The Role of Protein Oxidation in the Toxicity of Inorganic Nanoparticles

Wenjie Sun, Antonia Luna-Velasco, Reyes Sierra-Alvarez, Jim A. Field

Dept. Chemical & Environmental Engineering

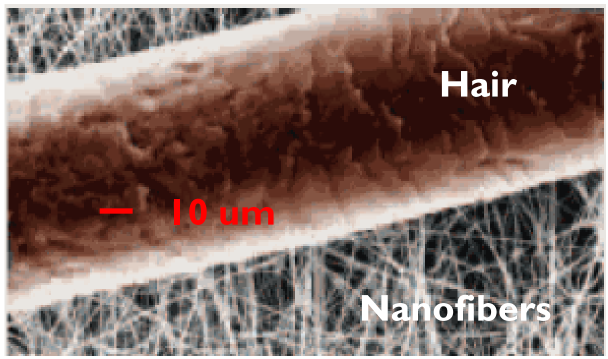
The University of Arizona

E-mail: alexsun@email.arizona.edu

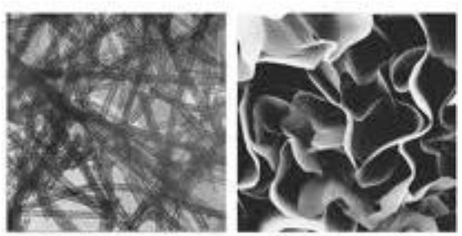
NSF/SRC Engineering Research Center for Environmentally Benign Semiconductor Manufacturing

Nanoparticles

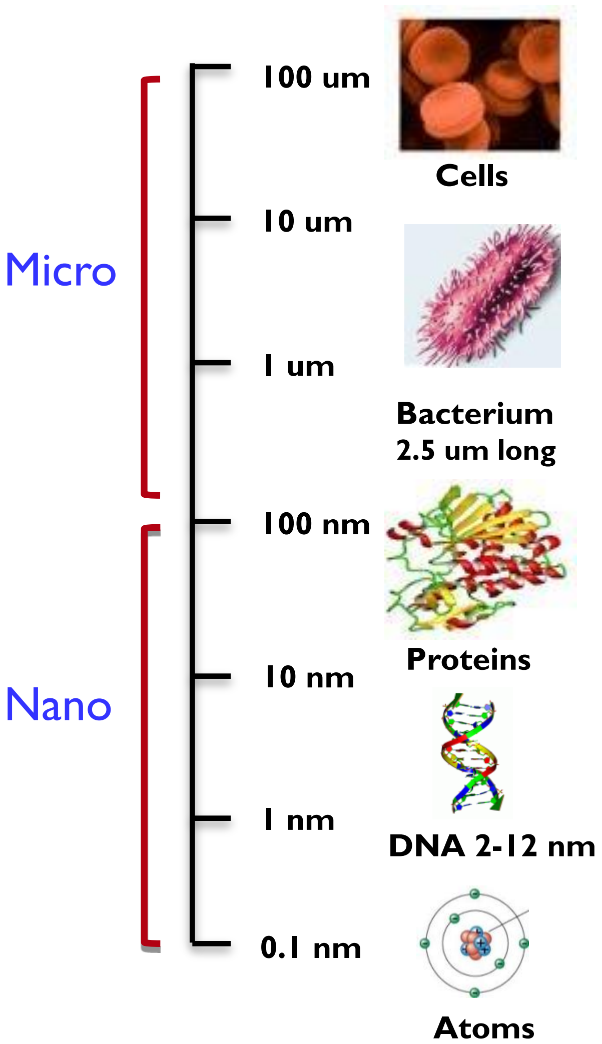
Nanoparticles (NPs): Nano-sized materials (1-100 nm)



LARTA newsletter, June 24 ,2002



1D- Nanotubes 2D- Nanowalls

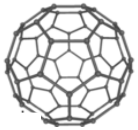


Nanoparticles in the Environment

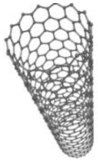
Natural
- Volcanic activity
- Erosion and dust
- Biological processes

Incidental
- Combustion
- Construction
- Mining

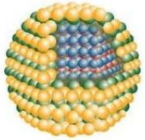
Engineered
- Carbon-based
  (fullerene, carbon nanotubes)
- Inorganic
  (metal oxides & metals)
- Hybrid structures
  (Quantum dots, core shell structures)



