IMPACT OF VARIOUS METALLIC NANOPARTICLES ON METAL HOMEOSTASIS

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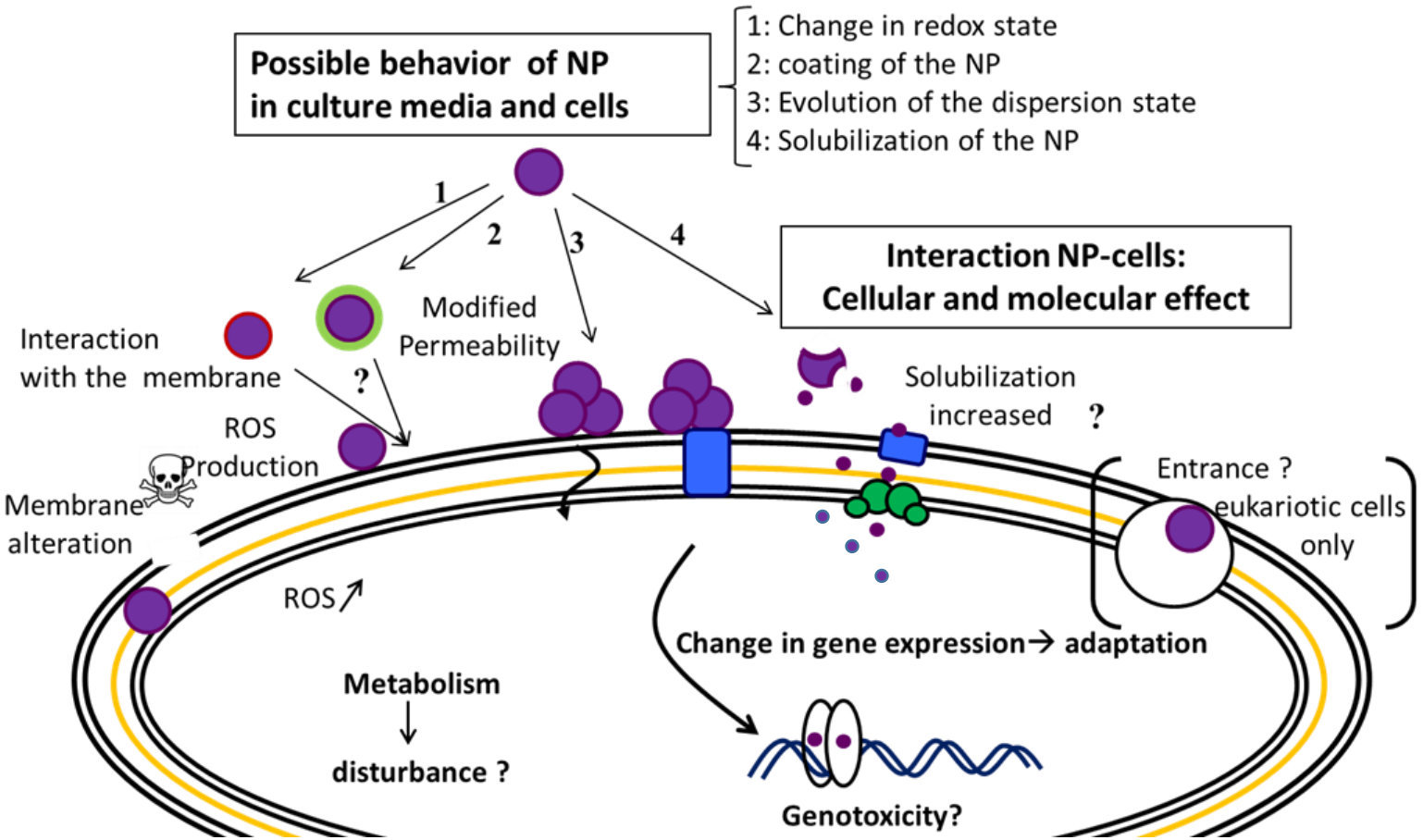
Interactions of Nanoparticles with media and cells



we postulated that metallic NP should interfere with metal homeostasis, which is closely related to oxidative stress

Adapted from Herlin, Michaud-Soret, Fauquant, Armand and Carriere, (2013) BioFutur (347) 39-41

Interferences between Nanoparticles and metal homeostasis

Labile metallic nanoparticles : CuO, ZnO, Ag..

cellular model: hepatocytes (HepG2) because metals end up in liver

1) Characterization of the NP in the medium
2) Dissolution studies
3) Cell viability
4) Q-PCR on genes involved in redox and metal homeostasis in subtoxic conditions
5) Visualization, localization, quantification (TEM; µXRF; XAS; ICP-MS)

- **Goal**

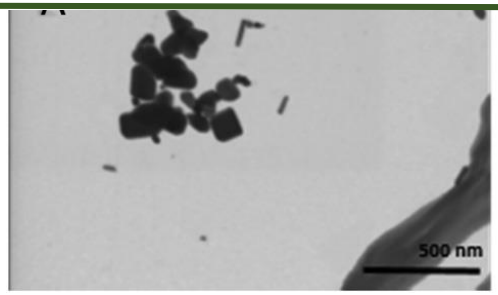
Decipher the mechanisms of disruptions at the cellular and molecular levels

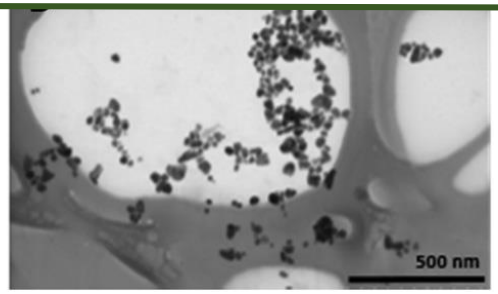
- **Main result:**

metallic NP interfere with metal homeostasis even in subtoxic conditions

but not always using the same mechanisms

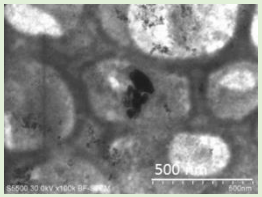
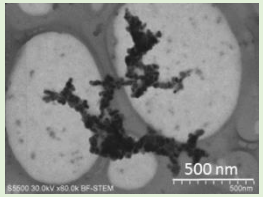
NP characterization solid/dispersion in medium by DLS, EM





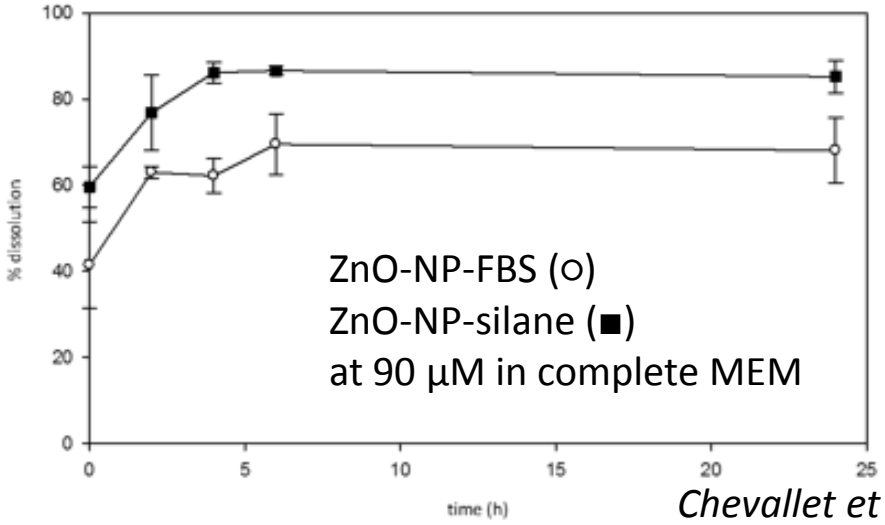
ZnO-NP case study

2 ZnO-NPs < 100nm from Sigma

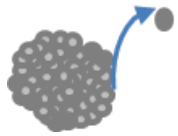
	ZnO-NP-SVF	ZnO-NP-silane
Size distribution of ZnO-NP in medium (DLS)	Multimodal, not measurable	Multimodal, not measurable
SEM images of ZnO-NP at **900µM** after 24h incubation in complete medium		

Best dispersion Conditions

(Cup-horn sonication)



Dissolution study



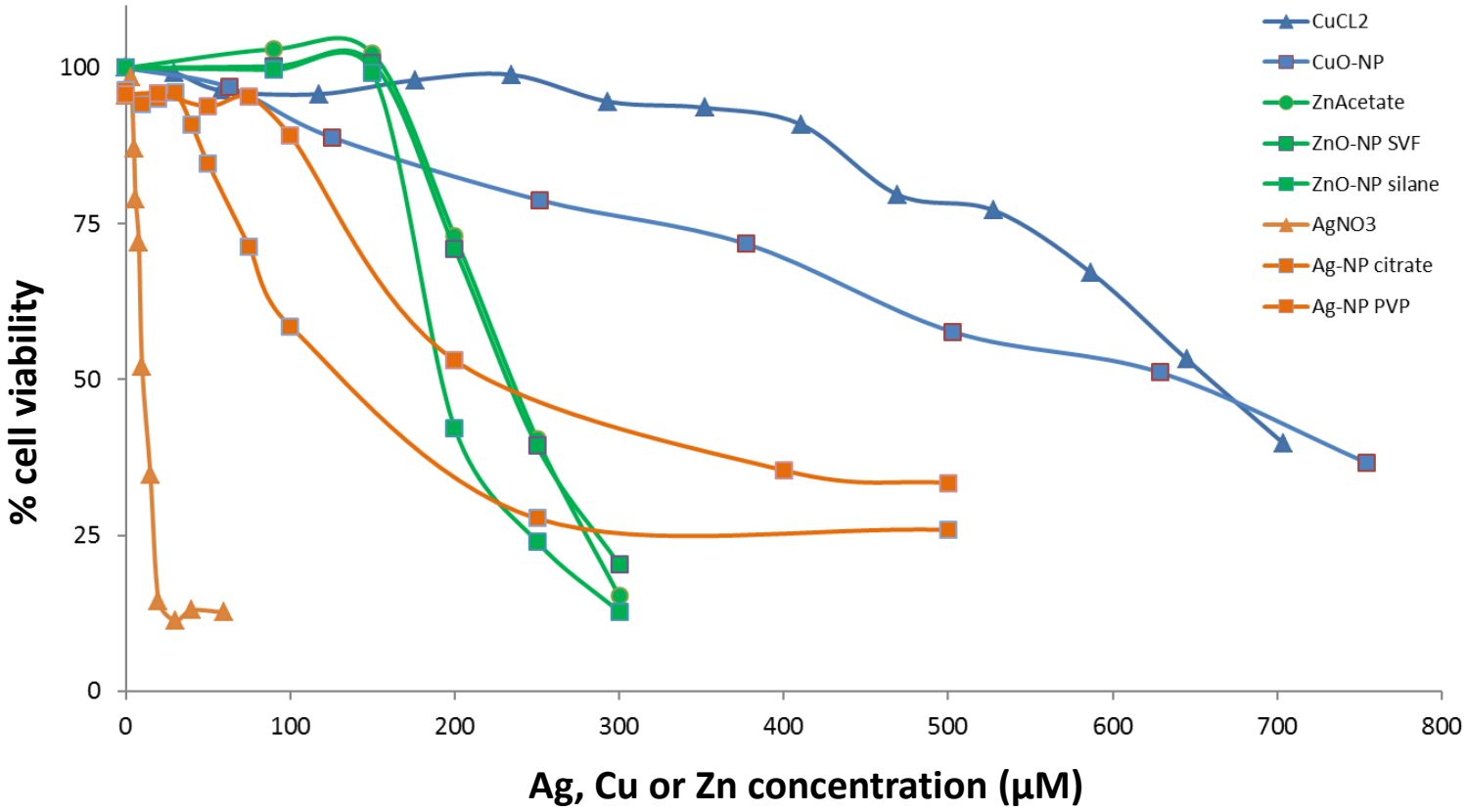
ions

(pH dependency)

