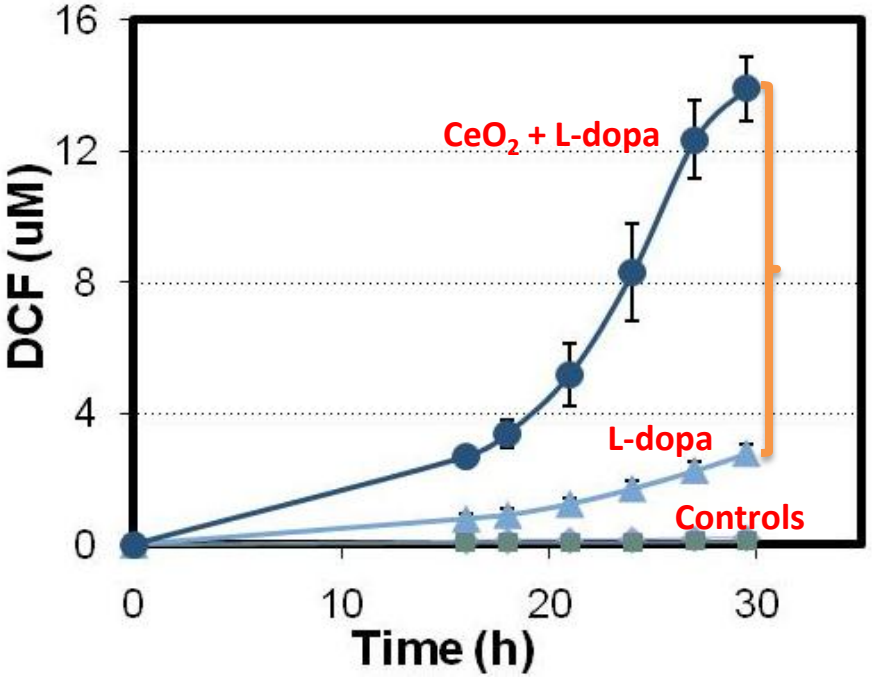
Hypothetical Mechanisms for ROS Reaction

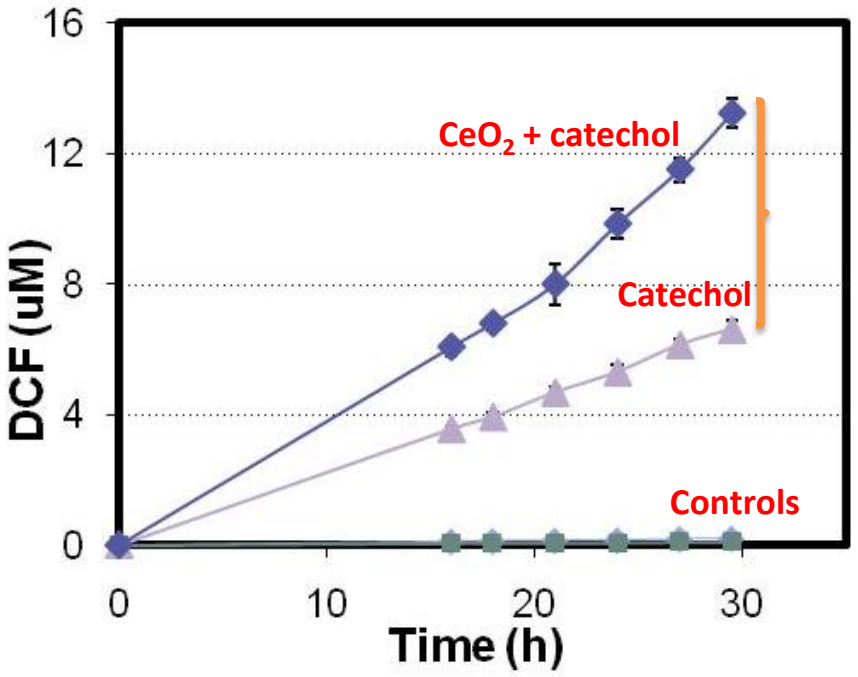
Formation of ROS by NPs in the cell-free assays



- Interaction of phenolic biomolecules with NPs increases ROS formation
- ROS formation requires oxygen