Fullerene



Carbon nanotube



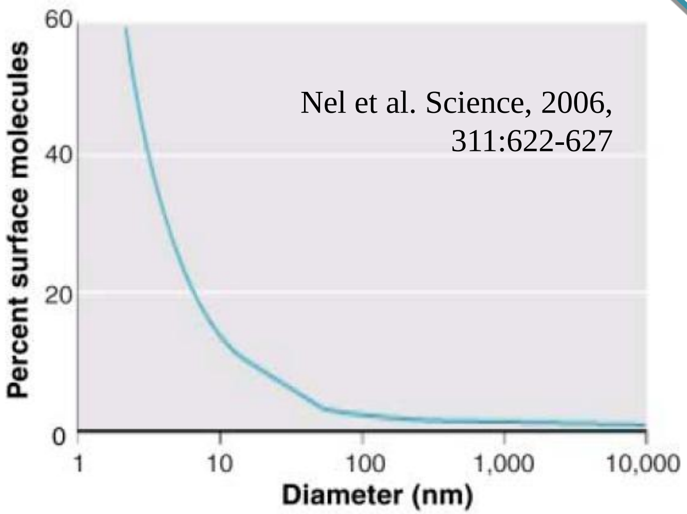
Quantum dots

Metallic oxide

Properties & Applications

Unique properties of NPs

- Purity
- Small size
- High surface area
- Crystallinity
- Shape



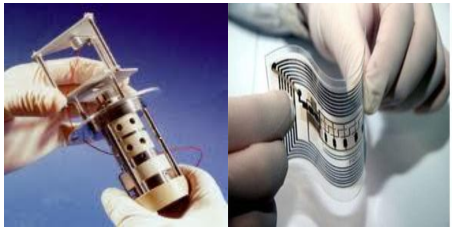
Nel et al. Science, 2006, 311:622-627

Nanotechnology applications

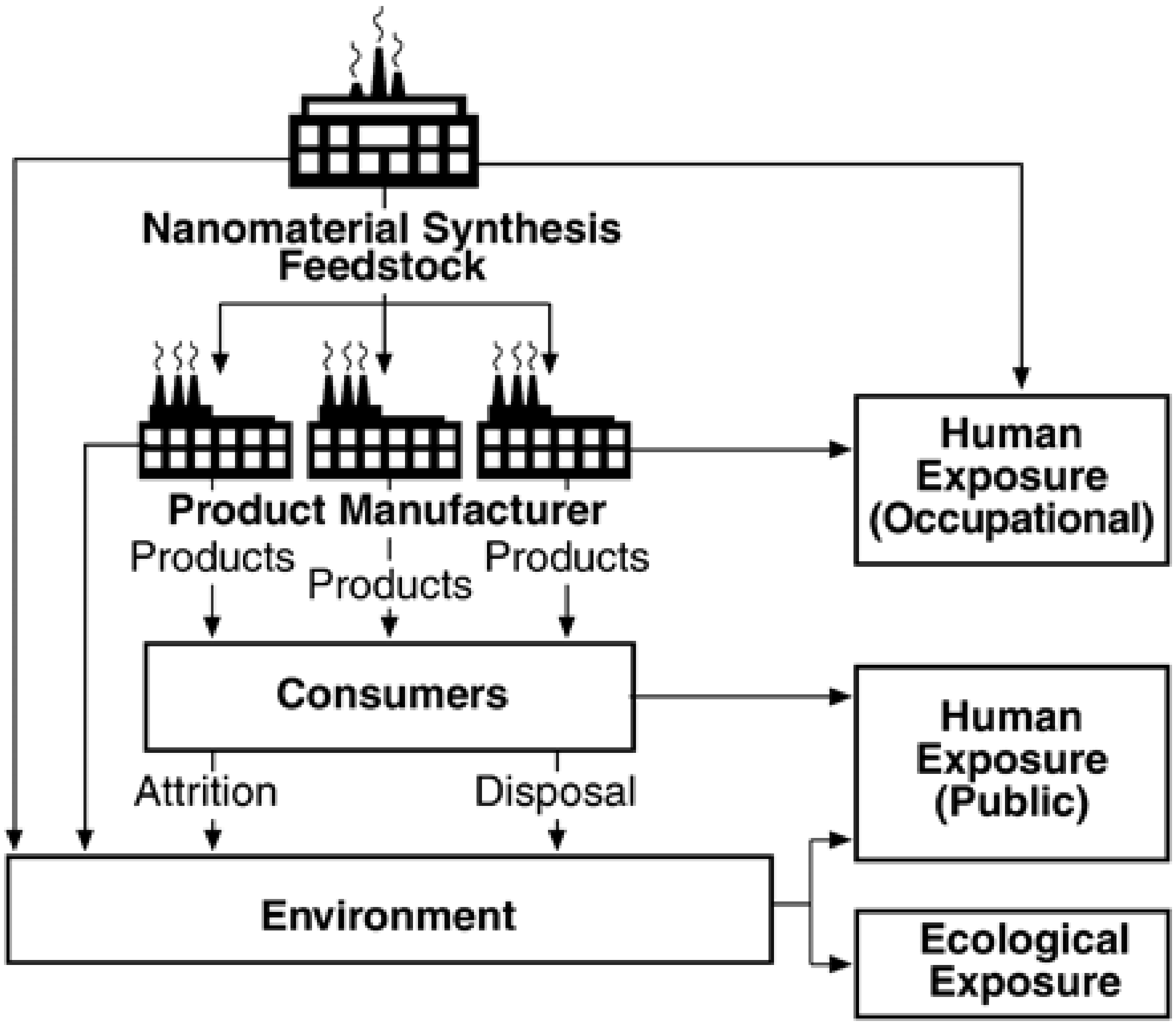
- Pharmaceuticals
- Cosmetics
- Materials science

- Biomedicine

- Electronics



Environmental Health & Safety Concerns



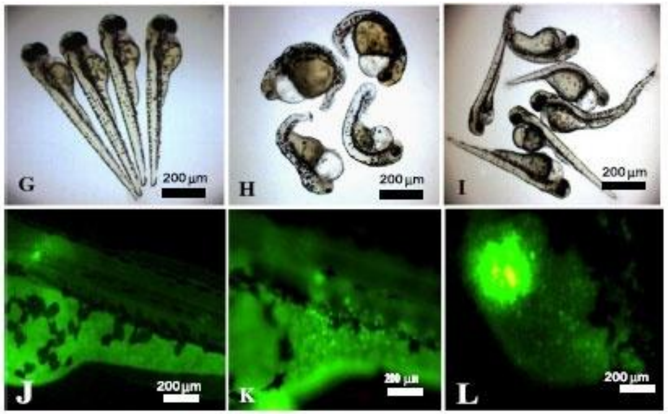
Inhalation

Ingestion

Dermal

Toxicity of Nanoparticles

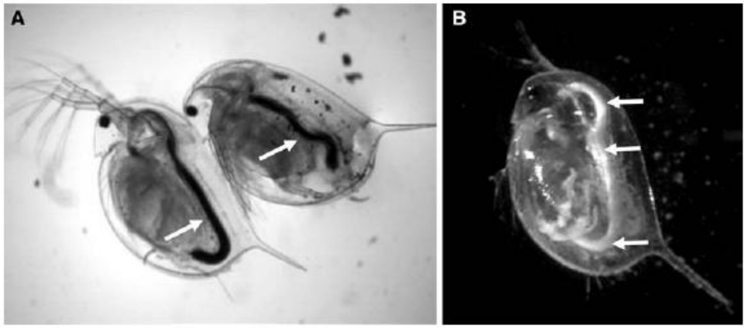
Not exposed



[2]**Zebrafish embryos exposed to Ag**

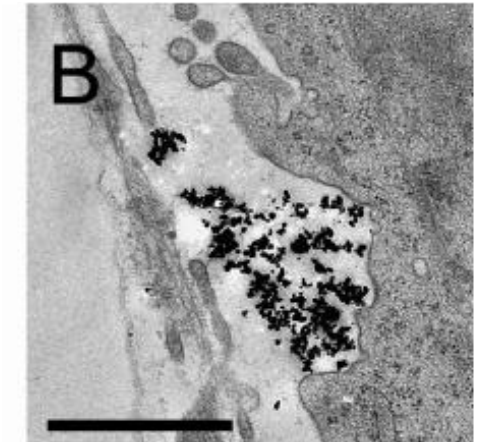
NPs deposition

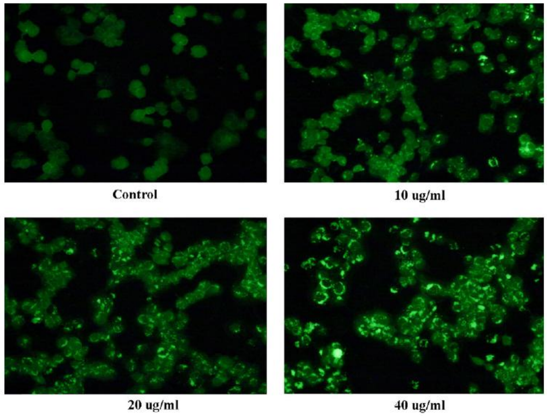
Deformation & apoptosis



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

NPs intake & translocation.





[3]**Lung epithelial cells** (BEAS-2B)