Chevallet et al (2016) Nanoscale, 8(43):18495-18506 , just published

Hepatotoxicity of ZnO-NP, CuO-NP and Ag-NP

HepG2 cells treated with different metal nanoparticles for 24h



Ag and Cu → Nano-effect

Zn → Fast dissolution

CuO-NP more toxic than Cu salt → Trojan horse

Ag-NP less toxic than Ag salt →Protective

Salt and NP same effect

Cellular responses induced by CuO-NP, ZnO-NP and Ag-NP

mRNA fold increase after 6 h exposure

sub-toxic conditions	CuO-NP PVP	$CuCl_2$	ZnO-NP silane	ZnO-NP SVF	ZnAcetate	Ag-NP PVP	Ag-NP citrate	$AgNO_3$
	60 µM		90 µM			100µM	50 µM	25 µM
HSPA6	6.0	1.2	4.6	2.1	1.9	435	994	553
HMOX	13.8	1.9	4.1	2.9	4.6	34	46	34.5
MET	38.4	11.1	46.9	44.5	56.4	362	539	30.5
GCLM	1.6	2.1	4.4	3.7	4.1	12	13	-
ZnT1	2.2	1.6	4	3.8	3.4	6	9	-

In all cases → Met, GCLM, ZnT1 overexpression → Metal homeostasis control
→ Weak oxidative stress response → HMOX only (not SOD or CAT)

Specificities: → ZnO-NP same result than Zn salt

→ CuO-NP more effect than Cu salt

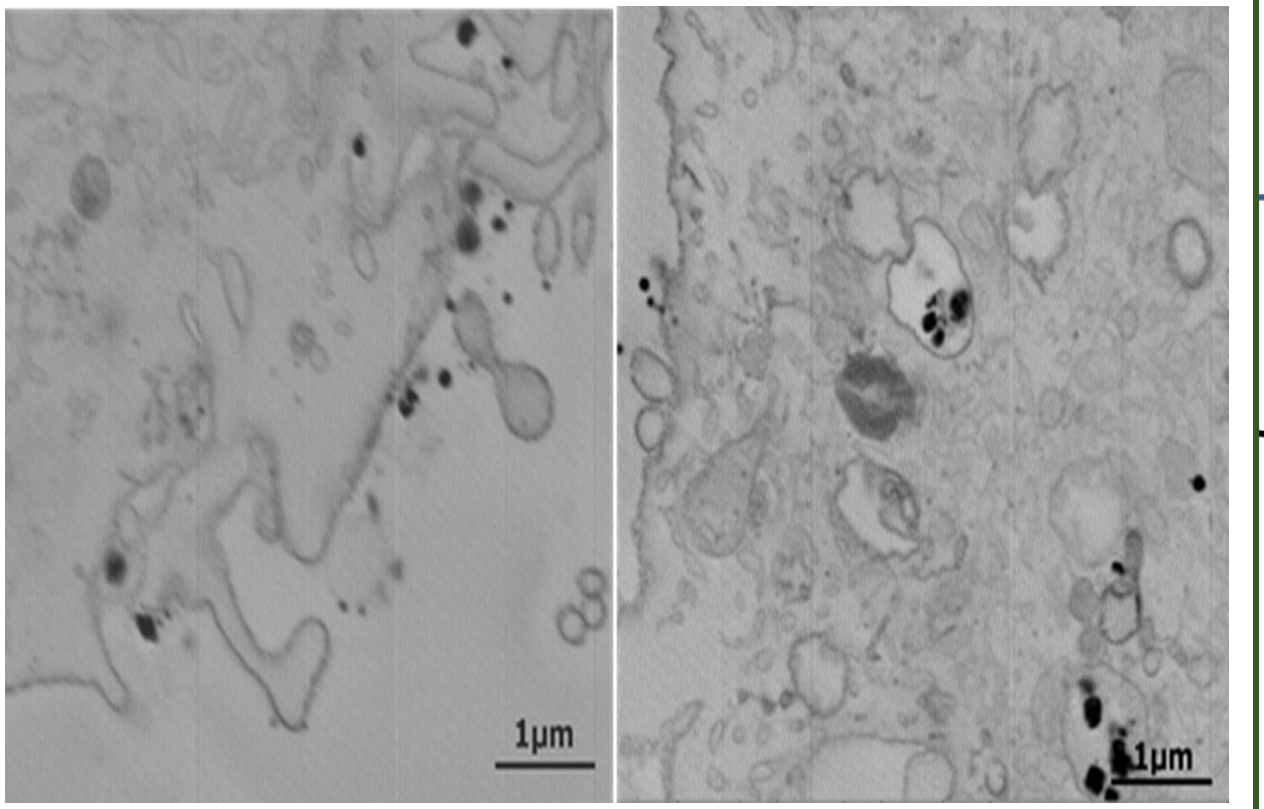
→ Ag stronger expression for all targets
Very high overexpression for HSPA6--> protein folding problem

HSP6: heat shock protein
HMOX: heme oxygenase
MET : Metallothionein
GCLM: in GSH synthesis
ZnT1: Zn exporter

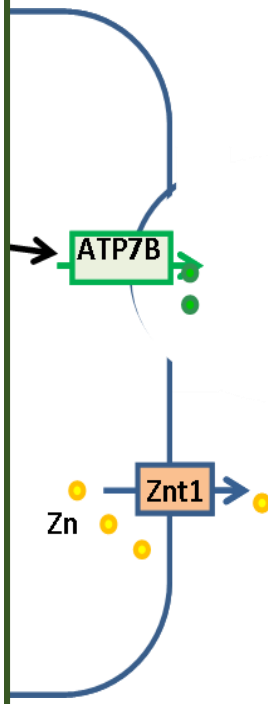
CuO-NP trigger disruption of Cu and Zn homeostasis under sub-toxic conditions

NP-CuO
a **Trojan**
to bring co
vesicles wh
condit
may favo
release ir

60µM [Cu] in



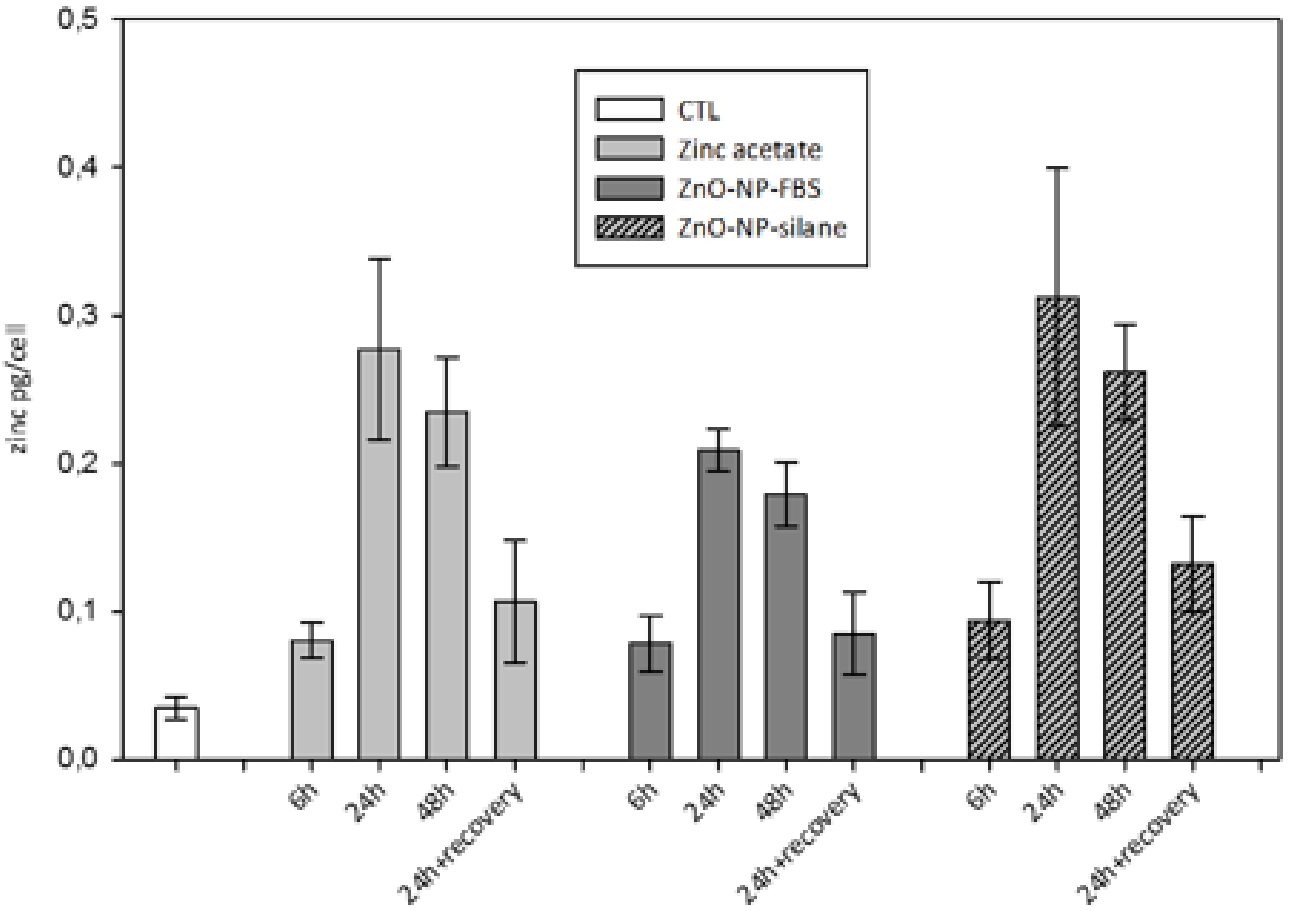
HepG2 cells after 24h-incubation with CuO-NP 250 µM



