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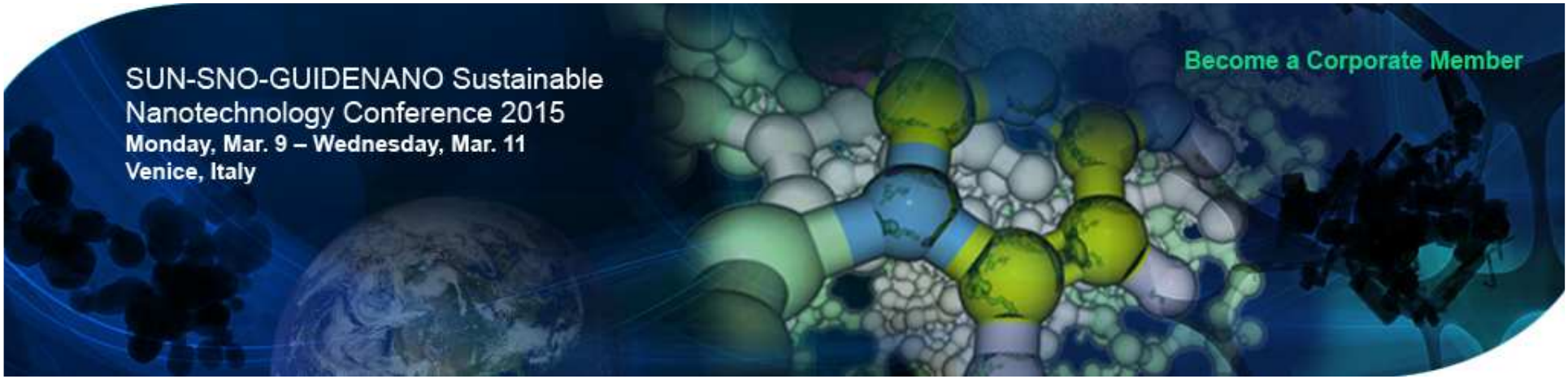

Surface reactivity of CuO NPs is responsible for the early oxidative damages to A549 cells: a Trojan-horse independent mechanism

Elisa Moschini, Paride Mantecca

CENTRO DI RICERCA Università degli Studi di Milano-Bicocca
POLARIS
POLVERI IN AMBIENTE E RISCHIO PER LA SALUTE

Presentation overview

1- Toxicity and mode of action of commercial forms of CuO NPs in human lung cells

CuO NPs are highly toxic to lung cells

Cytotoxicity is driven by abundant NP internalization and a Trojan horse-mediated autophagic cell death

2- A new copper oxide sonochemically synthetized showing enhanced antibacterial properties… and toxicity?

… what happen to human cells? A comparative study between a commercial and a sonochemical form of CuO NPs

3- Oxidative damage as key phenomenon that drives cytotoxicity of sonochemical CuO

Protein and lipids as precocious targets of CuO: - SH oxidation

- Protein carbonylation

- Lipid peroxidation

4- beyond the Trojan horse mechanisms

CuO intracellular dissolution

Cell-particle interactions

5- Conclusion and final remarks

What we know from literature about nCuO…

Chemical Research in Toxicology

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Article

Copper Oxide Nanoparticles Are Highly Toxic: A Comparison between Metal Oxide Nanoparticles and Carbon Nanotubes

Hanna L. Karlsson, Pontus Cronholm, Johanna Gustafsson, and Lennart Mo#ller

Toxicology Letters 188 (2009) 112–118

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Size-dependent toxicity of metal oxide particles—A comparison between nano- and micrometer size

Hanna L. Karlsson [1], Johanna Gustafsson [1], Pontus Cronholm, Lennart Möller

Toxicity and Metal Release From Copper and Copper Oxide Particles

small

Copper particle toxicity

Surface Characteristics … Release, and Toxicity of Nano- and Micromet… Copper and Copper(II) Oxide Particles: A Cross…inary Study

Klara Midander, Po…olm, Hanna L. Karlsson, Karine Elihn, …

Toxicology Letters 197 (2010) 169–174

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Nanoparticle cytotoxicity depends on intracellular solubility: Comparison of stabilized copper metal and degradable copper oxide nanoparticles

Andreas M. Studer[a,b], Lu…
Lukas C. Gerber[a], Holger …

RESEARCH Open Access

Cytotoxicity and genotoxicity of nano - and microparticulate copper oxide: role of solubility and intracellular bioavailability

Annetta Semisch, Julia Ohle, Barbara Witt and Andrea Hartwig*

Toxicology in Vitro 23 (2009) 1365–1371

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Copper oxide nanoparticles induce oxidative stress and cytotoxicity in airway epithelial cells

Baher Fahmy [1], Stephania A. Cormier *

OPEN ACCESS Freely available online

PLOS ONE

Copper Oxide Nanoparticles Induced Mitochondria… Mediated Apoptosis in Human Hepatocarcinoma…

Maqsood A. Siddiqui[1], Hisham A. Alhadlaq[2,3], Javed Ahmad[1], Abdulaziz A. Al-Khedh… Javed Musarrat[4], Maqusood Ahamed[2]*

Biochemical and Biophysical Research Communications 396 (2010) 578–583

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communica…

journal homepage: www.elsevier.com/locate/ybbre…

Genotoxic potential of copper oxide nanoparticles in … epithelial cells

Maqsood Ahamed[a,*], Maqsood A. Siddiqui[b], Mohd J. Akhtar[c], …itya B. Pant[d], Hisham A. Alhadlaq[a,e]

M… Responses of Human Lung …ial Cells to the Toxicity of Copper …de Nanoparticles Inferred from …hole Genome Expression Analysis

Nobutaka Hanagata,[†,‡,*] Fei Zhuang,[‡,§] Sarah Connolly,[†,∥] Jie Li,[†] Nobuhiro Ogawa,[§] and Mingsheng Xu[⊥]

OPEN … available online

PLOS ONE

Copp…r Oxide Nanoparticles Induce Autophagic Cell Death in A549 Cells

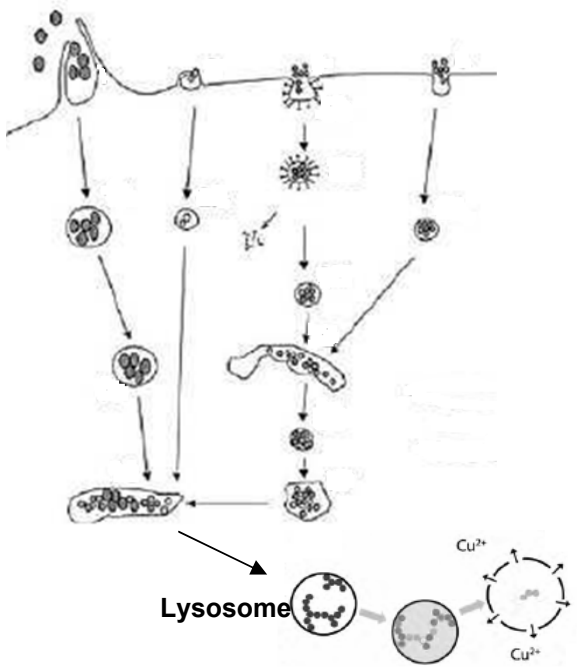
Tingting Sun[9], Yiwu Yan[9], Yan Zhao, Feng Guo, Chengyu Jiang*