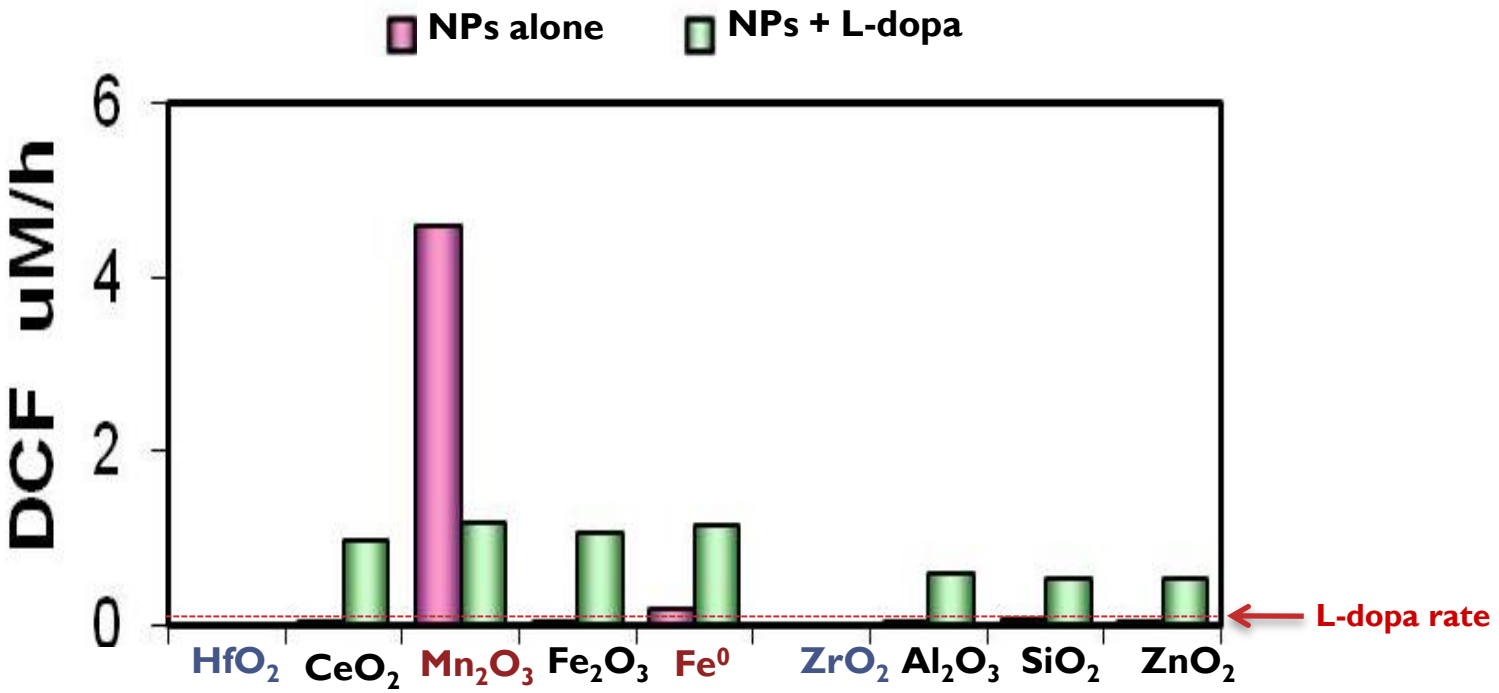
ROS Formation: CeO_2 NPs (20 nm)





CeO_2 NPs increased ROS production during oxidation of L-dopa & catechol with dissolved oxygen.