Cuillel *et al* (2014) *Nanoscale*, 6: 1707

Cellular zinc content measured by ICP-AES

after 6 h, 24 h and 48 h incubation with 90 µM Zn compounds for 24 h followed by a 24 h recovery.



protection by EDTA against Zn toxicity → Zn dissolution in the medium

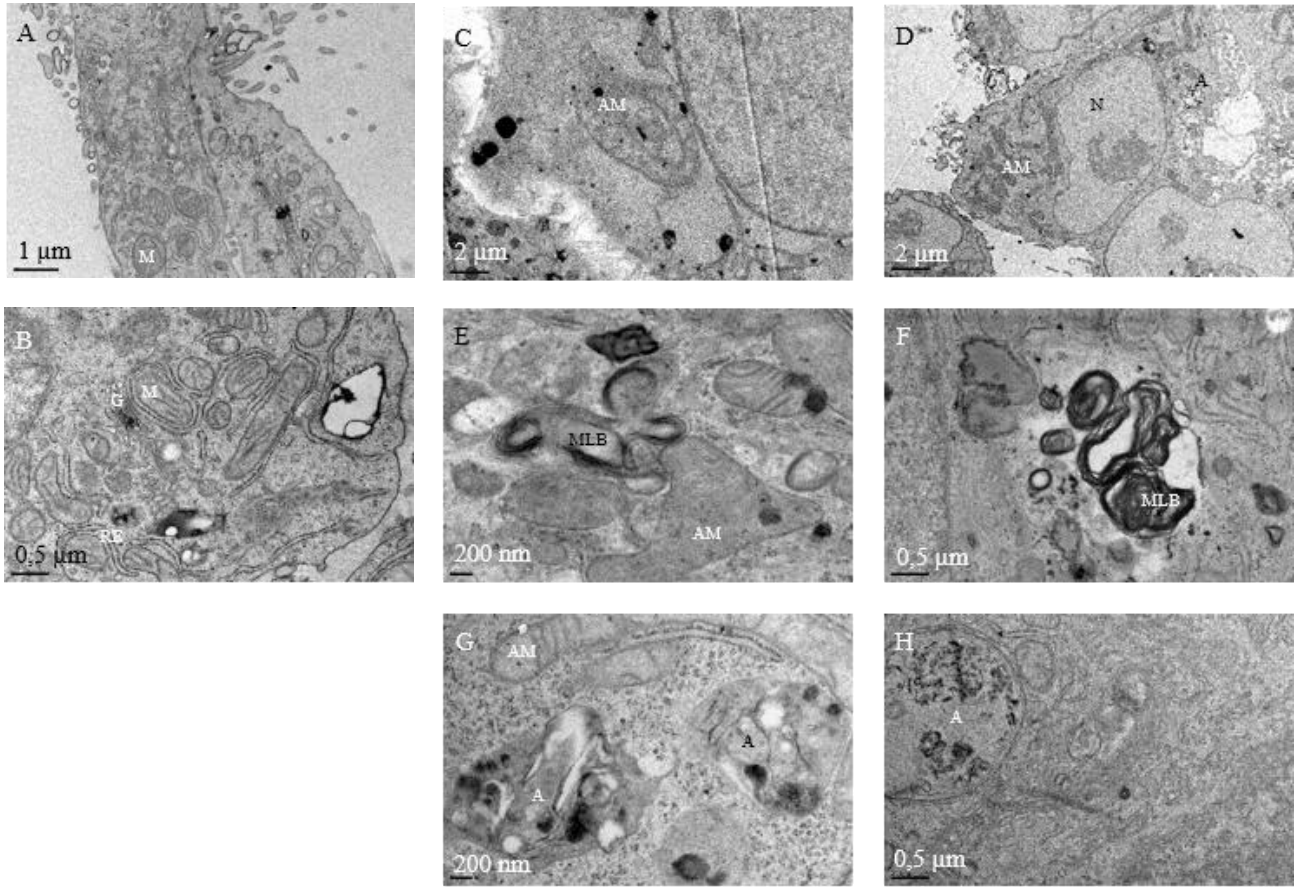
Chevallet et al (2016) Nanoscale, 8(43):18495-18506 , just published

TEM observation of ZnO-NP treated HepG2 sections

control

ZnO-NP-FBS
90 µM, 6 h

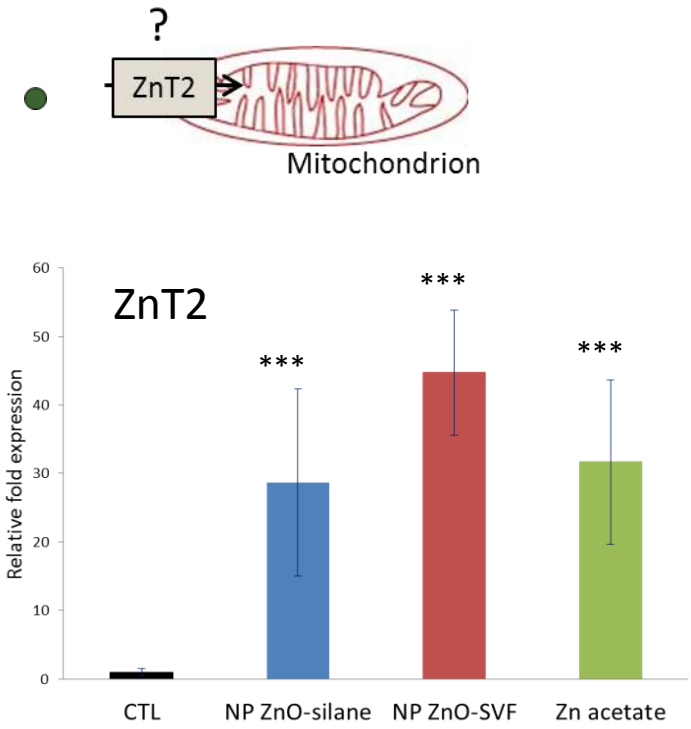
ZnO-NP-silane
90 µM, 6 h



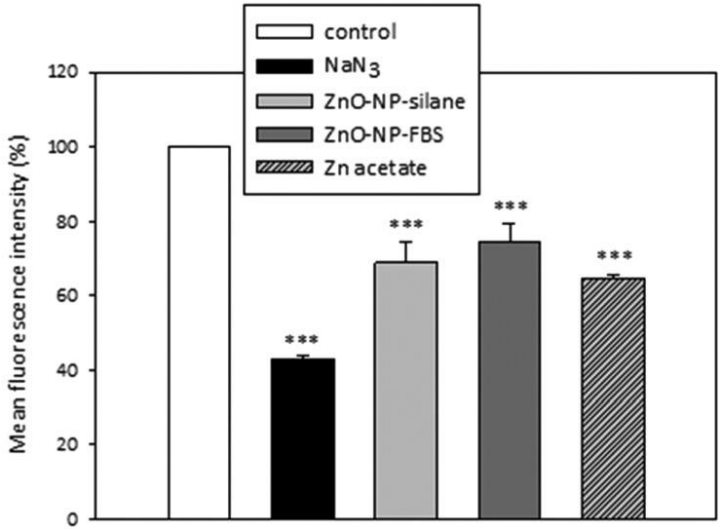
M: mitochondria, N: nucleus, G: Golgi apparatus, RE:endoplasmic reticulum, AM: abnormal mitochondria, MLB: multilamellar body, A: autophagosome.

Chevallet et al (2016) Nanoscale, 8(43):18495-18506 , just published

ZnT2 upregulated and decrease mitochondrial transmembrane potential



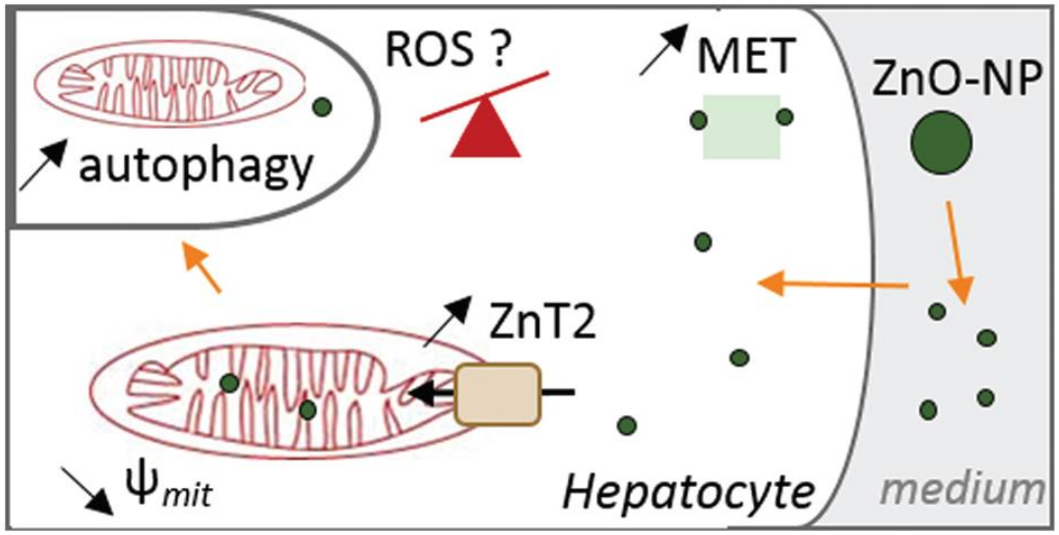
6 h incubation with Zn compounds
at **90 µM (sub toxic dose)**



after 24 h incubation
with 90 µM Zn compounds

**Suggesting a storage of zinc in mitochondria,
mitochondria alterations**

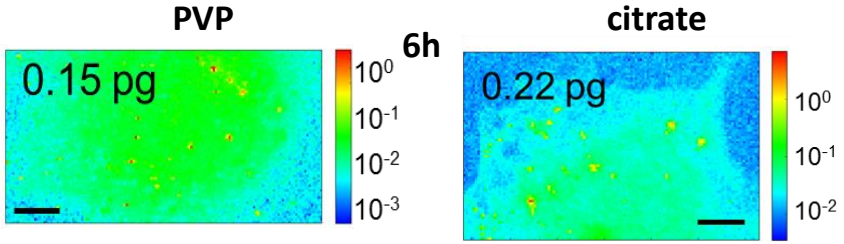
Sub-toxic doses of both ionic and nanoparticulate forms of zinc induce zinc homeostasis disruption, mitochondria alterations and increased autophagy



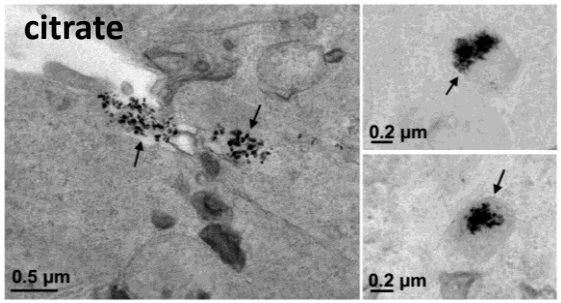
Ag-NP Conclusion (as described in G. Veronesi talk)

- Ag-NP dissolve intracellularly in acidic vesicles

single cell Analysis !