State Key Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Peking Union Medical College, Tsinghua University, Beijing, China

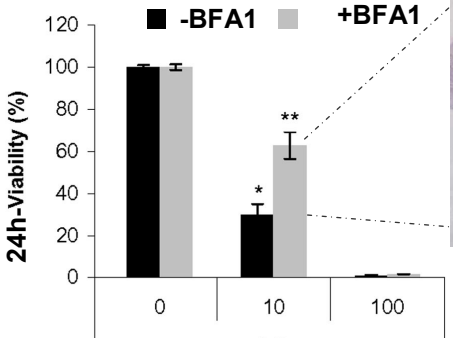
Chemical-physical characteristics

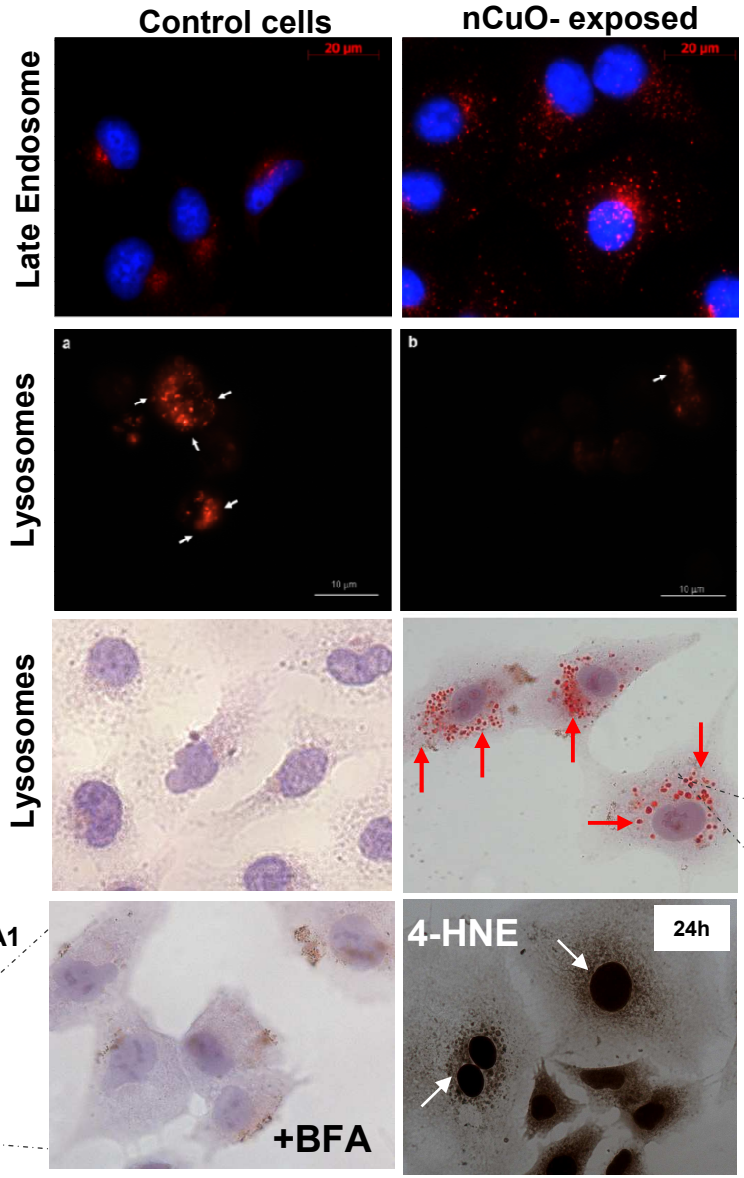
Molecular mechanisms and toxic effects

Toxicity and mode of action of nCuO-BSA in human lung cells



Trojan horse mechanism works at late exposure time promoting cell death



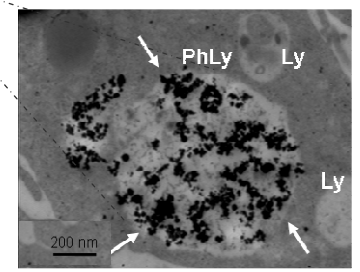


Viability recovery when NP dissolution was inhibited

1-..-nCuO enter cells bv endocytosis

2-engulfes lysosomes and induces lysosomal destabilization..

3- .by intracellular ion dissolution



4- that induce oxidative damage and autophagy

Moschini et al. 2013. Toxicology Letters 222: 102– 116

Aim of the work

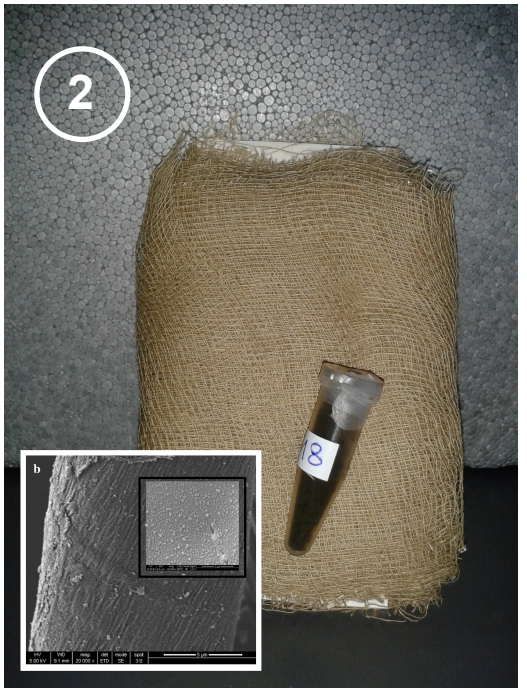
Comparative study of the precocious cytotoxic effects on A549 cells induced by two CuO NPs with similar morphology and primary size but different crystalline structure and reactive oxygen species generation potential

1.- Commercial CuO < 50nm
(sigma-aldrich)