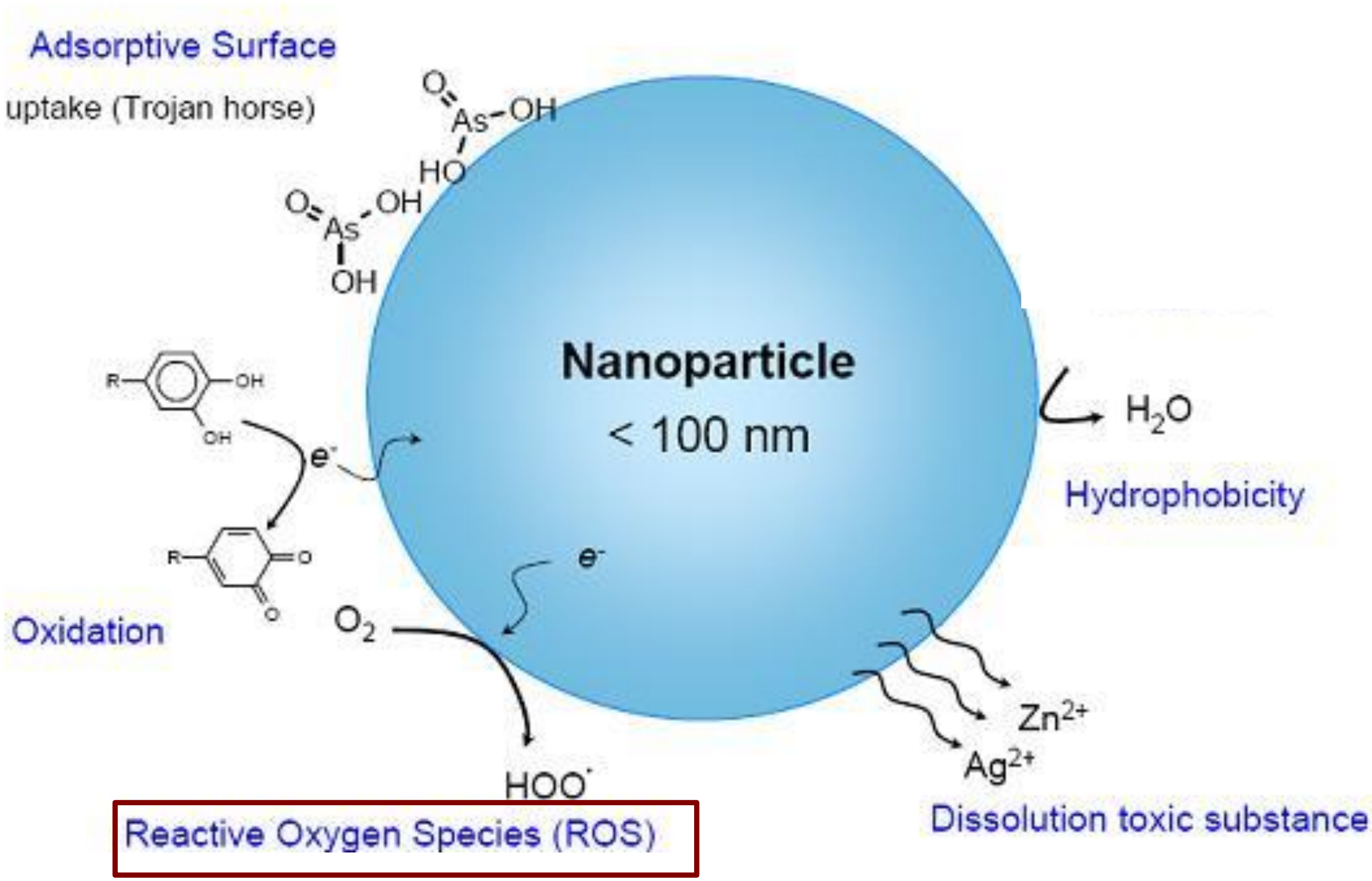
exposed to CeO_2

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.

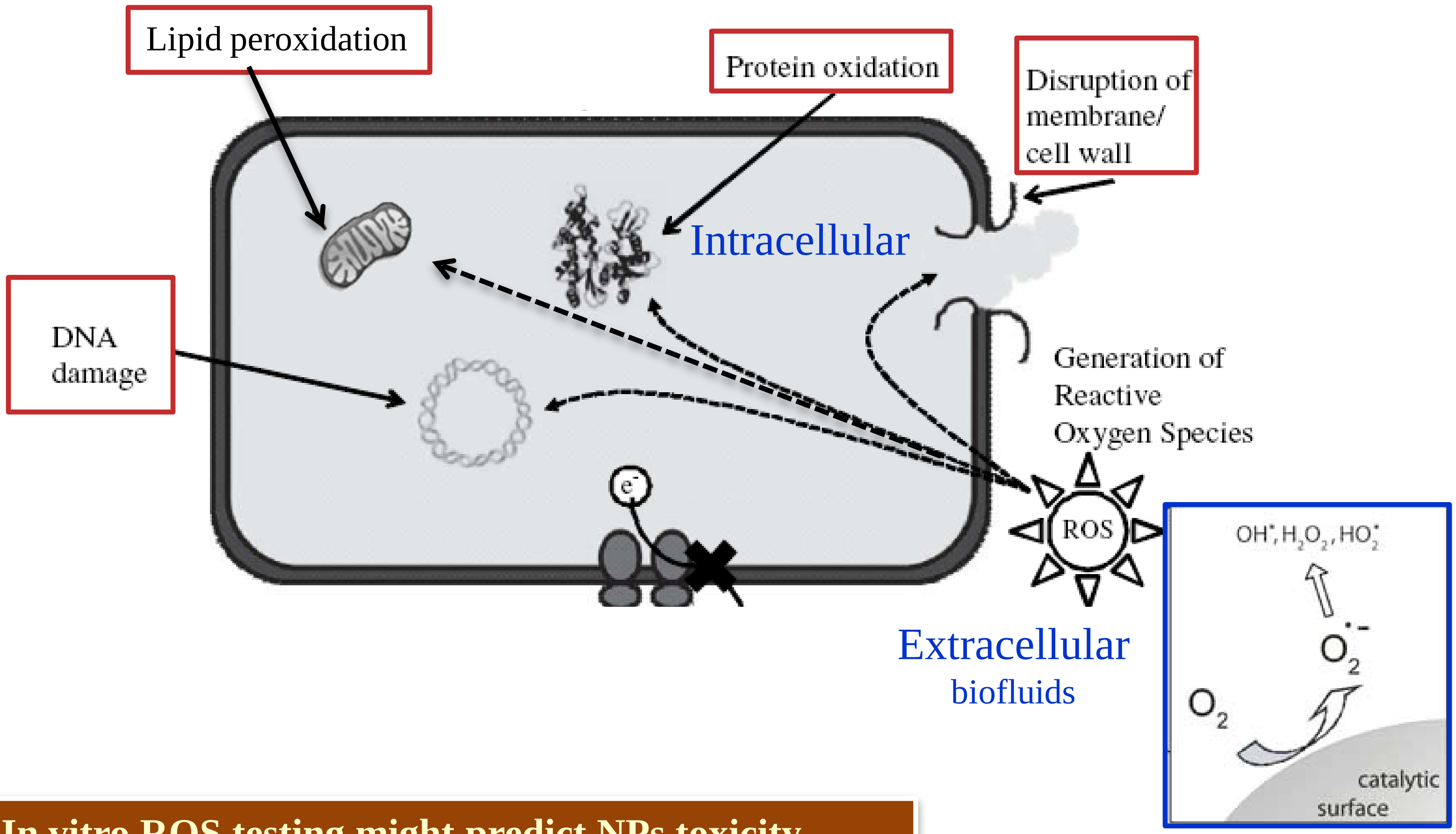
Toxicity Mechanisms



Superoxide ($O_2^{\cdot -}$)
Hydroxyl radical ($HO^{\cdot}$)
Hydrogen peroxide (H_2O_2)

Toxicity by Reactive Oxygen Species (ROS)