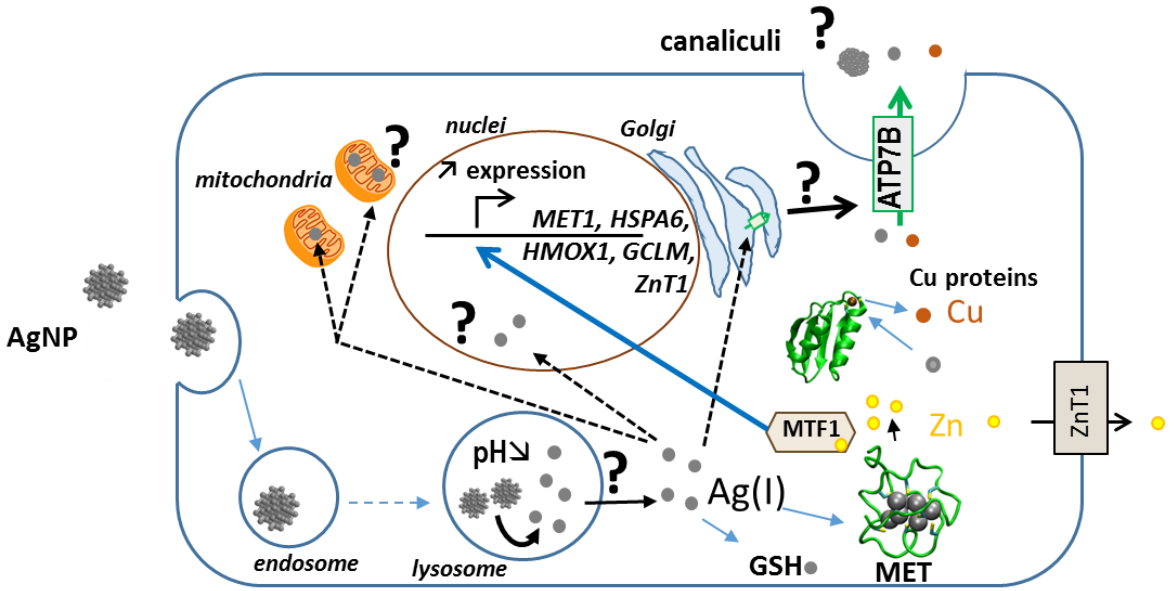


nanoXRF on ID16B and TEM



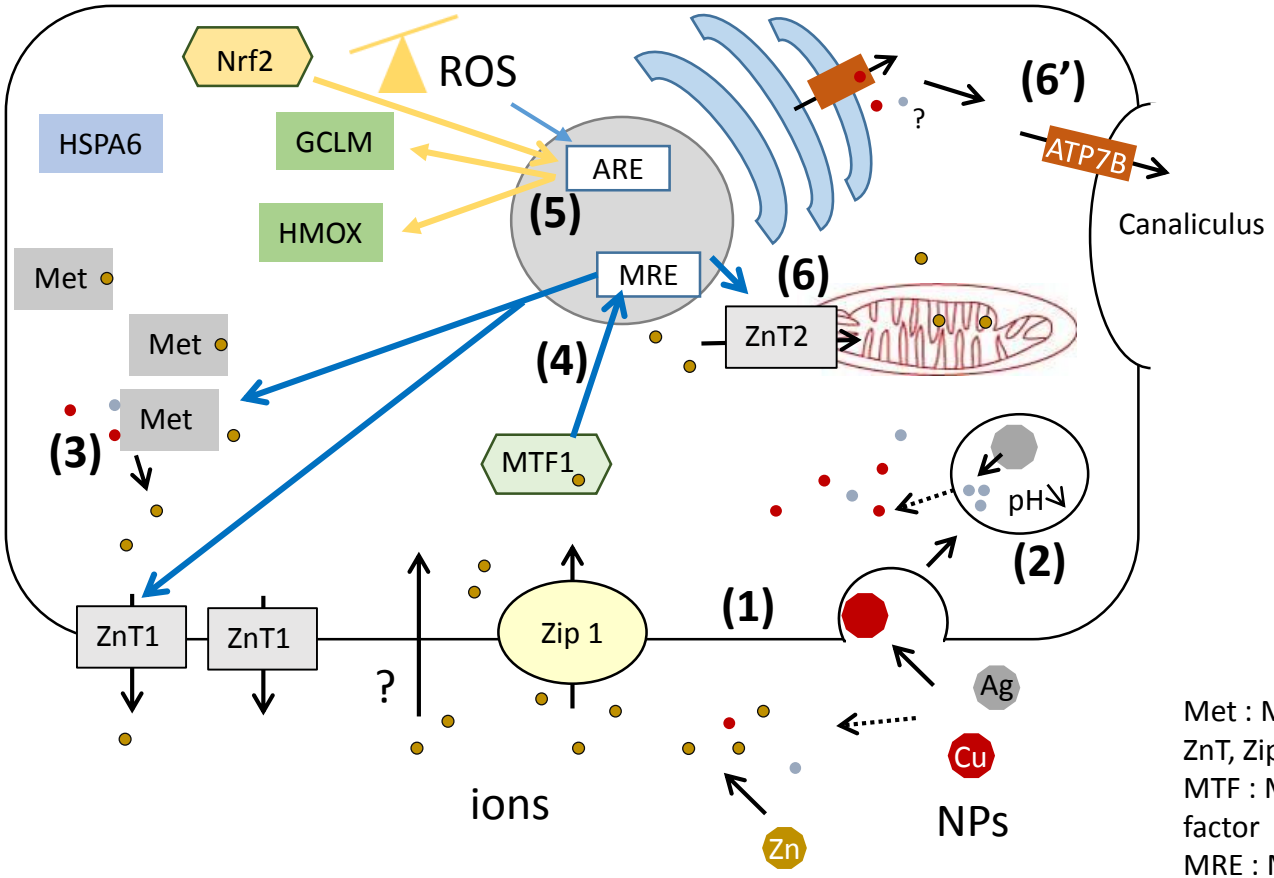
- redox and metal homeostasis disruptions

- Ag(I) forms complexes with thiol-containing biomolecules as AgS_2 (GSH) and AgS_3 (Met) complexes



Veronesi, Deniaud *et al* (2016) *Nanoscale,* ,8(38):17012-17021

General conclusion : mechanisms of disruptions by labile MeNPs in hepatocytes



Met : Metallothionein
ZnT, Zip : Zinc transporter
MTF : Metal regulatory transcription factor
MRE : Metal response element

M Chevallet, G Veronesi, A Fuchs, E Mintz , I Michaud-Soret & A Deniaud (2016) BBA review submitted

Conclusions : from predictive toxicology to safer-by-design

Predictive toxicology

- Interferences between metal homeostasis and metallic NP
- Nano-effect due to endocytosis and dissolution (NP-CuO)
- no general correlation between NP and cytotoxicity

  Metallothionein biomarker of metal ion exposure AgNP>ZnNp & CuONP
  release of Zn(II)→MTF activation/translocation→ Zn(II) exporter Znt1
  GSH major player
  late induction of a moderate oxidative stress

  Labile NP→ higher inflammatory responses than other NP (literature)
  → Redox and metal homeostasis disruption

- Biological chelators assisted-dissolution of metallic NP (AgNP; I. Worms talk)

Perspectives : Safer-by-design approach

- Control of the dissolution by the coating
- Bio-inspiration for eco-conception

(posters Marchioni S3.2-P2 & Laisney S3.2-P4)

Serenade
Safe(r) Ecodesign Research and Education applied to Nanomaterial DEvelopment.

Acknowledgments

lcbm

BioMet team

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I. Worms

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Serenade Safe(r) Ecodesign Research and Education applied to nAnomaterial DEvelopment.

Institut of Metals in Biology of Grenoble

Serenade
Safe(r) Ecodesign Research and Education applied to NAnomaterial DEvelopment.

7th International IMBG meeting on Metallic Nanoparticles: Health, Environment, and Safer-by-Design

September 11 – 15th 2017
Villars de Lans, France

Advanced courses
Sept 11 – 12th

Practical methodologies and analyses:

- Analytical chemistry, imaging, spectroscopy & molecular biology techniques
- NPs in complex media: tricks and pitfalls
- Safer-by-design approach

Conference
Sept 13 – 15th

Behavior, fate and impacts:

- in the environment
- on health

Safer-by-design, coating and surface reactivity

Applications (health, agriculture, textile, ...)