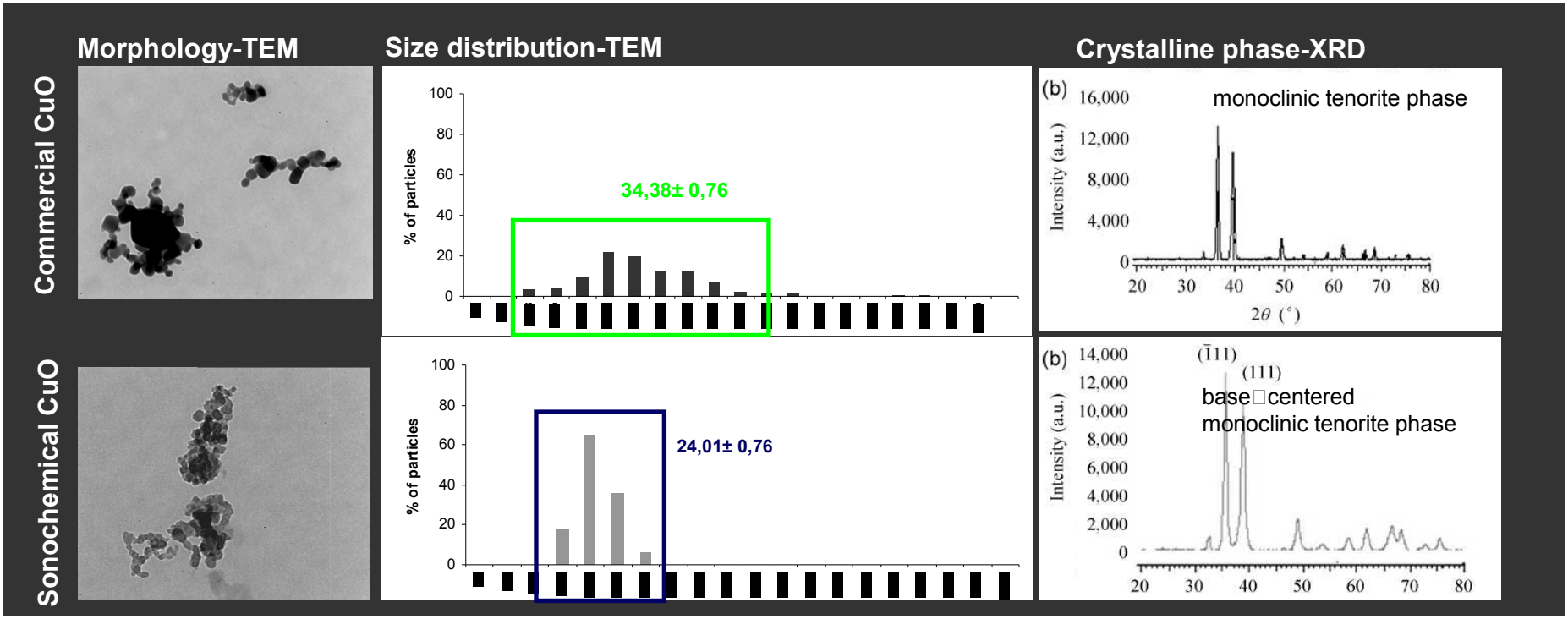
2.- Sonochemical CuO < 50nm

sonochemically synthesized, antimicrobial,

used for textile coating *(Bar-Ilan University, Israel)*



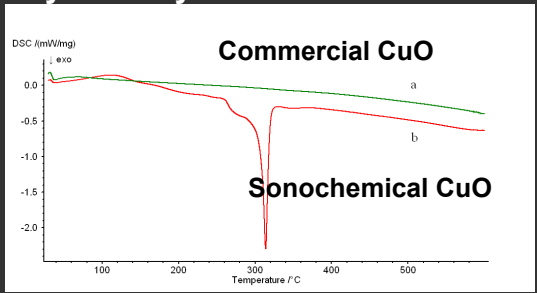
Particle characterization



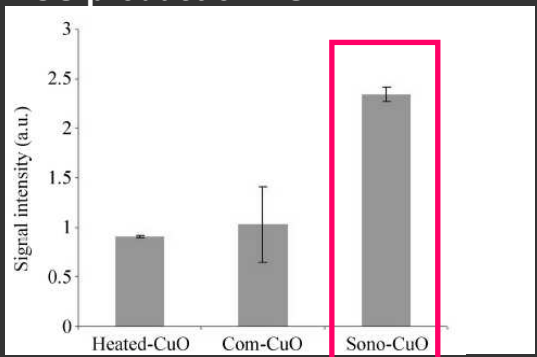
- Both nCuO appeared with an irregular morphology
- commercial CuO presented a mean diameter of 34,38± 0,76 nm while and a broad size distribution
- sonochemical CuO has a mean of diameter 24,01± 0,76 with a NP size distribution between 15 and 30 nm

Particle characterization

Crystallinity - DSC



ROS production-ESR

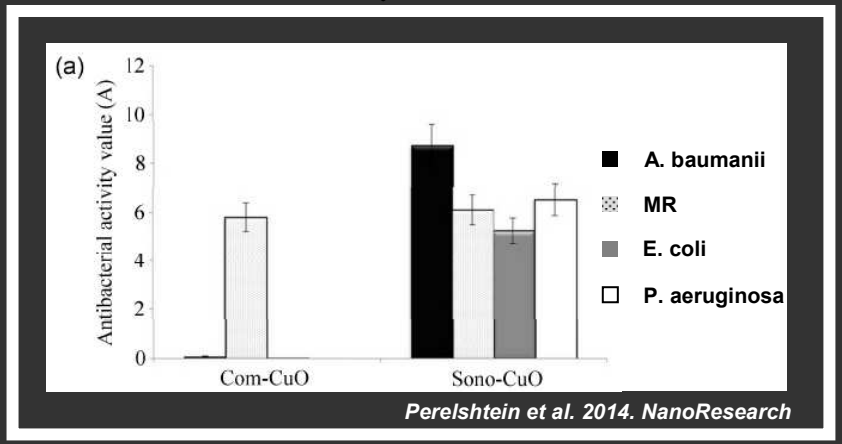


sonochemical CuO NPs

are more toxic to the bacteria…

sonochemical CuO NPs are crystallites with more defects and less organized structure than commercial CuO

different ability to produce ROS in a cell free system



Perelshtein et al. 2014. NanoResearch

Perelshtein et al., 2014. NanoResearch.

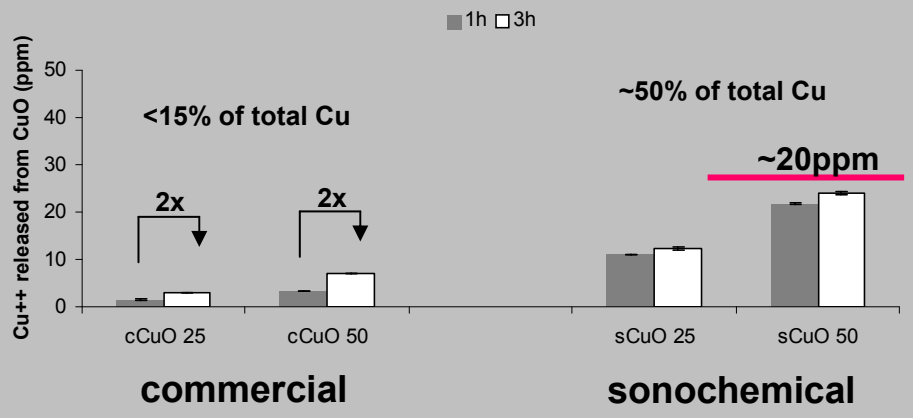
NP suspension characterization in culture medium