Oxidative damage

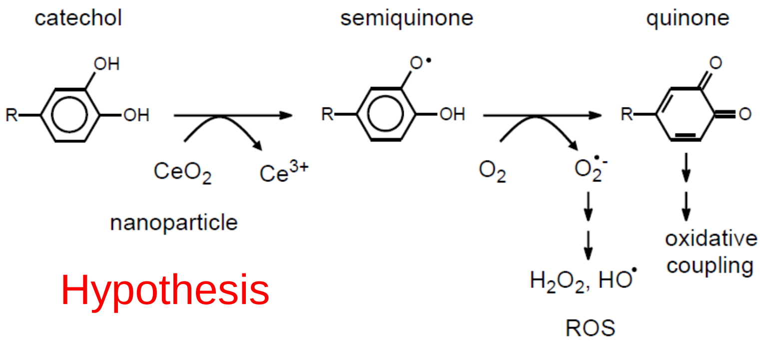


In vitro ROS testing might predict NPs toxicity

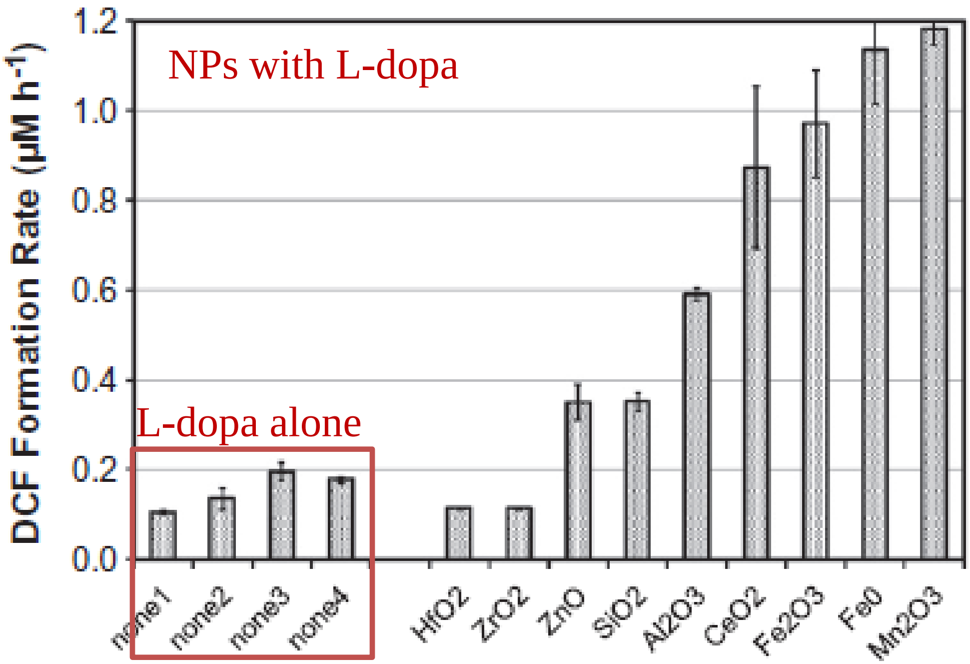
Adapted from Li et al. 2008, Water Res, 42:4591

ROS Formation by Inorganic NPs

Monitor chemical ROS production caused NP oxidation of L-dopa



Hypothesis



Summary Results

Mn_2O_3 , CeO_2, Fe_2O_3 and Fe(0) react with L-dopa and O_2 to greatly enhance ROS formation

ZnO, SiO_2 and Al_2O_3 react with L-dopa and O_2 to enhance ROS formation to a lesser extent

HfO_2, ZrO_2 do not enhance ROS production (inert)

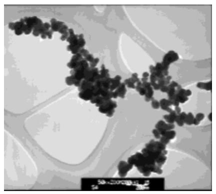
Luna-Velasco et al. 2011, Chemosphere, 85:19-25

Objectives

- Determine if the NPs studied can cause oxidation of the protein BSA.
- Evaluate the effect of particle size on the oxidation of the protein BSA.
- Evaluate the role of oxygen in the oxidation of the protein BSA.
- Evaluate the chemical ROS production by Cu(0) and CuO NPs.

Nanomaterials and Methods

- Hafnium oxide (HfO_2)



HfO_2

- Cerium oxide (CeO_2)

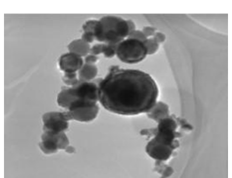
  Silicon oxide (SiO_2)

  Aluminum oxide (Al_2O_3)

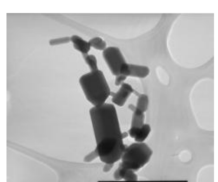
- Others:

  Fe(0), Fe_2O_3, ZrO_2, ZnO,

  Mn_2O_3, Ag, Cu(0) and CuO.



Mn_2O_3



ZnO

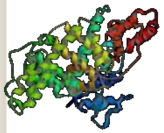
- <u>ICP- OES:</u>
  To determine very precisely the elemental composition of samples

- <u>Scanning and Transmission Electron Microscopy (SEM and TEM)</u>
  University of Arizona Spectroscopy and Imaging Facilities

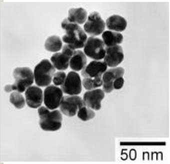
- Particle Size Distribution (PSD) and Zeta Potential

Measurement of BSA Protein Oxidation by NPs

BSA (Bovine Serum Albumin)

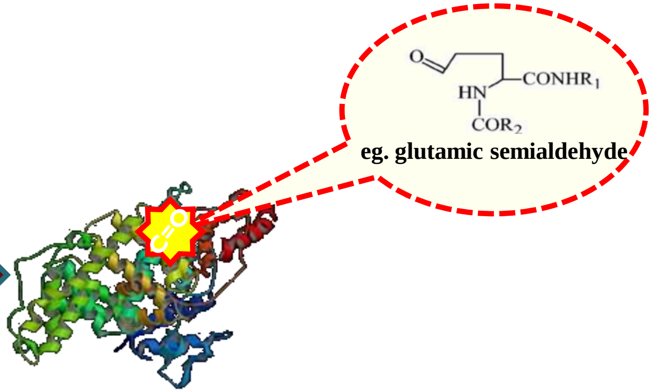


BSA (100 mg L^{-1})
Stock 20x in PBS, pH 7.4



NPs (200 mg L^{-1})
Stock 10x dispersed in PB 5 mM (pH 7.4) & sonicated 5 min at 75% amplitude

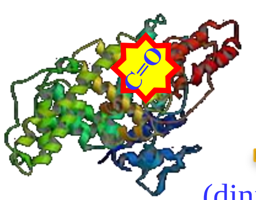
Incubations in PB
pH 7.4 at 37 ºC



Protein Oxidation
Carbonyl (CO) groups on side protein chain mainly from: Pro, Arg, Lys, and Thr

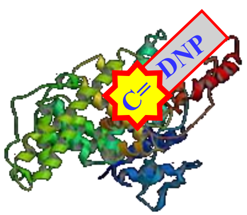
Protein carbonyls measurement by ELISA test

Rapid detection/quantification as an index of oxidized proteins (Kit OxiSelect)



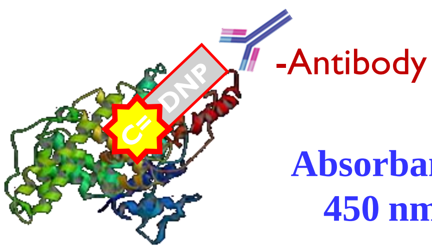
Oxidized protein

DNPH
(dinitrophenylhydrazine)



DNP-protein
DNP (Dinitrophenyl hydrazone)