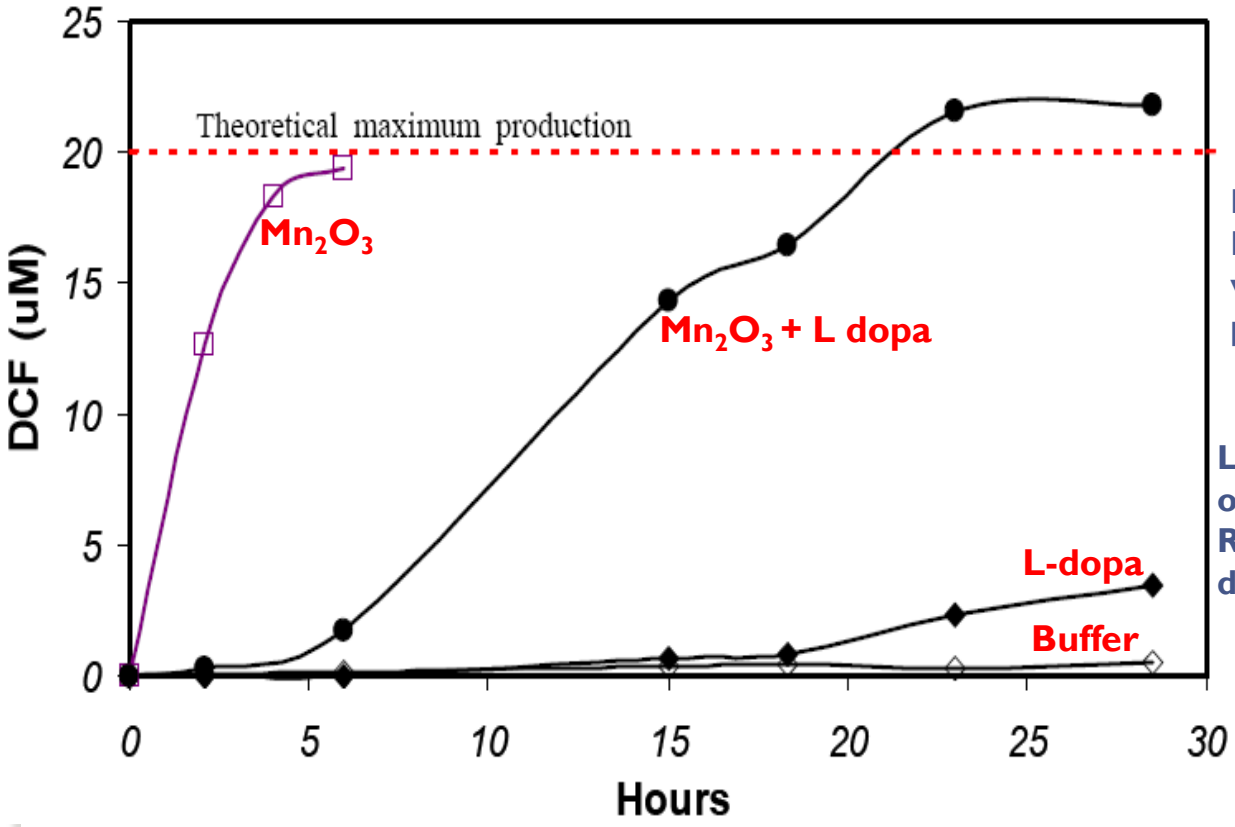
ROS Indicator-Dye Oxidation by Inorganic NPs



CeO_2, SiO_2,Al_2O_3 ZnO & Fe_2O_3 increased ROS production rate during the oxidation of L-dopa.

Direct reaction of Mn_2O_3 and Fe^0 NPs with either ROS-dye or with dissolved Oxygen to produce ROS.