DLS analyses

		z-average (nm)	pdi
commercial CuO	25 ug/ml	342,7	0,248
	50 ug/ml	691,2	0,359
sonochemical CuO	25 ug/ml	157,7	0,376
	50 ug/ml	230,3	0,298

- dose dependent particle aggregation
- sonochemical CuO finely dispersed than commercial CuO

ICP-OES analyses



- dose dependent Cu^{++} release for both CuO

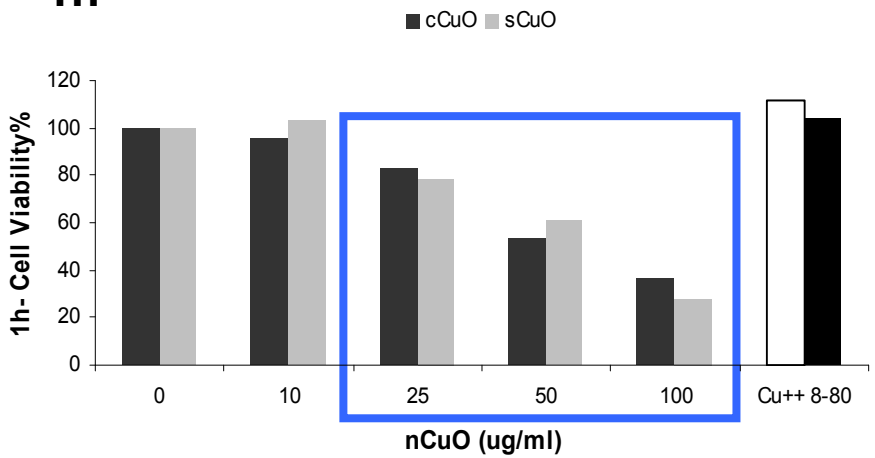
  About 50% of sonochemical-CuO dissolves within the first hour of incubation in culture medium

- time dependent release of copper ions from commercial CuO that reach the 15% of the total Cu

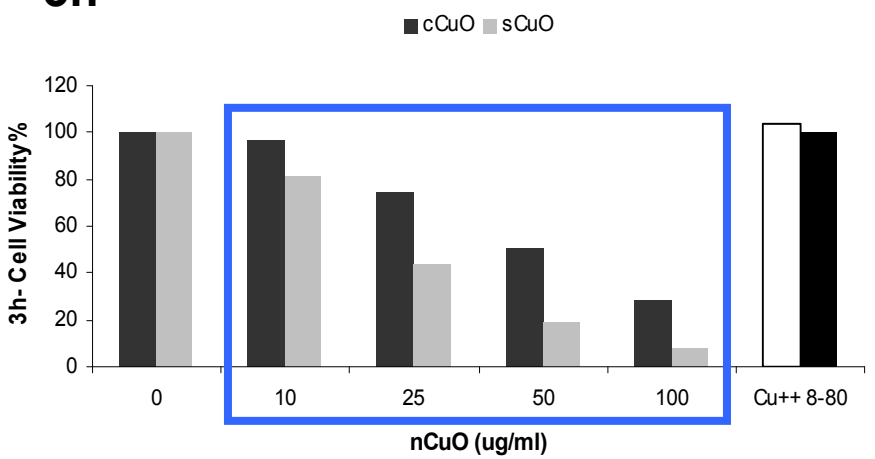
..what about toxicity in human cells??

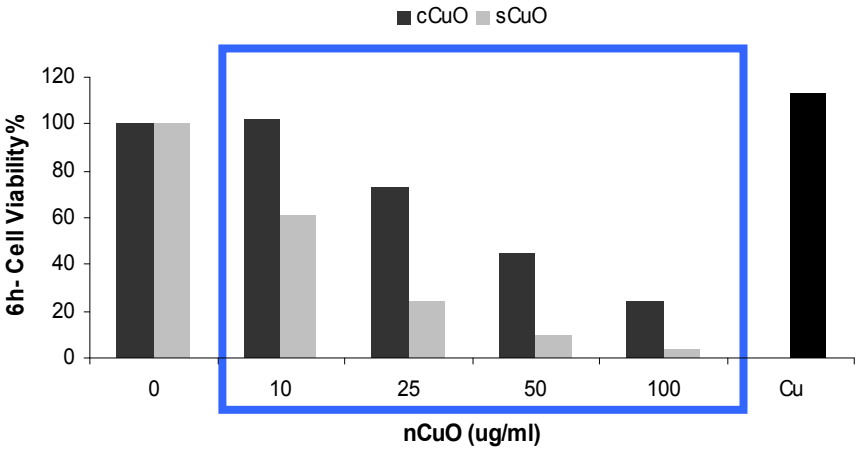
Cell viability (1h,3h,6h) [MTT ASSAY]