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Scientific committee:
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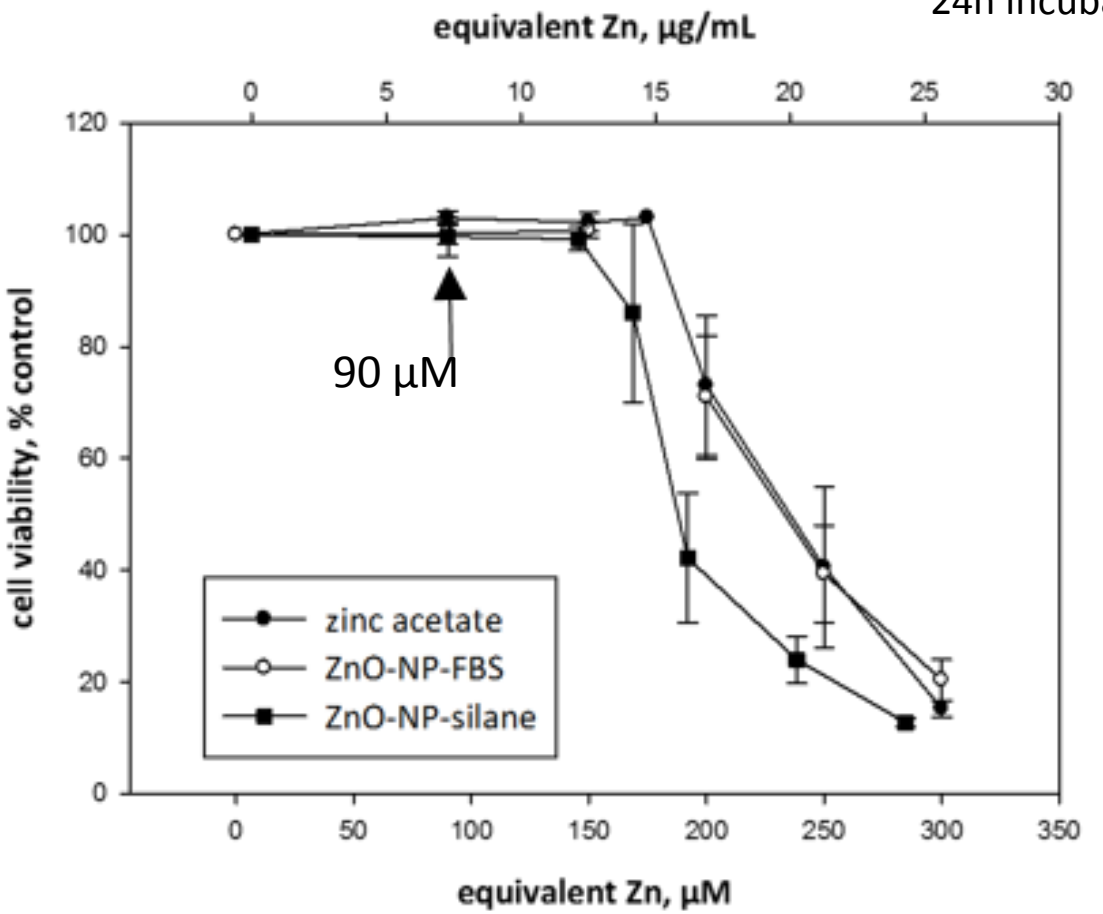
Organizers:
Géraldine Sarret, Marie Carrière & Isabelle Michaud-Soret

website: http://imbg-grenoble.fr/

Study of ZnO-NP toxicity

Viability of HepG2 cells after a 24h incubation



Sub-toxic dose

90 µM

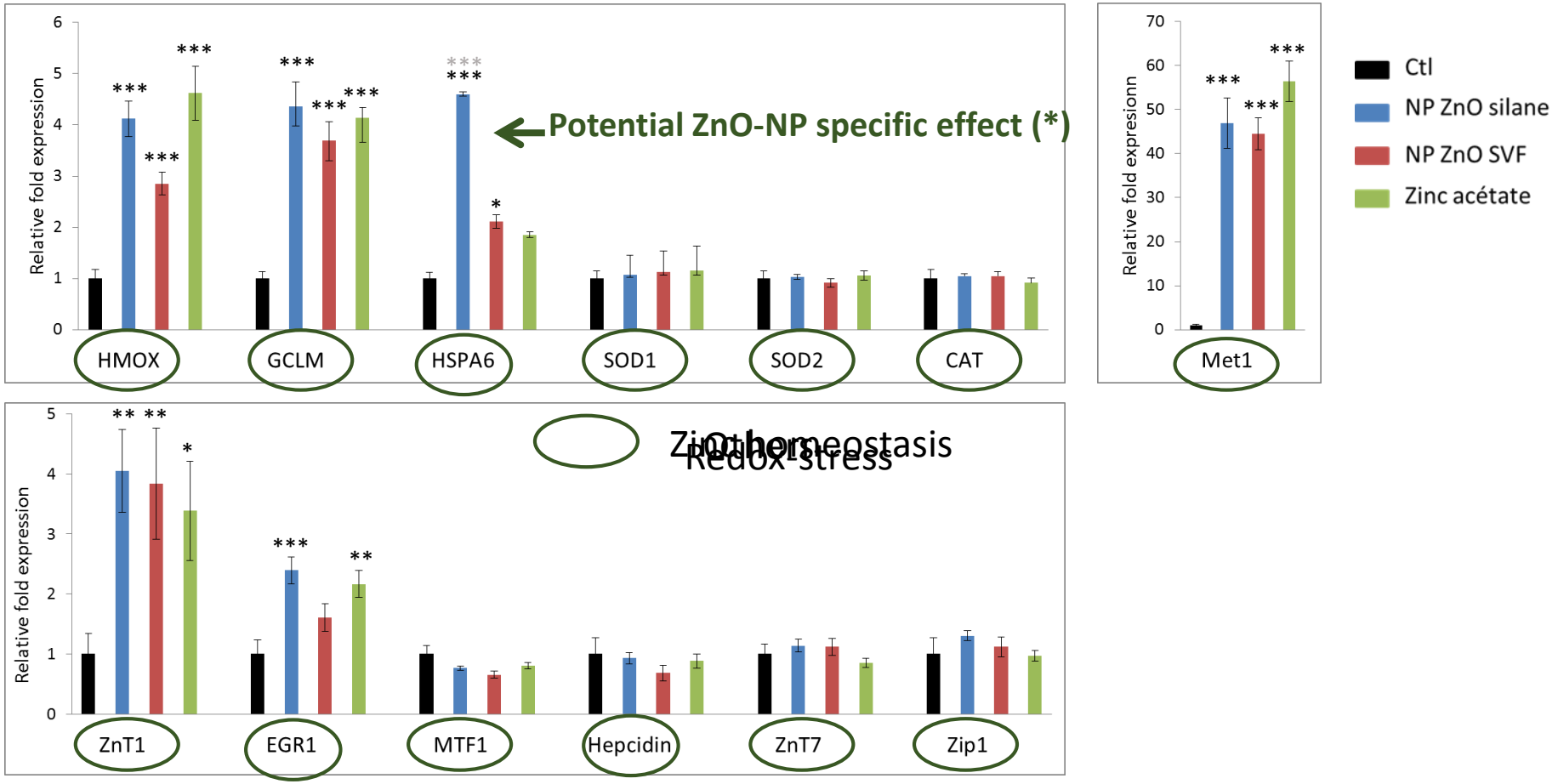
(7.3 µg mL^{-1})

gene expression studies

ZnO-NP Toxicity is equivalent to Zinc salt in Hepatocytes

mRNA expression of Zn- and redox-stress genes

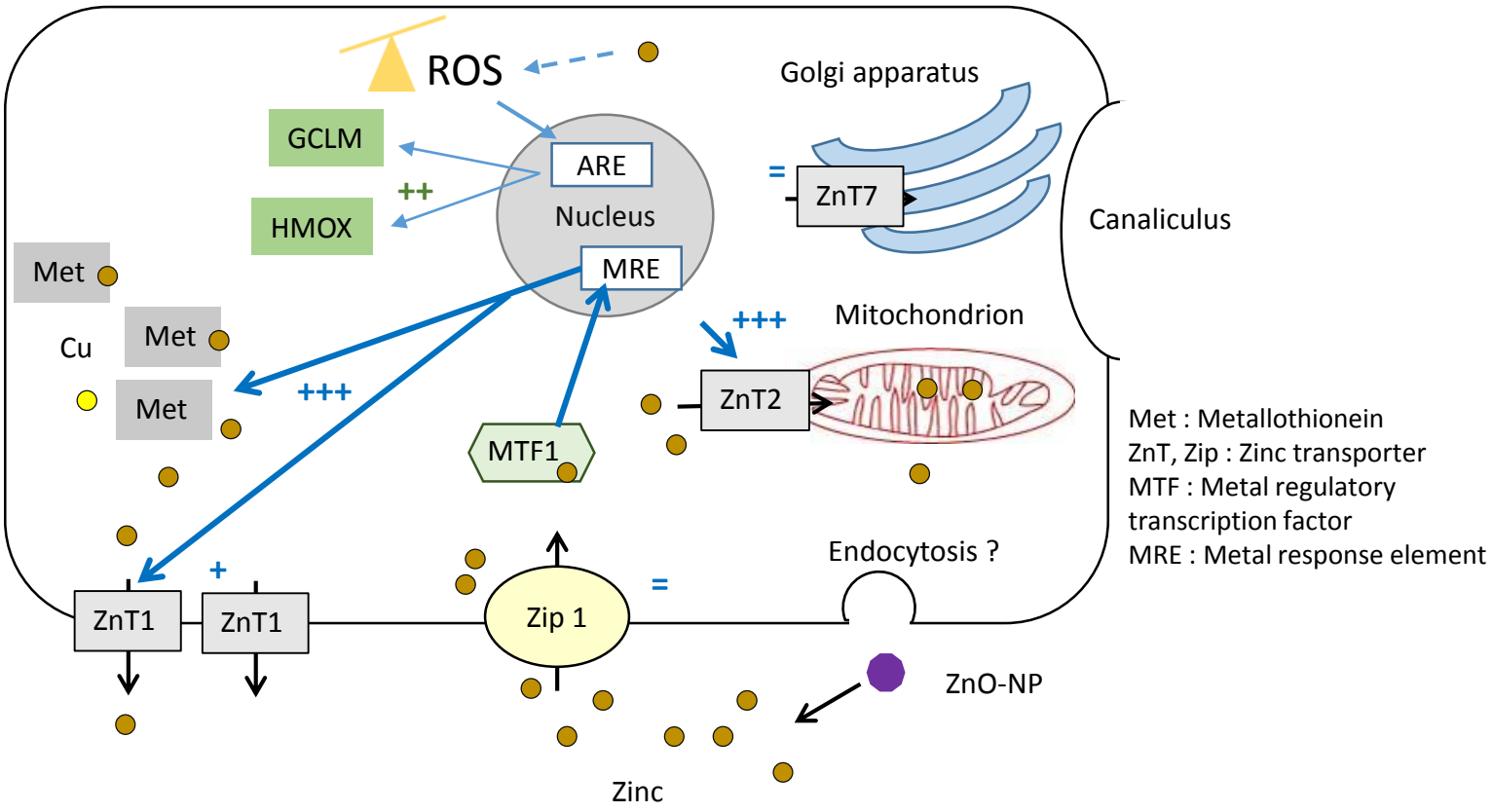
after a 6 h incubation with Zn compounds at **90 µM (sub toxic dose)**



Globally similar results between NP and salt

(*) Moos et al (2011) *Metallomics* 3(11), 1199-211

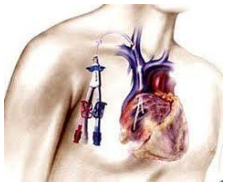
ZnO-NP Conclusions



ZnO-NP toxicity seems to a great extent a direct consequence of zinc dissolution and subsequent increase in intracellular and mitochondria zinc concentrations.