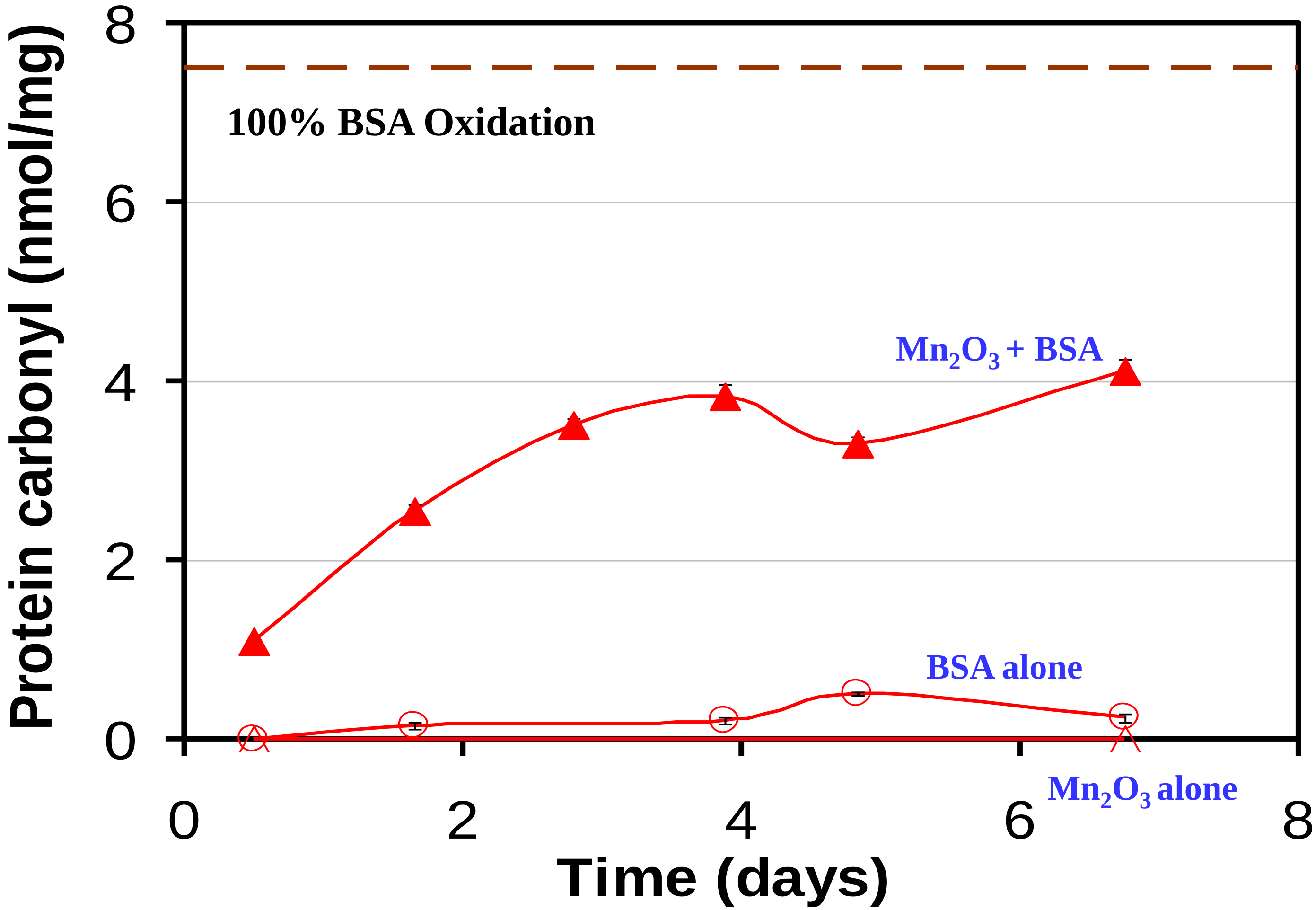
Anti-DNP Antibody



DNP-protein

Absorbance 450 nm

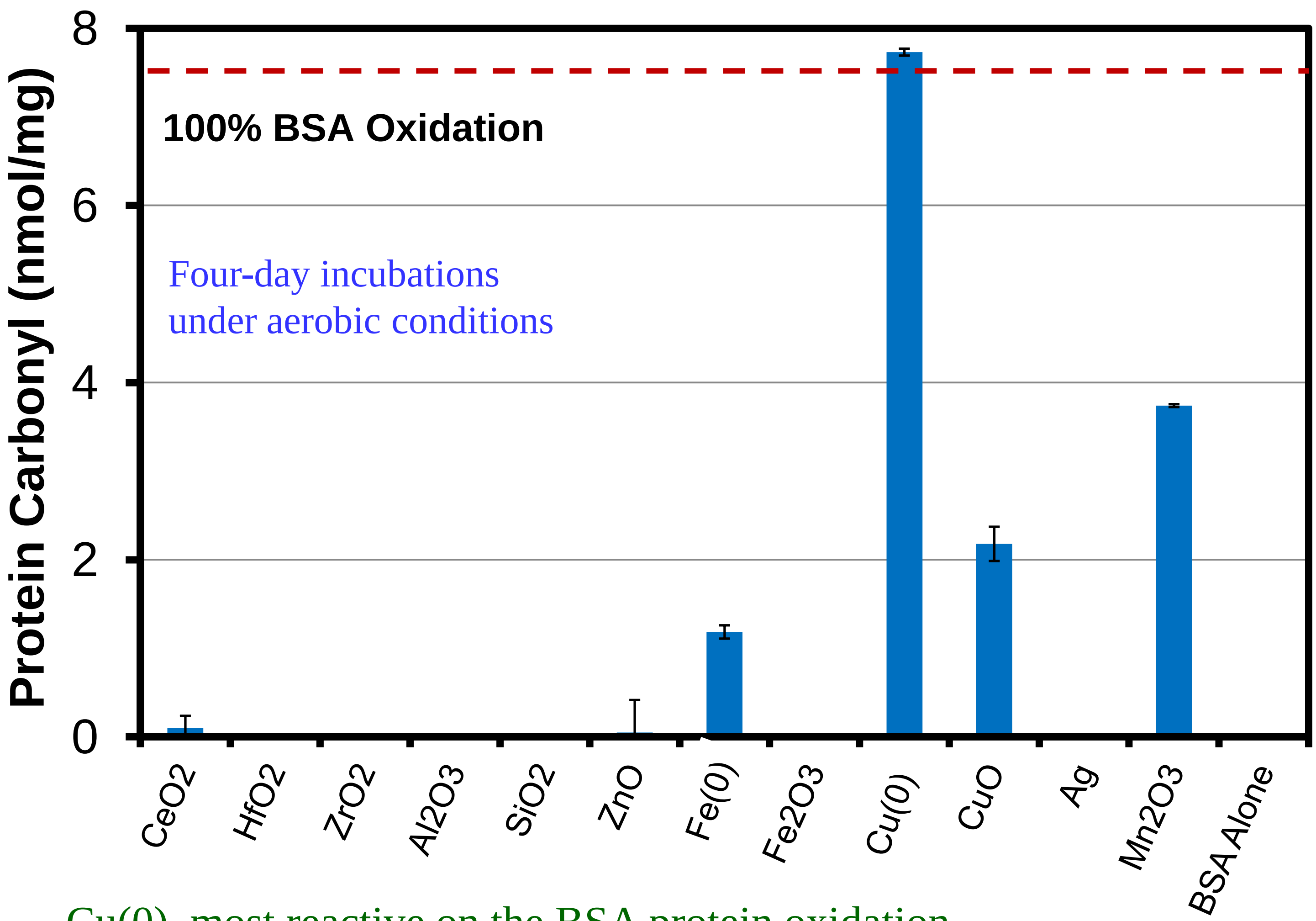
BSA Protein Oxidation by Mn_2O_3 NPs



Oxidized standard BSA: 7.5 nmol protein carbonyl/mg protein (= 100% oxidized BSA).

~ 50% of BSA was oxidized by Mn_2O_3 NPs at the rate of 1.06 nmol protein CO/mg protein/d within a week exposure under aerobic conditions.