1h-



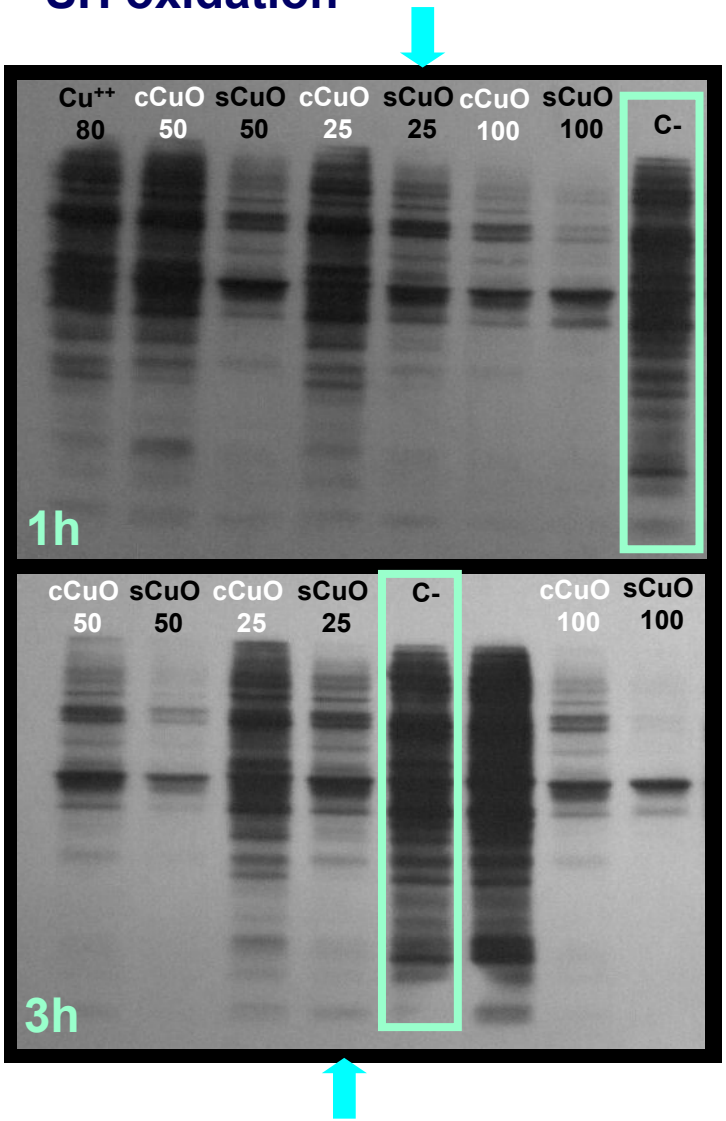
3h-





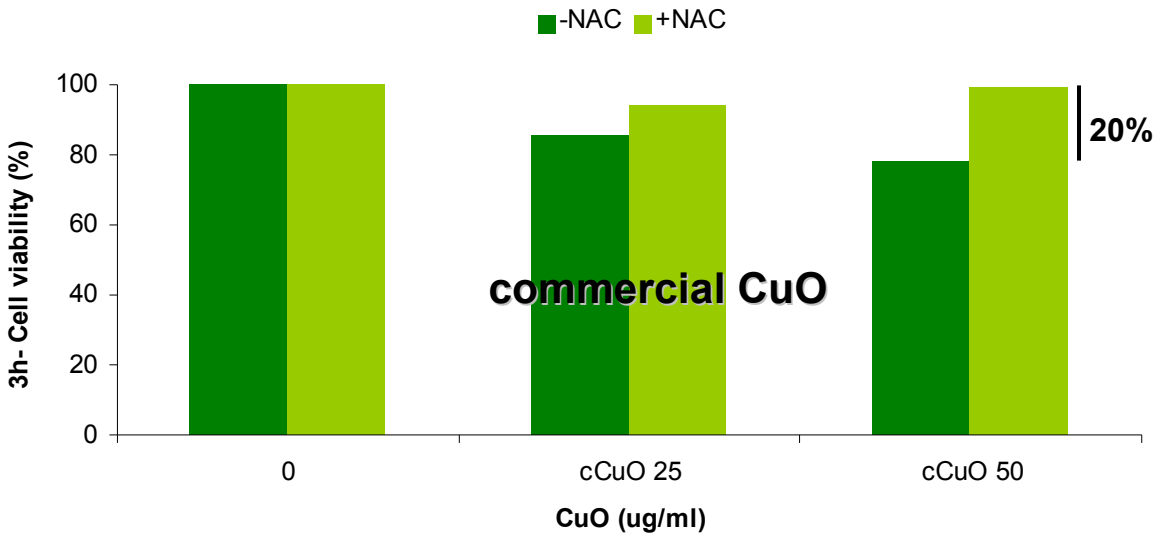
- dose and time dependent viability reduction observed after exposure to both CuO NPs
- precocious cytotoxicity detected after 1h
- sonochemical CuO more toxic than commercial CuO

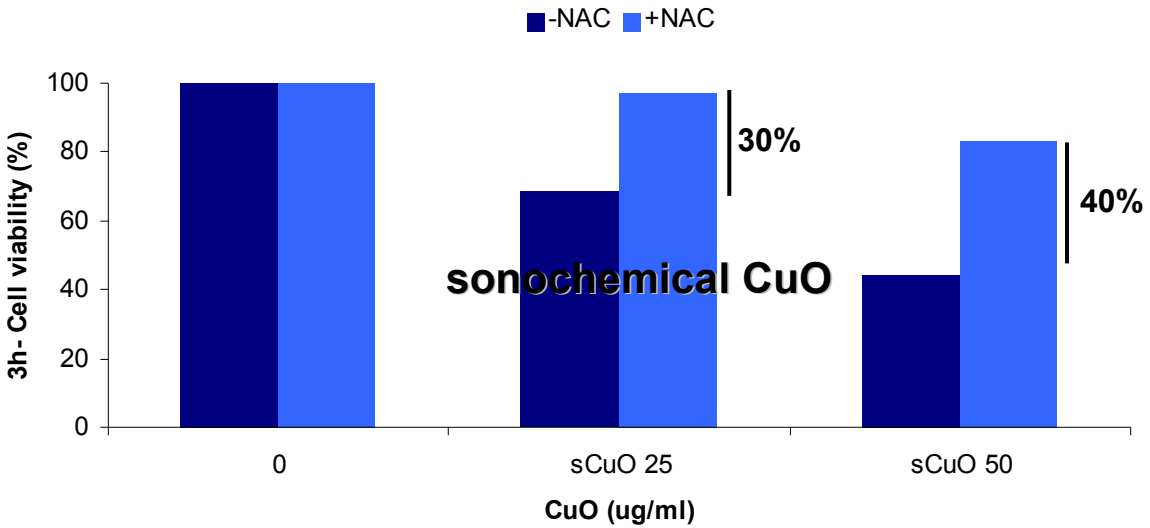
- SH oxidation



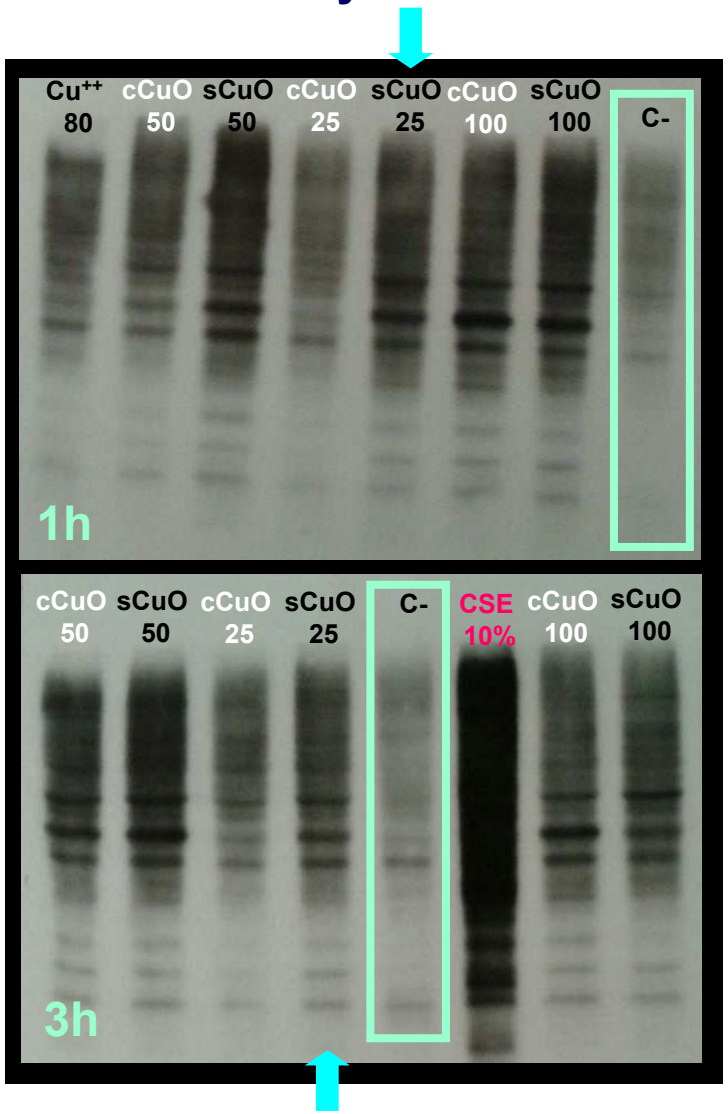
❖ significant decrease in reduced thiol content induced by both CuO NPs (clear profiles)