Nanomaterials in commercial products

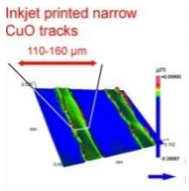
Ag-NP



1000 to 5000 tonnes/year (2015)
Woodrow Wilson Institut

>442 products containing Ag-NP

CuO-NP



ZnO-NP

TiO_2-NP

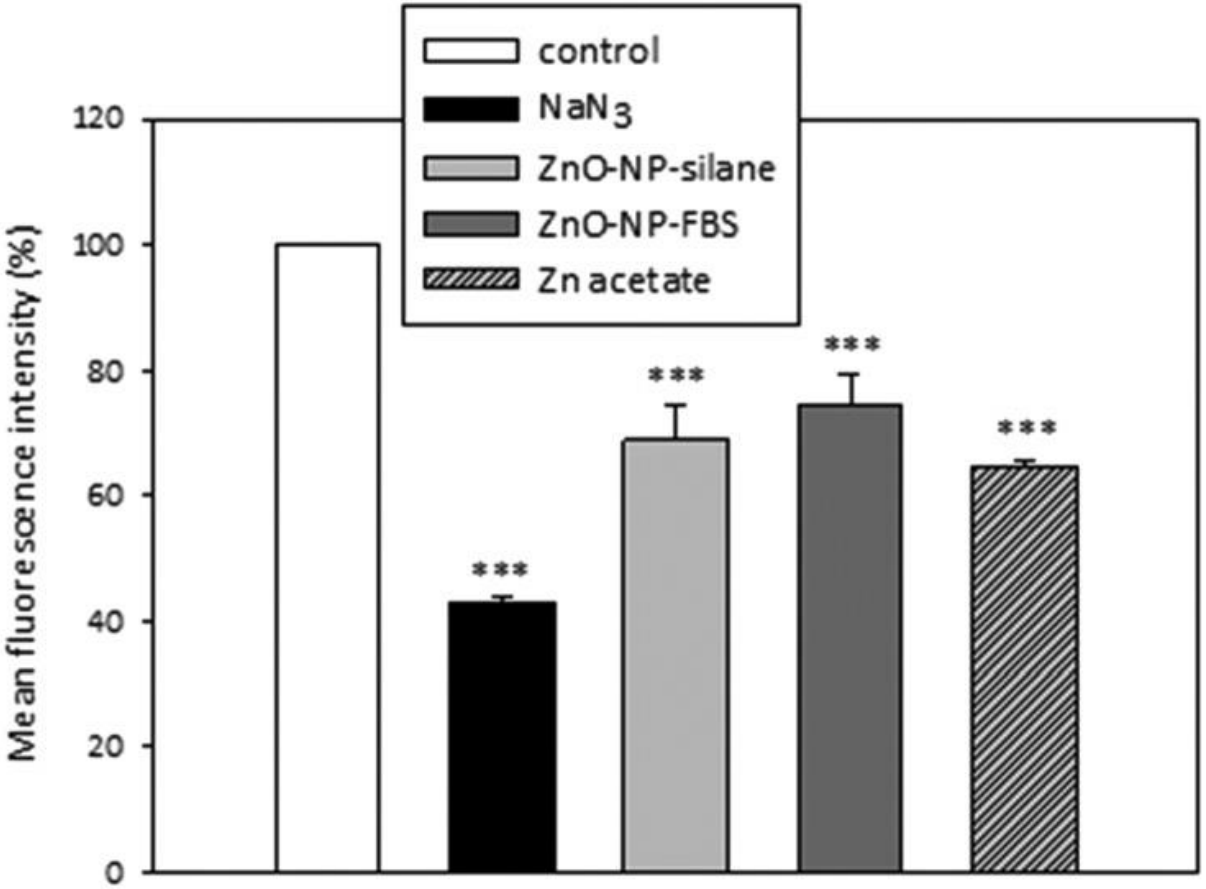
Weir et al., Env.science & Tech., 2012
Wilson Center (USA) (2013)
www.nanotechproject.org/cpi

Increased exposure of environment & humans to NP

→ mechanistic studies at the molecular and cellular levels were essential for predictive toxicology

Decrease mitochondrial transmembrane potential

after 24 h incubation with 90 µM Zn compounds

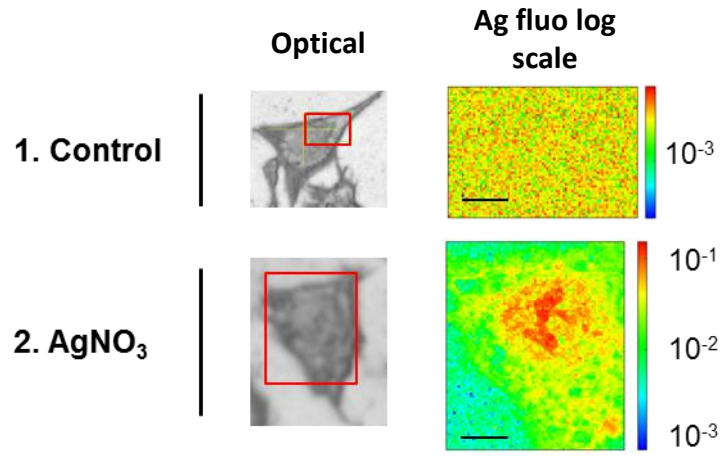


Amount of rhodamine 123 internalized in the cells expressed as a percentage of the mean fluorescence of control cells (three independent experiments). ***: $p < 0.001$.

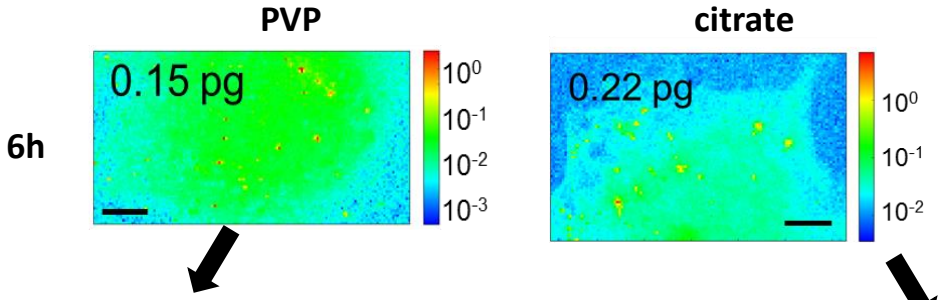
Chevallet et al (2016) Nanoscale, 8(43):18495-18506 , just published

AgNP intracellular dissolution (as described in G. Veronesi talk)

X-ray fluorescence microscopy on ID16B on whole cells coupled with cell section observed by TEM

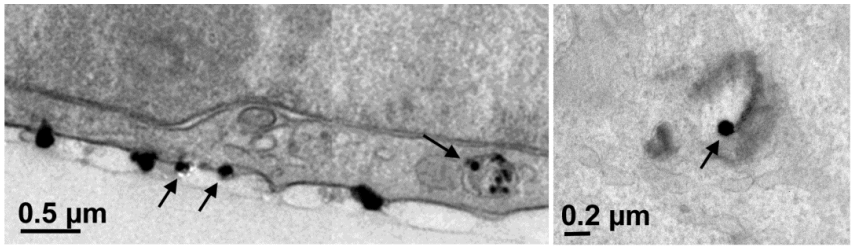


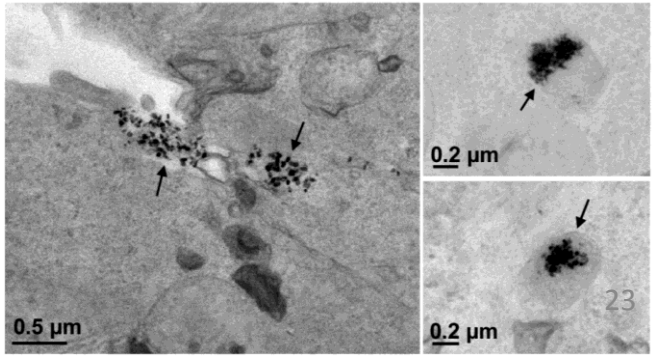
High sensitivity → allows Ag(I) detection