BSA Protein oxidation by inorganic NPs

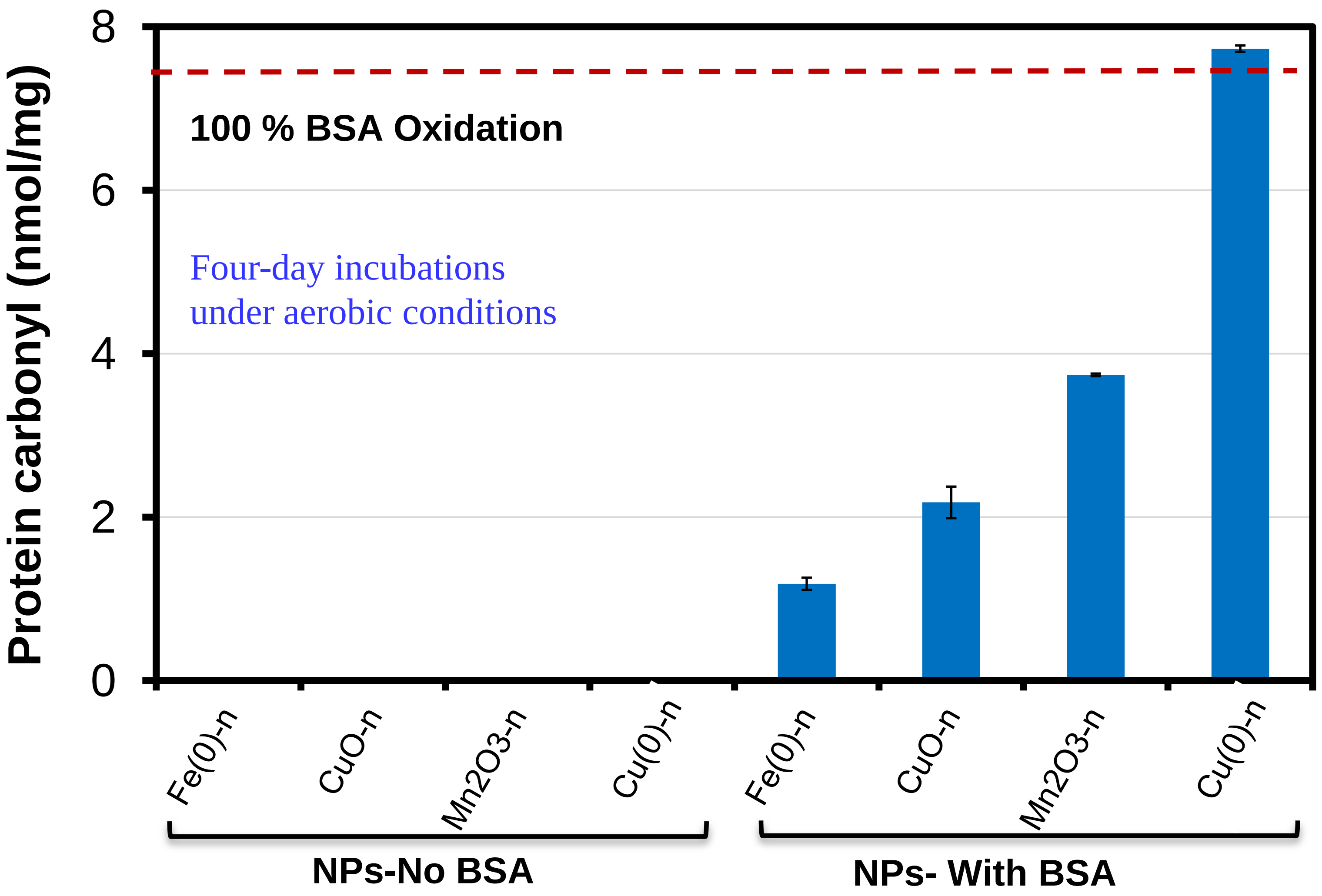


Cu(0), most reactive on the BSA protein oxidation.

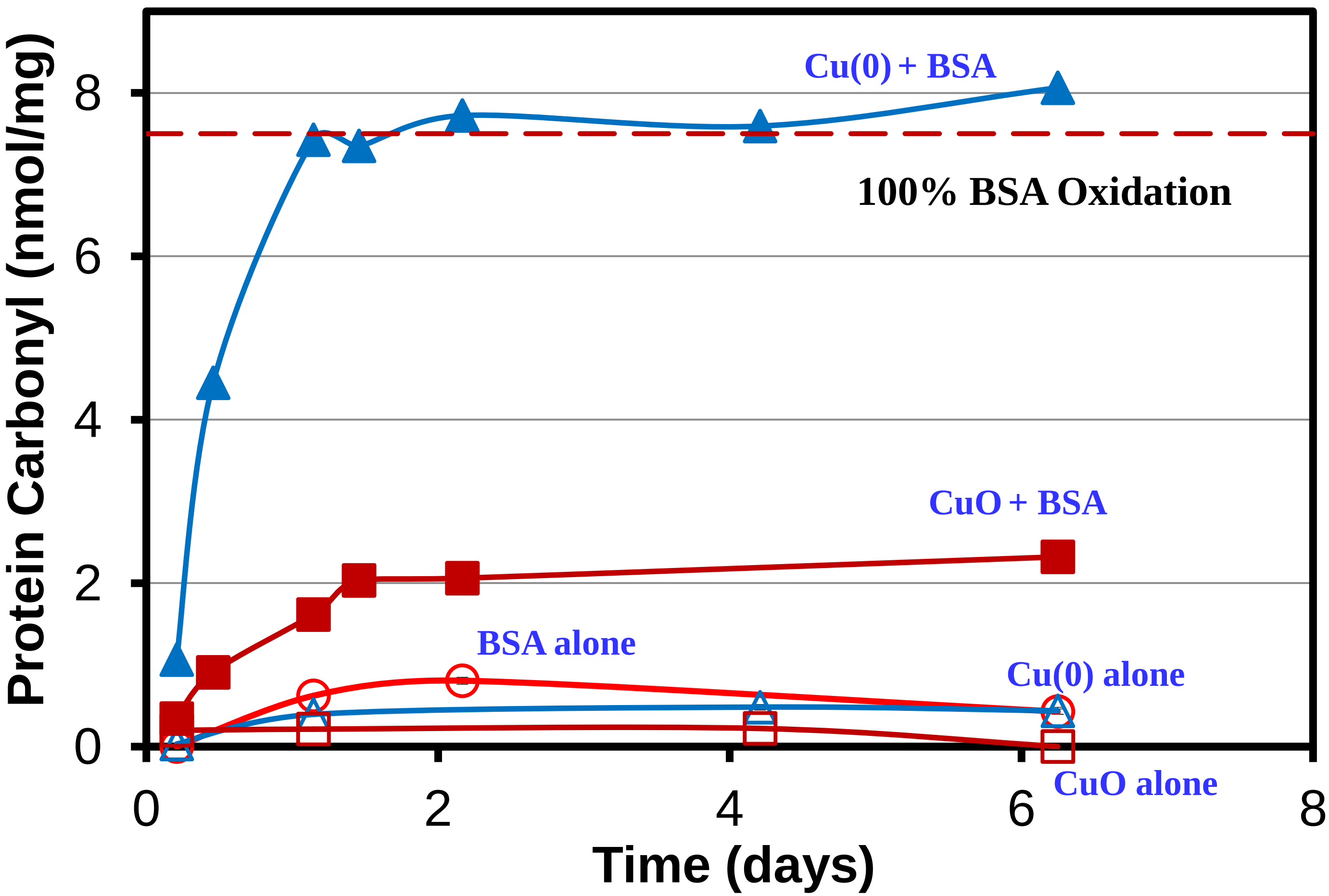
Mn_2O_3, CuO and Fe(0) moderate on the BSA protein oxidation.

All other NPs are not reactive or very slow reactive on the BSA protein oxidation (inert).