❖ preincubation of cells with NAC significantly reduces citotoxicity



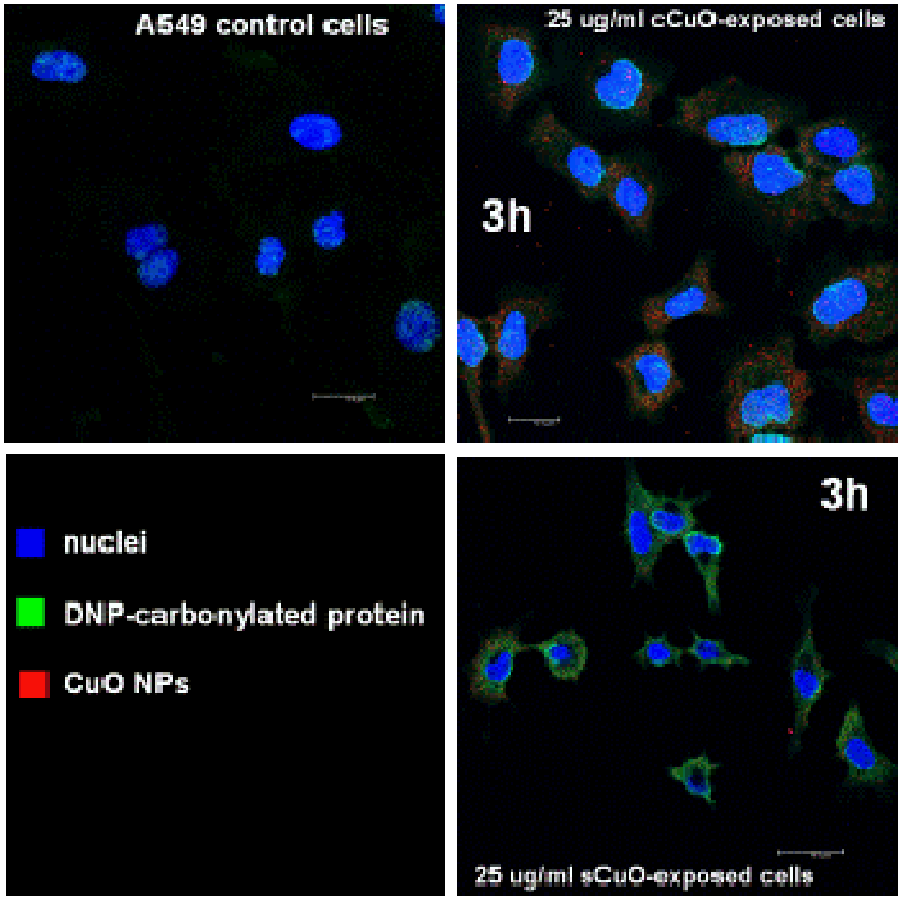


Protein carbonylation



❖ significant increase of protein carbonylation induced by both CuO NPs (dark profiles)

Immunocytochemistry of protein carbonylation

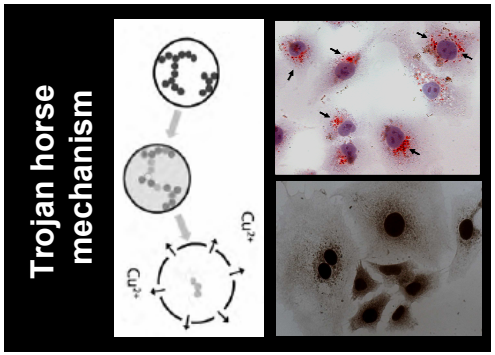


❖ some evidence of protein carbonylation (green) in A549 exposed to commercial CuO; significant presence of particles was also detected

❖ significant protein carbonylation in A549 exposed to sonochemical CuO

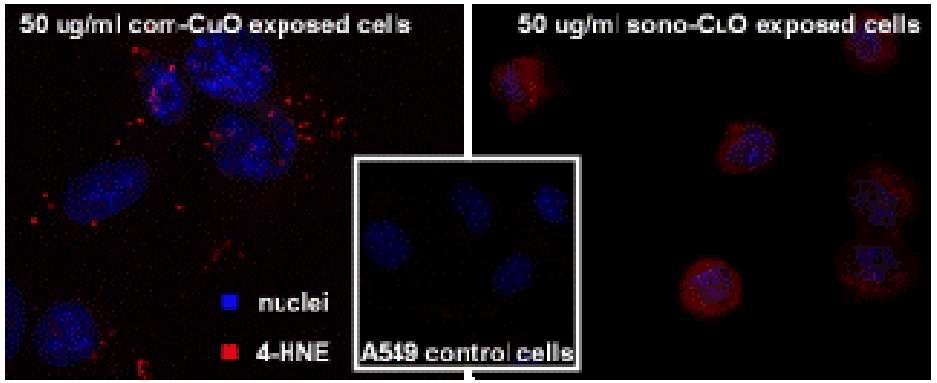
Lipid peroxidation – intracellular dissolution