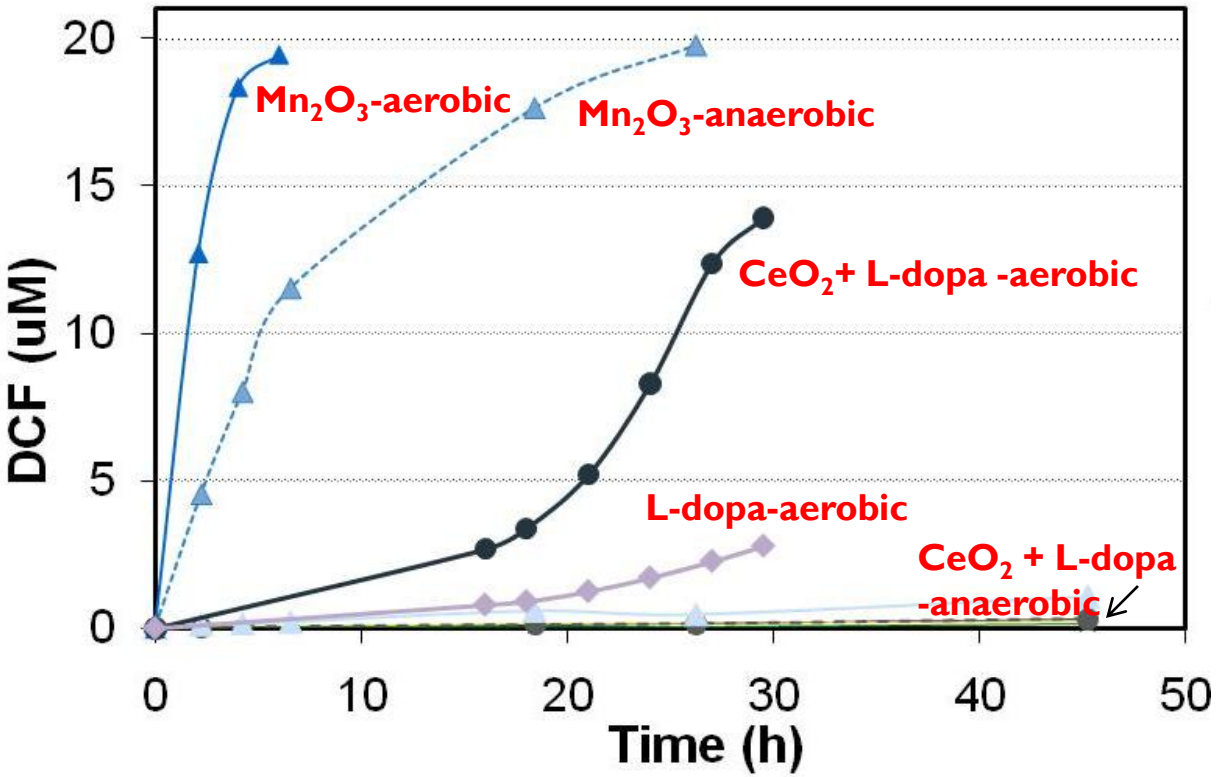
ROS Indicator-Dye Oxidation by Mn_2O_3 NPs (40-60 nm)



Direct reaction of Mn2O3 NPs with either ROS-dye or with dissolved oxygen to produce ROS.

L-dopa inhibits the formation of ROS or oxidation of ROS-dye by Mn_2O_3, possibly due to competitive oxidation.

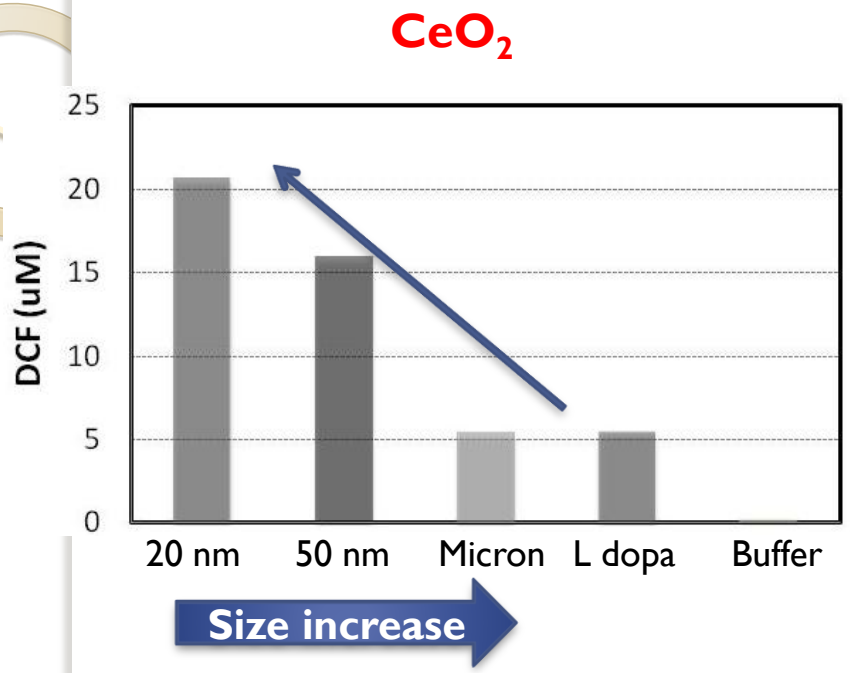
Aerobic *vs* Anaerobic ROS Indicator-Dye Oxidation by Inorganic NPs