BSA Protein oxidation by inorganic NPs



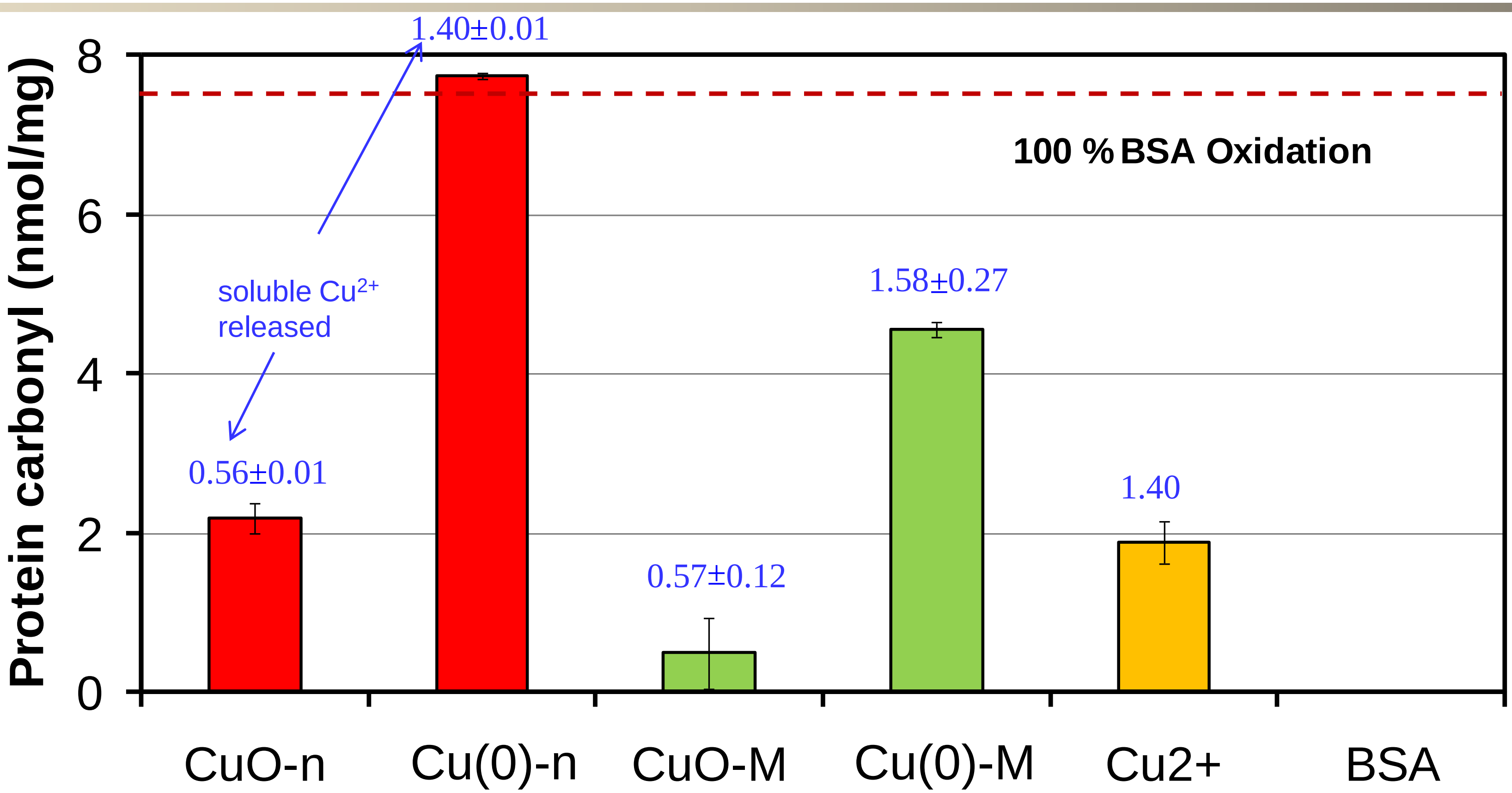
BSA Protein Oxidation by Cu(0) and CuO NPs



Oxidized standard BSA: 7.5 nmol protein carbonyl/mg protein (=100% oxidized BSA).

BSA protein was oxidized by Cu(0) and CuO NPs at the rate of 6.26 and 1.27 nmol protein CO/mg protein/d , respectively, with a week exposure under aerobic conditions.

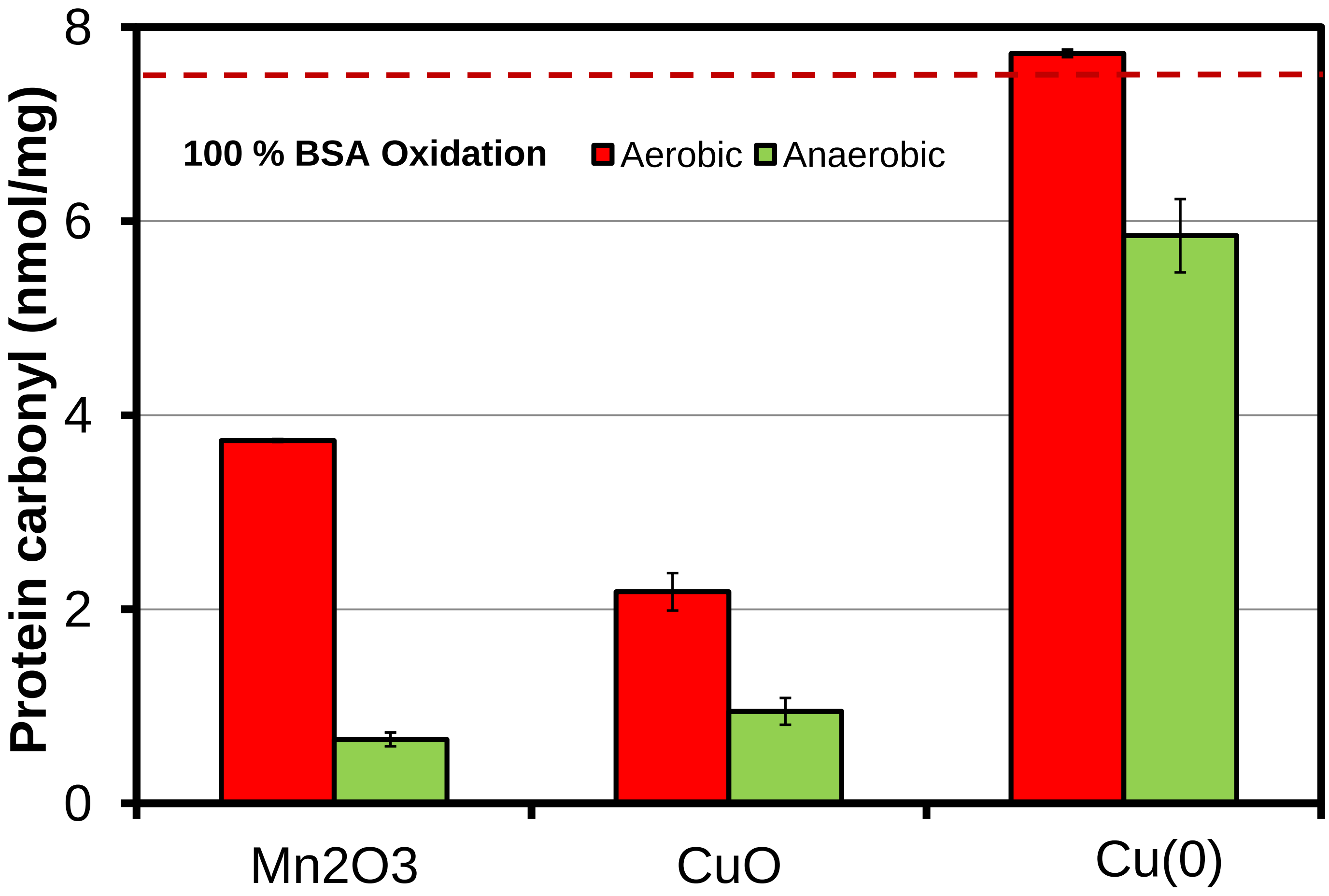
Effect of Size on the BSA Protein Oxidation by Cu(0) and CuO NPs



Nano-sized Cu(0) and CuO demonstrated significant increase of BSA protein oxidation compared to micro-sized counterpart with four days exposure under aerobic conditions.