...remember…

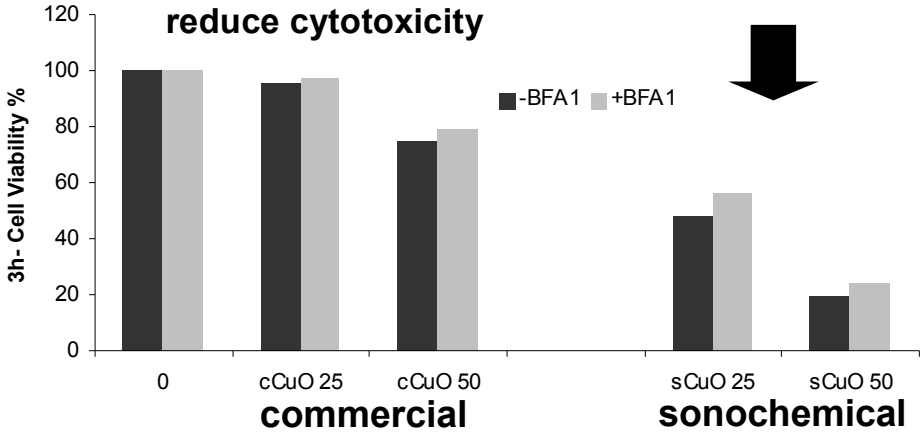


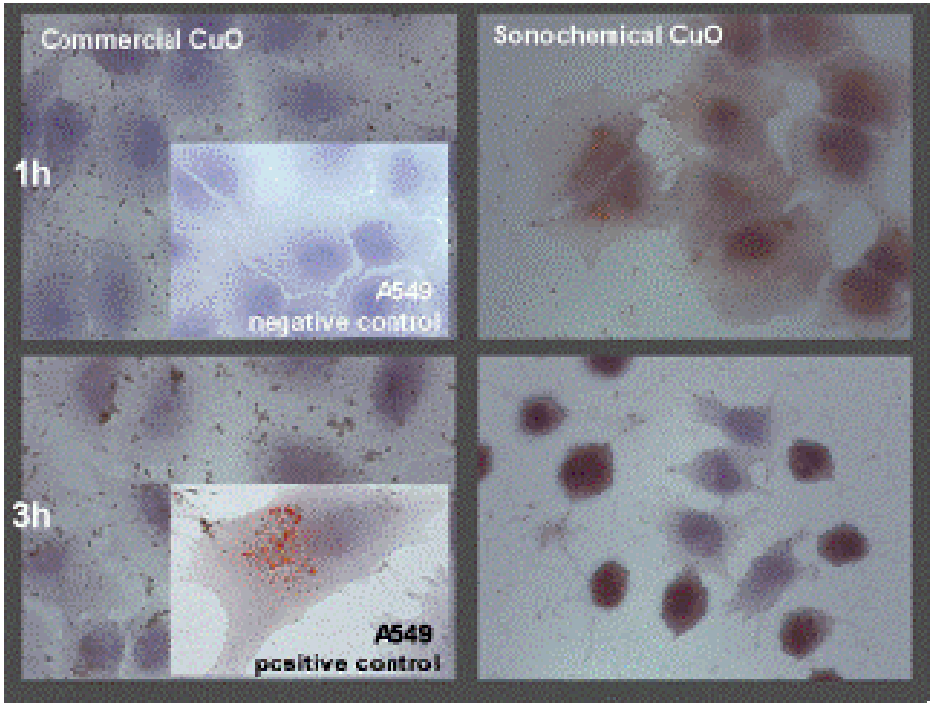
..NOW

- sonochemical CuO induces significant lipid peroxidation in A549

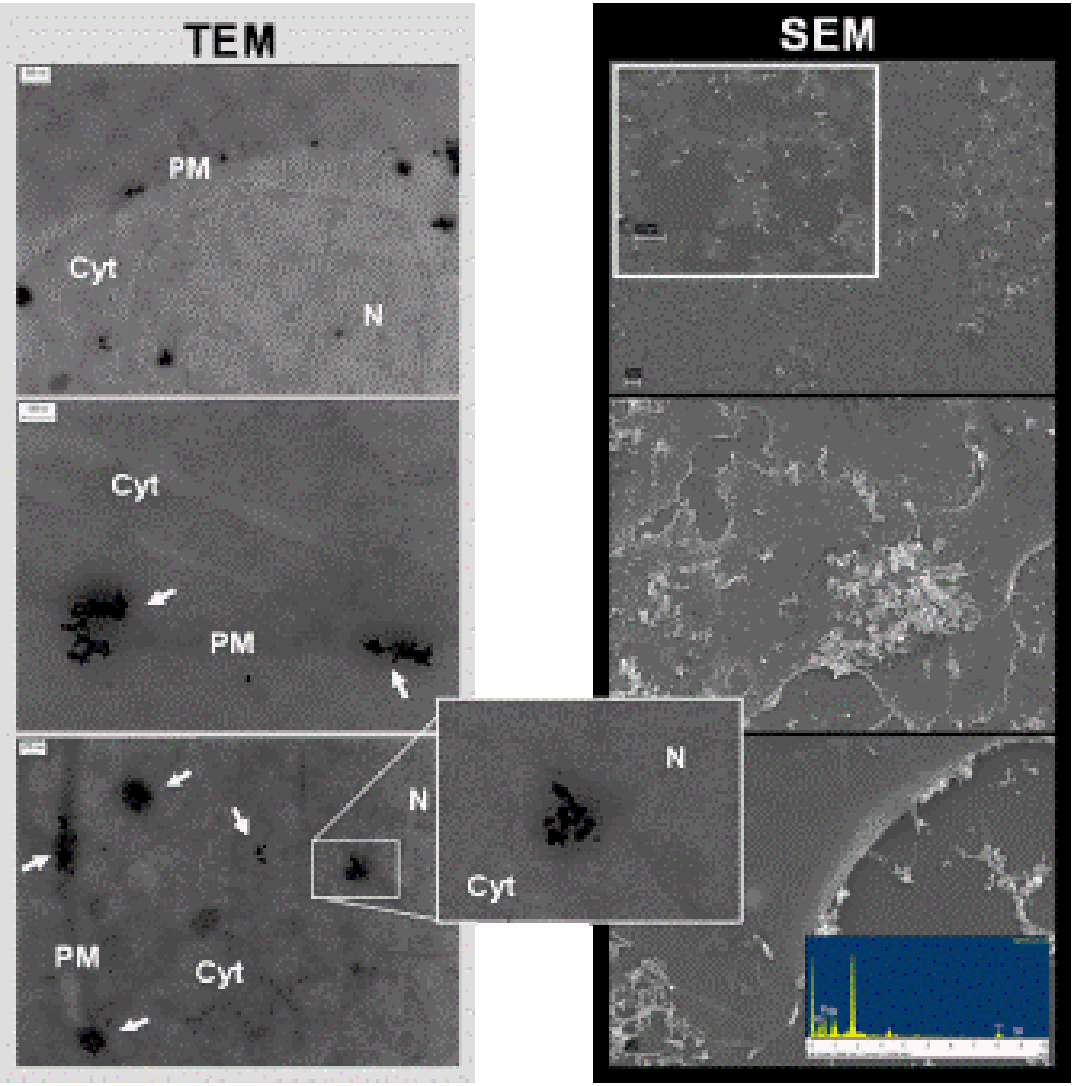


- no evidences of intracellular dissolution in A549 exposed to cCuO despite the presence of NP aggregates
- significant morphological alteration in A549 exposed to sCuO even NP dissolution
- inhibition of lysosomal acidification didn't reduce cytotoxicity