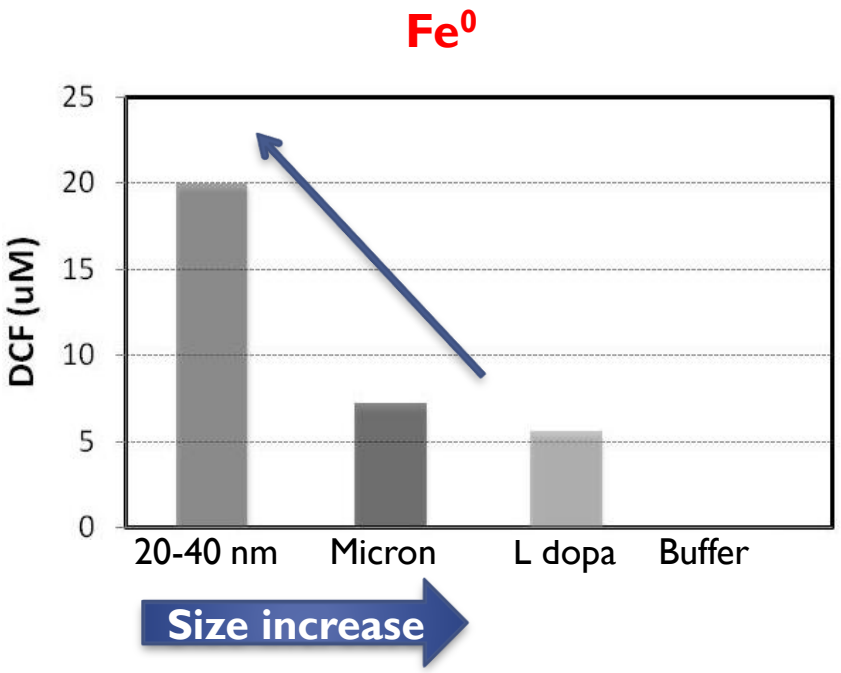


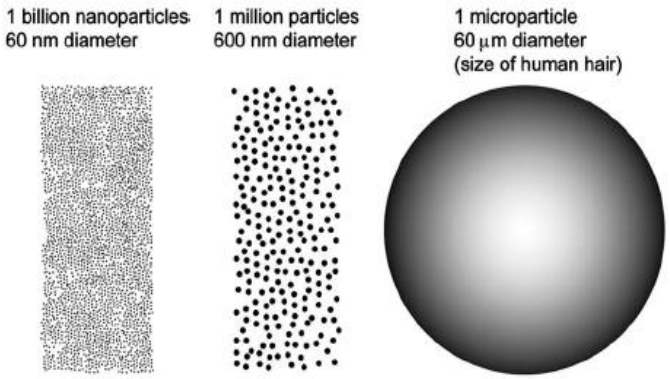
Mn_2O_3 NPs directly oxidizes ROS indicator-dye, dissolved oxygen not required.

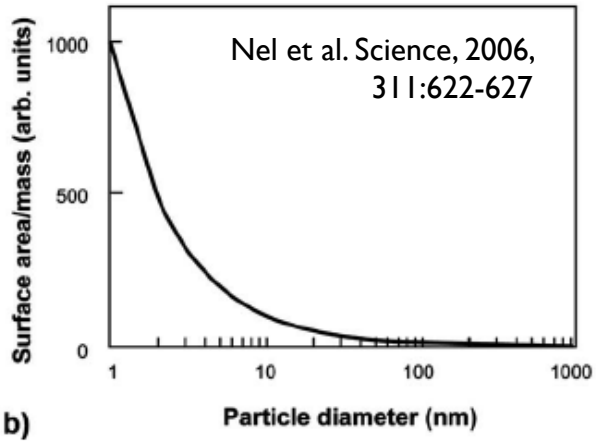
CeO_2 NPs produce ROS trough the oxidation of L- dopa in the presence of dissolved oxygen