Ag hot spots → Ag-NP
as well as diffuse signal → Ag(I) species

Larger hot spots with citrate → vesicles with agglomerated Ag-NP

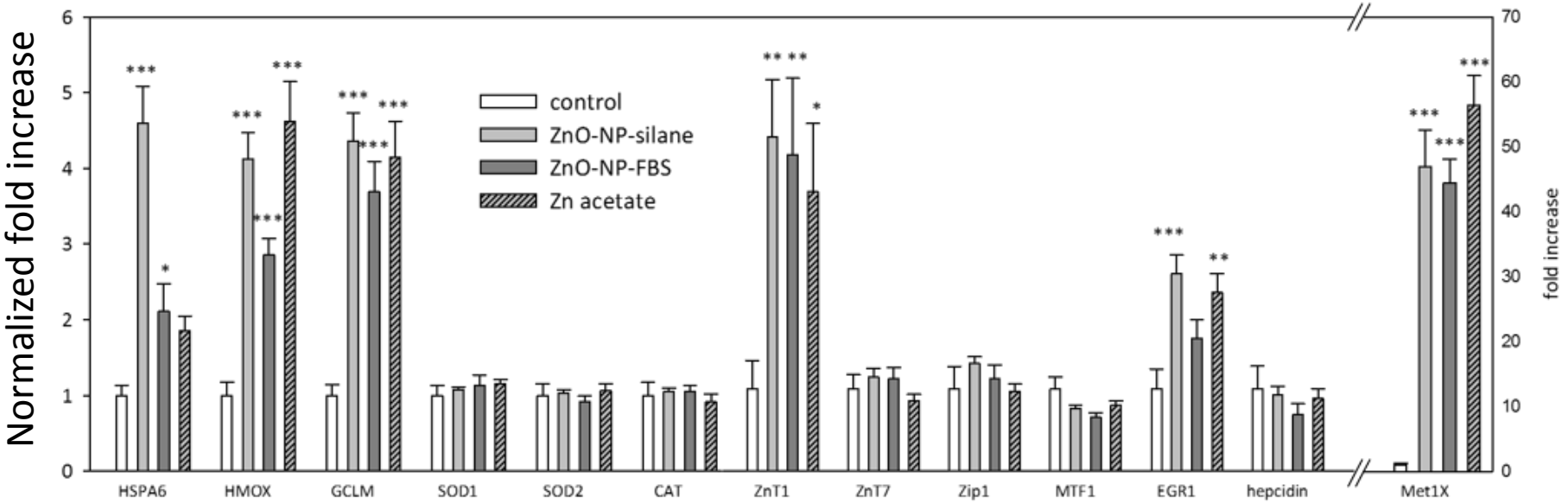


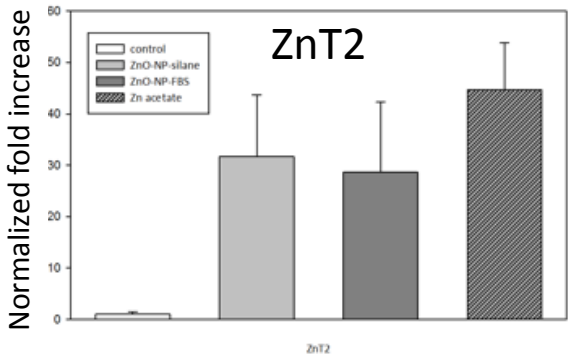


Veronesi, Deniaud *et al* (2016) *Nanoscale,* ,8(38):17012-17021

mRNA expression in HepG2 by quantitative PCR analysis

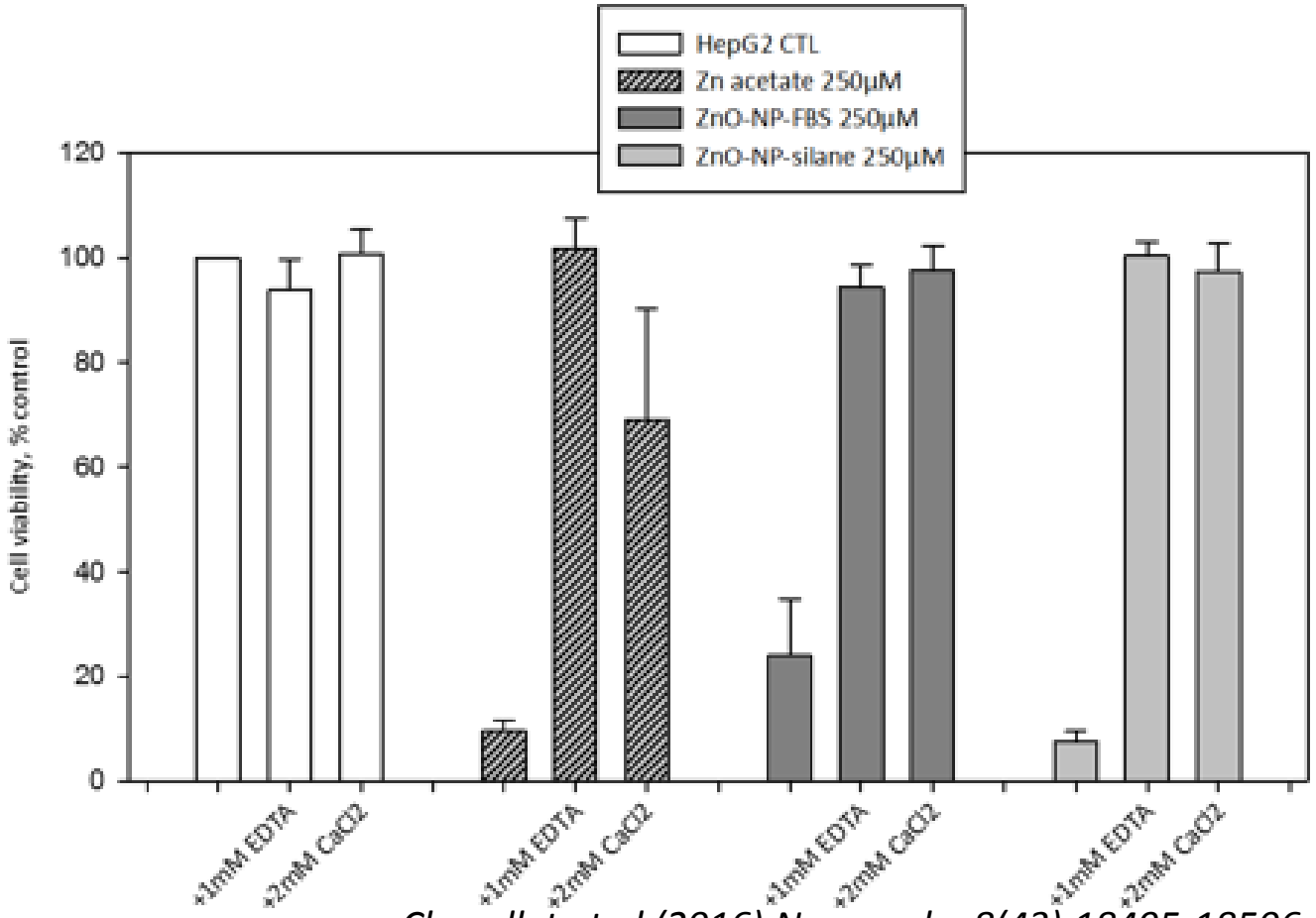
after a 6 h incubation with Zn compounds at 90 µM.





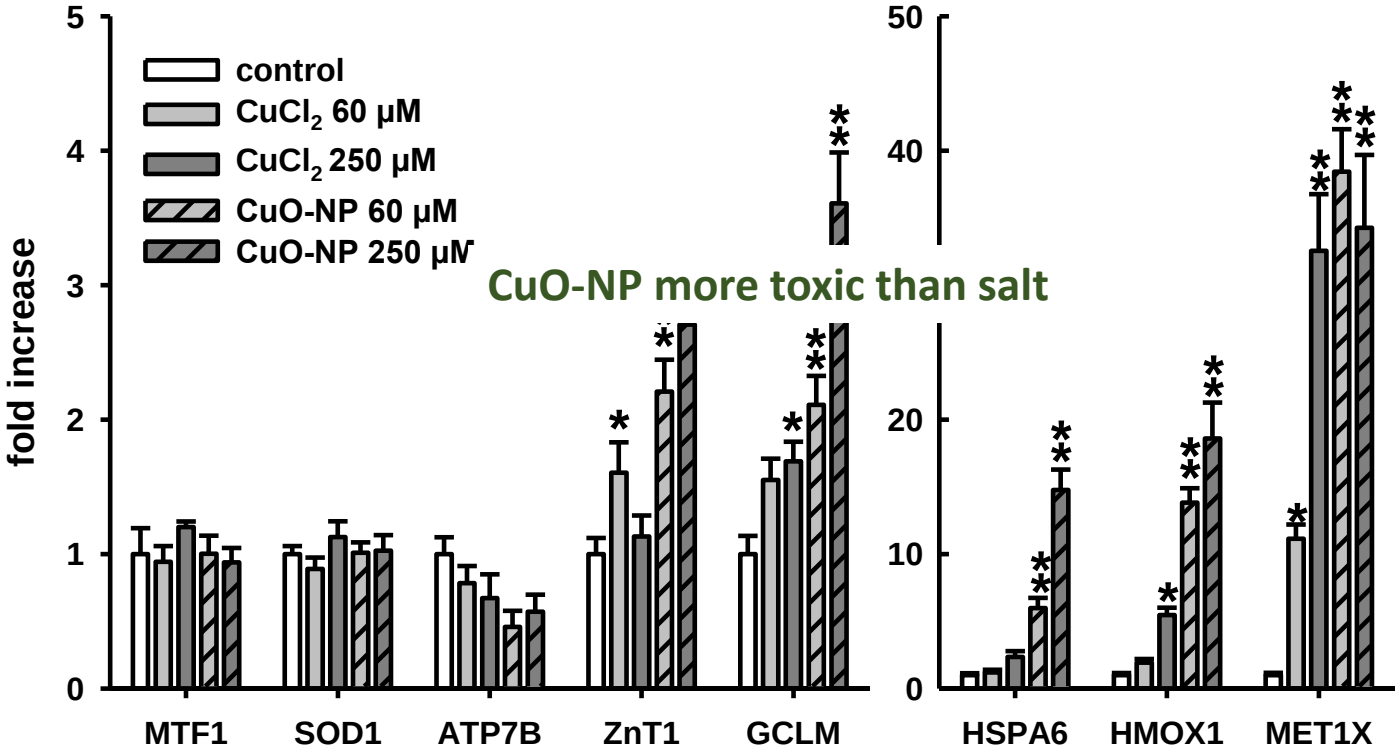
Protection by EDTA or Ca2+ against Zn toxicity

HepG2 pretreated for 15 min with 1 mM EDTA or 2 mM CaCl2 before adding Zn compounds at the toxic dose of 250 µM for 24 h.
EDTA or CaCl2 treatment alone had no effect on viability of HepG2 cells.



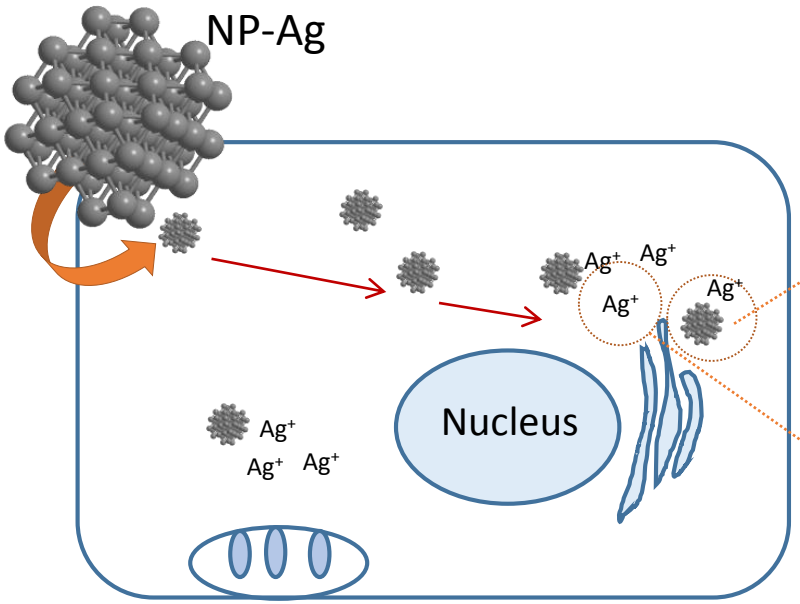
Chevallet et al (2016) Nanoscale, 8(43):18495-18506 , just published

Comparison with CuO-NP

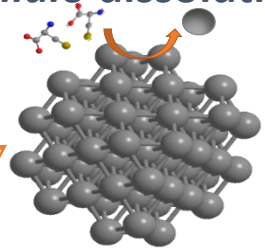


CuO-NP more toxic than salt

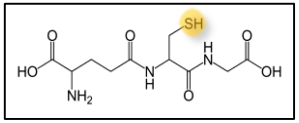
Influence of copper chelating proteins on the dissolution of silver nanoparticles and their toxicity



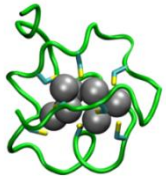
***In cellulo* dissolution**



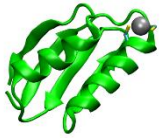
Ag^+ trafficking

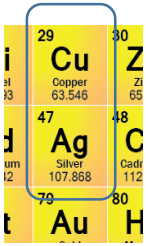


Intracellular Glutathione (GCLM)