Compared to the BSA protein oxidation caused by soluble Cu^{2+}, the Cu(0) (both nano and micro size) show much higher oxidation.

Role of Oxygen in BSA Protein Oxidation by Inorganic NPs

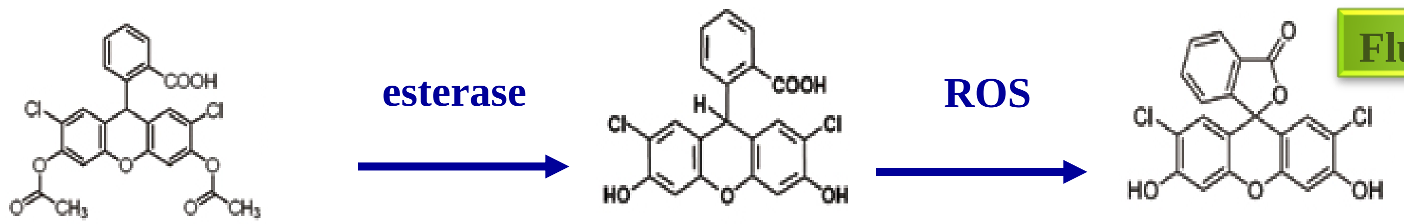


Mn_2O_3, Cu(0) and CuO could oxidize the BSA protein with four days exposure under both aerobic and anaerobic conditions.

The presence of oxygen enhanced the BSA protein oxidation by NPs.

Rapid Method for Chemical ROS by Inorganic NPs

Based on fluorescence of ROS-sensitive dye

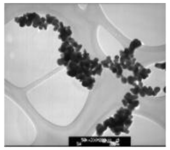


2′,7′-dichlorodihydrofluorescin diacetate **(DCFH-DA)**

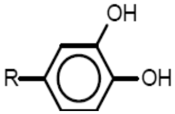
2′,7′-dichlorodihydrofluorescin **(DCFH)**

2′,7′-dichlorofluorescein **(DCF)**

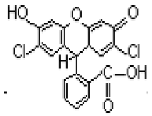
ROS Assay



NPs (200 mg L^{-1})
Stock 10x in water
sonicated 5 min, 75 % amplitude

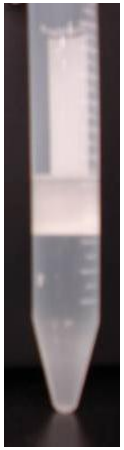


Phenolic biomolecules
L-dopa or catechol (500 mM)
Stock 4x in 3% methanol (v/v)



nonfluorescent

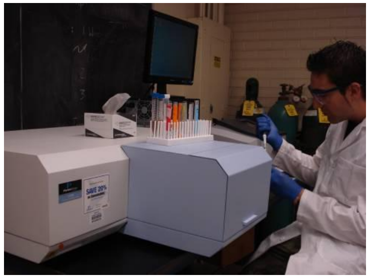
ROS-dye (DCFH 20 uM)
Stock 40 uM in phosphate buffer (PB), pH 7.4



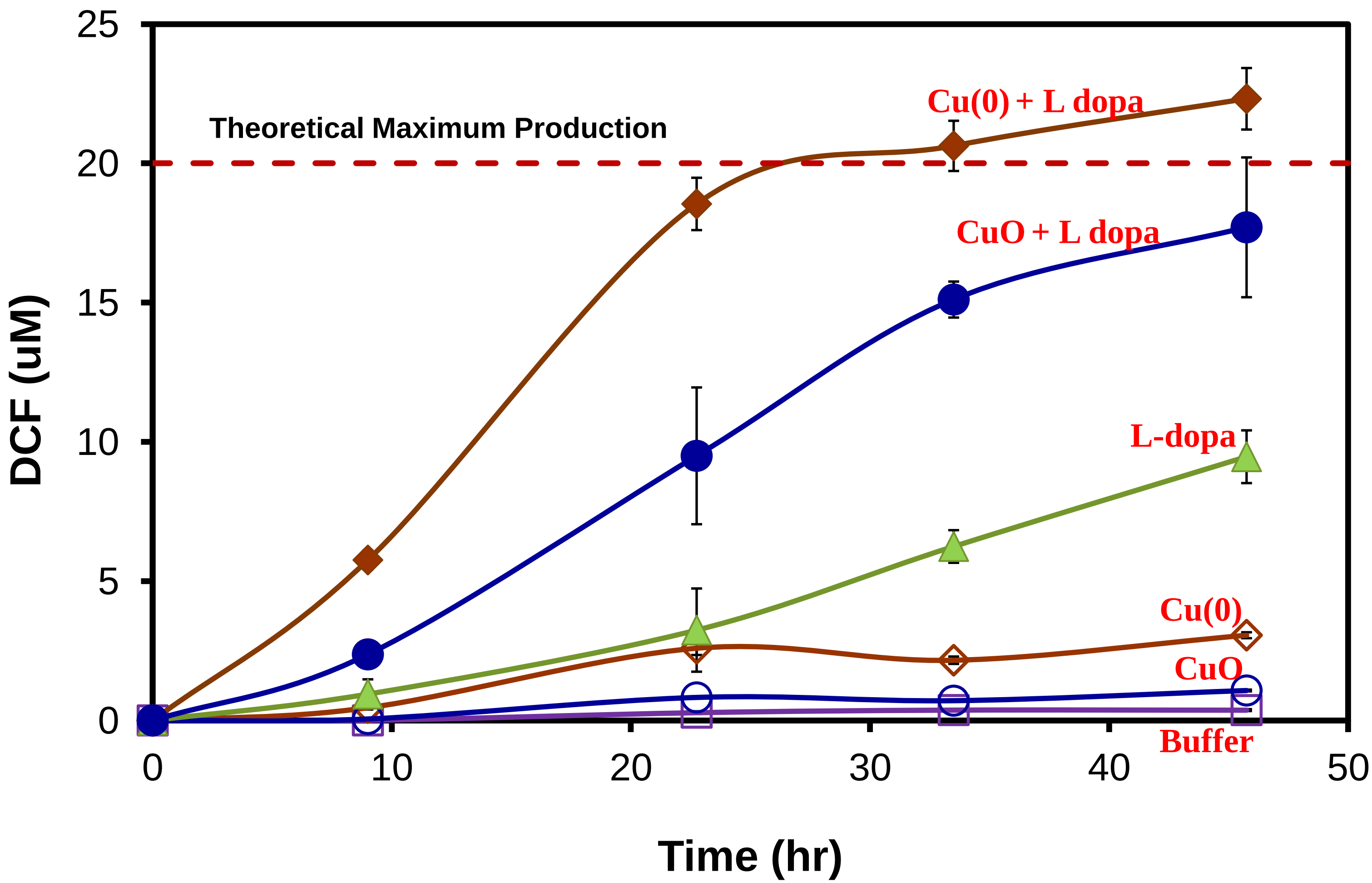
Incubated at 37 ºC
Masked from light.

Fluorescence Measurements

Controls:
NPs, L-dopa & dye, each alone



ROS Indicator-Dye Oxidation by Cu(0) and CuO NPs



Cu(0) and CuO NPs react with L-dopa and O_2 to greatly enhance ROS formation

Conclusions

- Mn_2O_3, Fe(0), Cu(0) and CuO NPs caused constant and significant oxidation of BSA protein under aerobic conditions; however, all other inorganic NPs are not reactive or very slow reactive with BSA protein.
- The nano-sized Cu(0) and CuO significantly increased the protein oxidation compared with micro-sized counterpart.
- The protein oxidation by NPs happened under both aerobic and anaerobic conditions; however, the presence of oxygen enhanced the protein oxidation.
- ROS formation or direct protein oxidation by NPs could be important mechanisms for the oxidative stress to cells.