Size Effect on ROS Formation: CeO_2 & Fe^0



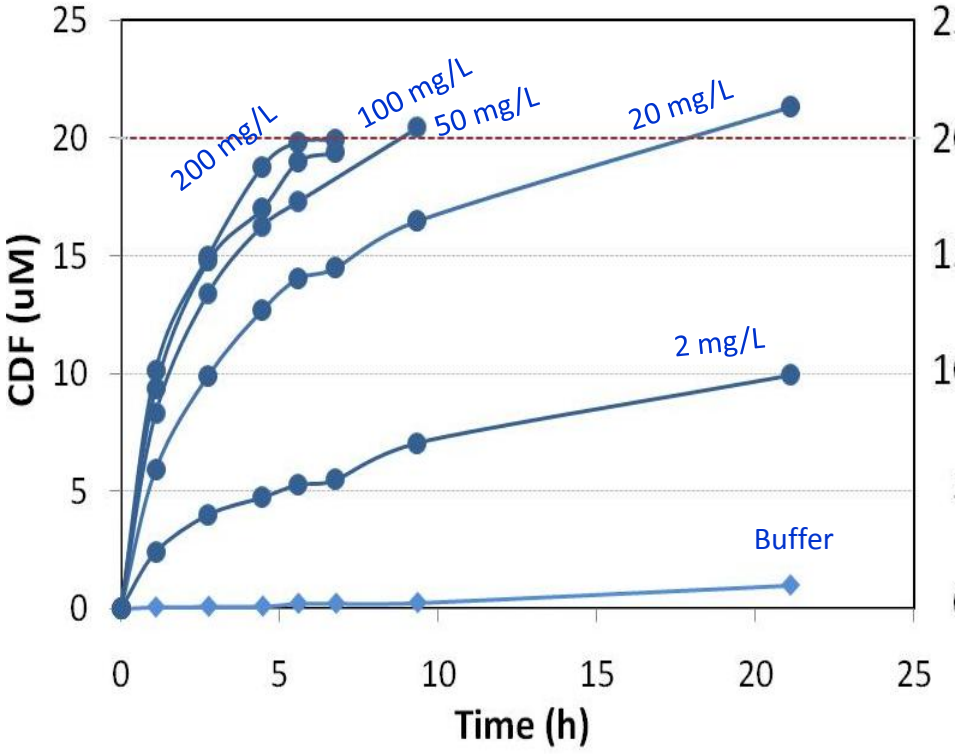




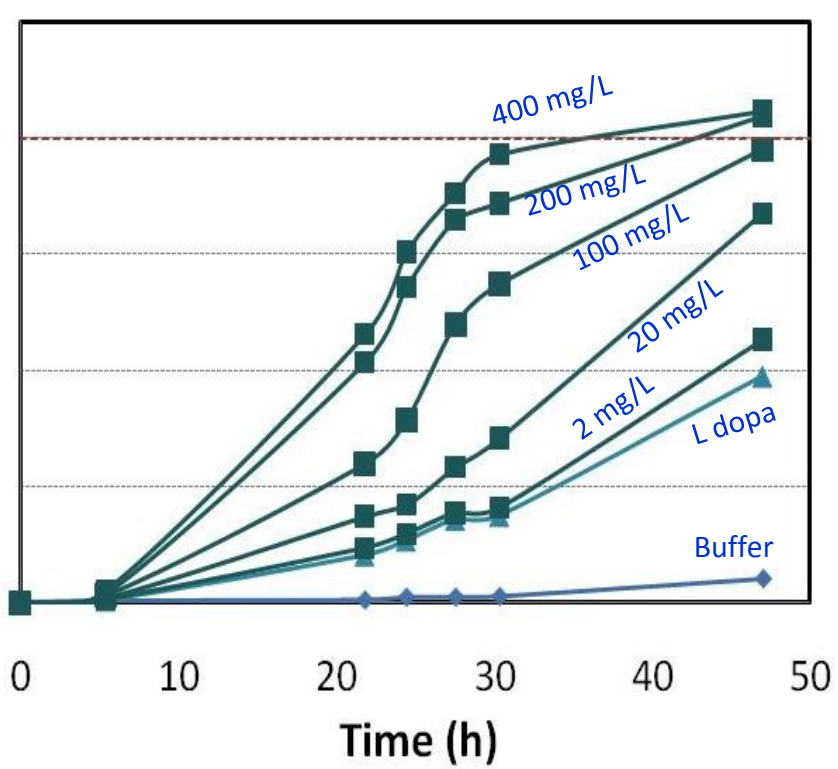


Dose-Response: Mn_2O_3 & CeO_2 NPs

Mn_2O_3 40-60 nm



CeO_2 20 nm + L dopa



Mn_2O_3 effectively oxidizes ROS-dye at low concentrations.