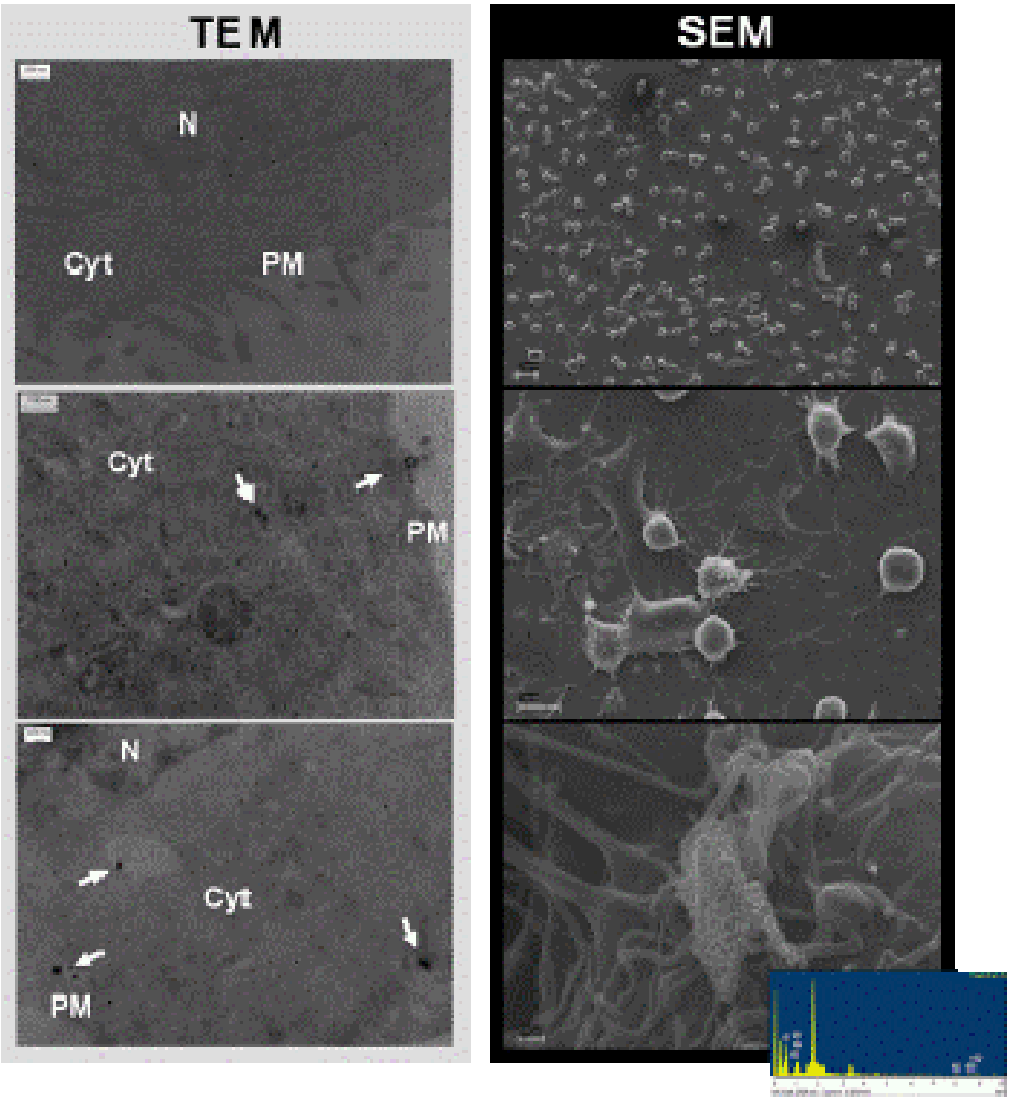


Cell-particle interaction: commercial CuO



- commercial CuONPs interact with cells at early exposure time
- commercial CuONPs was easily internalized by A549 and appear as aggregates free in the cytoplasm
- Particles cover entirely the cell surface and appeared as large aggregates
- No evidences of significant morphological changes were found

Cell-particle interaction: sonochemical CuO



- plasma membrane presents significant blebbing …
- particle were detected on plasma membrane as a very small aggregates and only few sono CuO enter cells during the first 3h of exposure
- sonochemical CuO induced precocious morphological changes in A549
- NP aggreagates deeply interact with PM

Surface reactivity and cell-NP interaction drive cytotoxicity of sonochemical CuO

Conclusions

- The contribute of extracellular copper ions release to cytotoxic effect is negligible for both the CuO NPs
- Oxidative damages to proteins and lipids occur very soon after CuO exposure and bring to cell death
- Sonochemical CuO NPs resulted more toxic than commercial CuO NPs
- The oxidative stress mechanism drives CuO cytotoxicity independently from intracellular dissolution of NPs
- Surface reactivity is the key factor to explain the very high cytotoxicity of sonochemical CuO at early exposure time beyond the trojan horse mechanism

Final remarks

- The knowledge of the modality of cell-NP interactions and the molecular pathways driving toxicity are crucial in assessing NM safety

- By coupling these toxicological data with the efficacy in killing unwanted bacteria, it will be possible to engineer nano-biocides with reduced impact on environmental and human health

- the cooperation among scientists (physicians, chemists, engineers, biologists, toxicologists…), and with stakeholders, is fundamental in order to reach the goal of the “safe development of nanotechnologies”.

Acknowledgements

…to the Nanotoxicology working group

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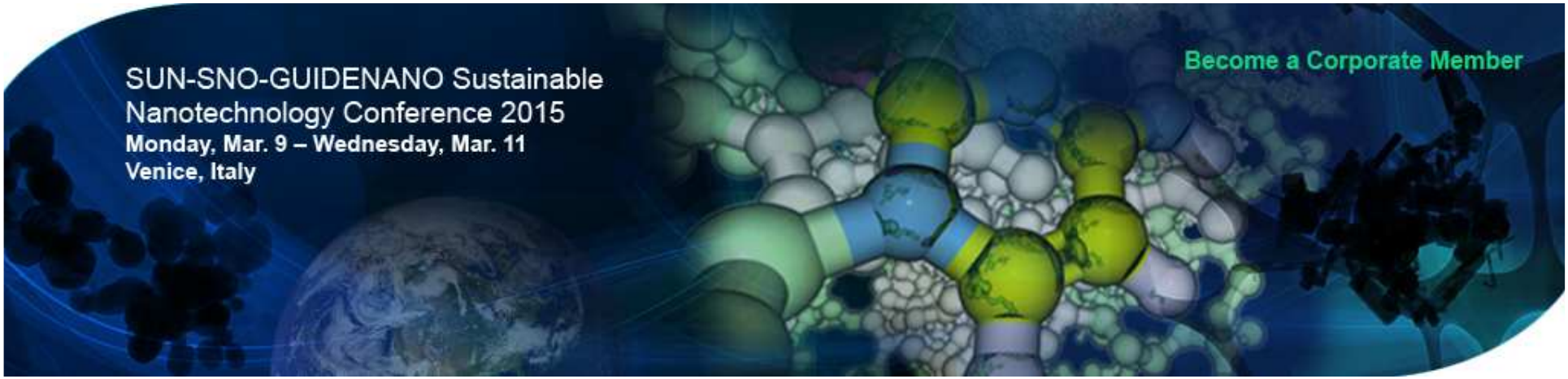

Thanks for your attention…

…any question?