Low mass of CeO_2 NPs did not produce significant ROS

Conclusions

- The interaction of CeO_2 NPs with biological molecules (L-dopa & catechol) increased the oxidation of the ROS indicator-dye.
- Inorganic NPs used in semiconductor industry (CeO_2, SiO_2, Al_2O_3) enhanced the oxidation of the ROS indicator-dye through its reaction with L-dopa and dissolved oxygen.
- Mn_2O_3 NPs caused direct oxidative reaction with the fluorescent dye (DCF), suggesting that the Mn oxide could potentially react with some cell components.

Conclusions

- The lack of oxygen totally inhibited the oxidation of the ROS indicator-dye by all NPs assayed (via oxidation of L-dopa), except in the case of Mn_2O_3 NPs.

- The enhancement of ROS formation by nano-sized CeO_2 and Fe^0 showed a direct correlation with the particle size.

Ongoing Work: Identification of ROS Produced by NPs

Inhibiting Oxidation of the ROS-indicator Dye

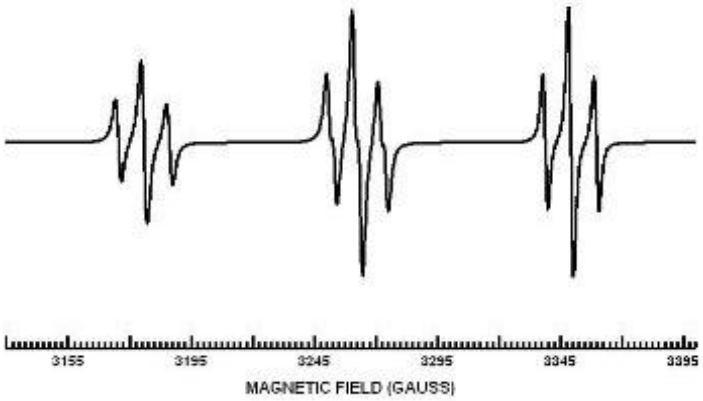
Scavengers/ Quenchers	ROS	ROS inhibition (%)
SOD & Catalase	Superoxide & H_2O_2	0
DMSO	Oxygen radicals	0
NaN_3	Singlet oxygen	0
*Ascorbic Acid	Antioxidant	100

*not specific

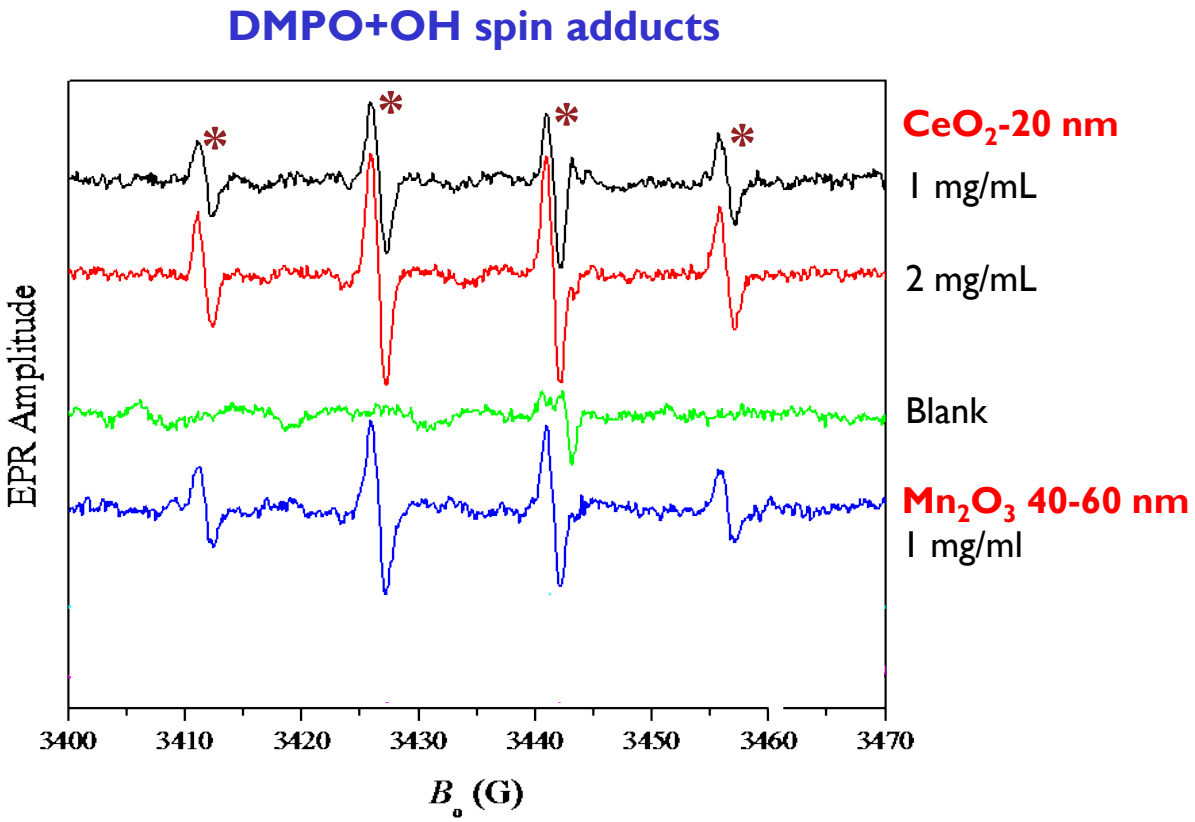
Ongoing Work: Identification of ROS Produced by NPs

Electron Paramagnetic Resonance (EPR)

Based on the resonant absorption of microwave radiation by paramagnetic ions or molecules, with at least one unpaired electron spin, and in the presence of a static magnetic field.



Ongoing Work: Identification of ROS Produced by NPs



"*" denote lines of the DMPO+OH spin adduct.

CeO_2 and Mn_2O_3 produce $OH^{\bullet}$ radicals in water

Future Work: Oxidation of Proteins by NPs

In vitro test

- **BSA protein**

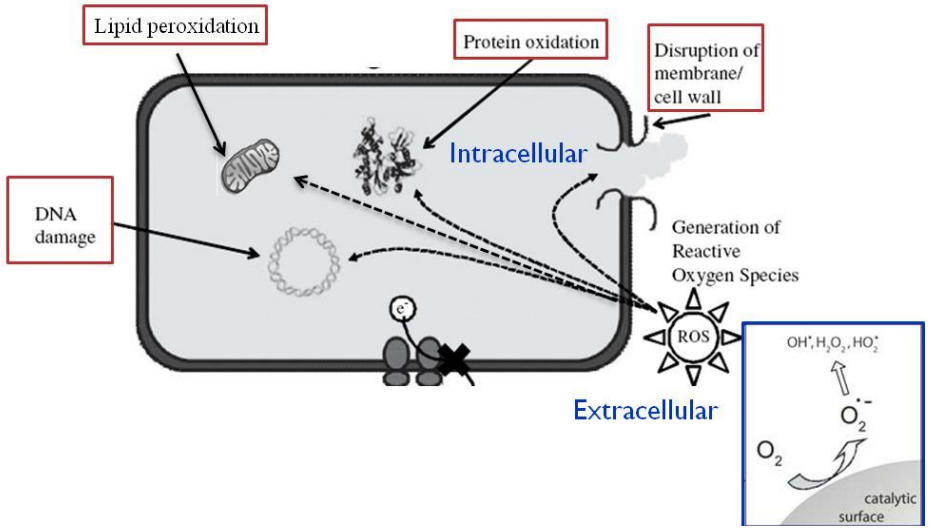
NPs

Incubation

Oxyblot test:

Sensitive and rapid detection of proteins (in a western blot format) modified by ROS and other reactive species.

ELISA (Protein carbonyl kit): Rapid detection and quantification of protein carbonyls.



Acknowledgments

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Thanks!