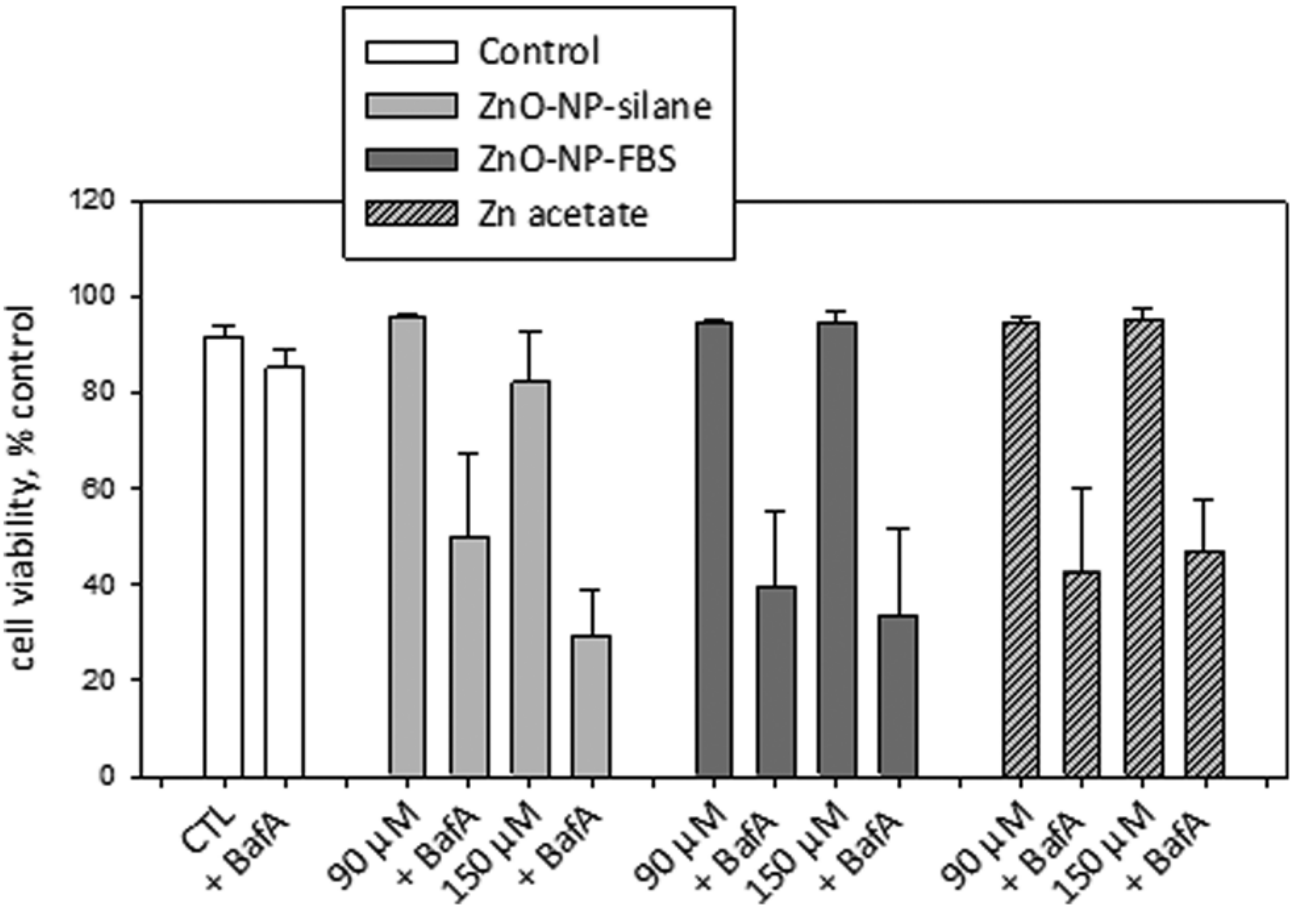


Metallothioneins (Cu storage) (Met)





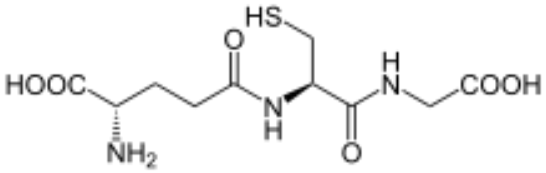
Effect of bafilomycin A on Zn toxicity

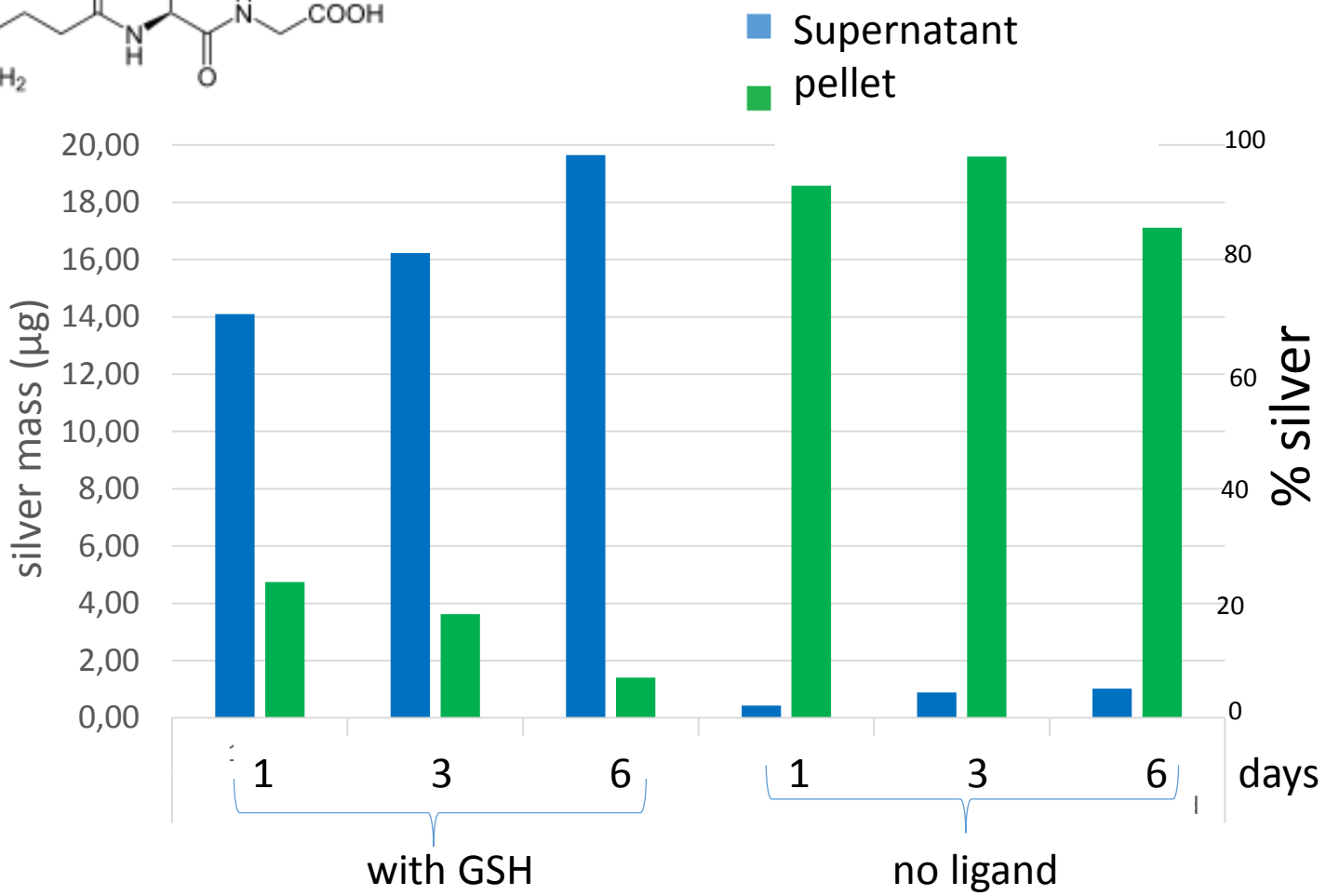


HepG2 cells were pretreated 1 h with 100 nM Baf A before adding Zn compounds for 24 h at subtoxic doses of 90 and 150 µM

Dissolution of Ag-NP: silver release quantified by ICP-AES

Ag-NP coated citrate





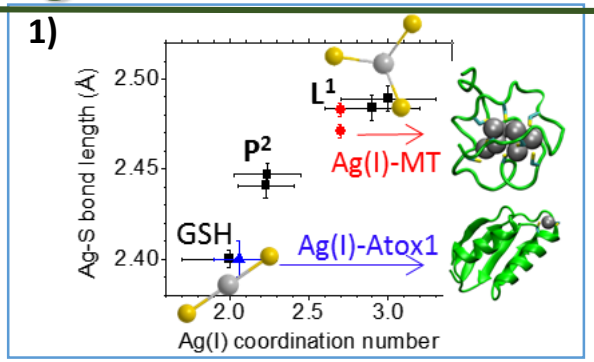
X-ray Absorption and fluorescence to understand the fate and toxicity of NP-Ag

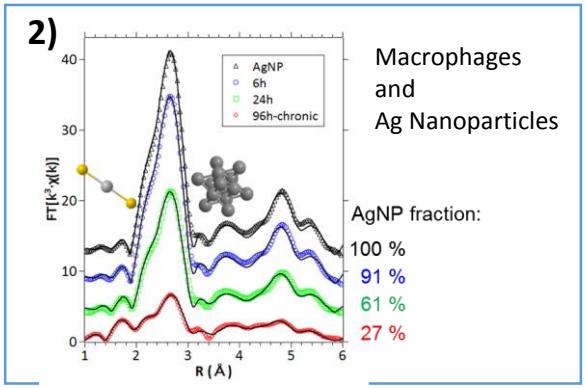
Main results

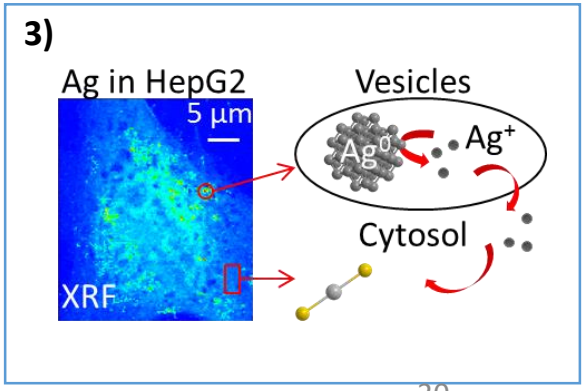
1) Correlation coordination number and d(Ag-S) for Ag-complex with bioinspired ligands and Ag-biomolecules containing 1 to 20 thiols (GSH, Atox1, metallothioneins (MeT))

2) Simultaneous measurements *in cellulo* of dissolved Ag(I) and remaining NP-Ag part
Ag(I) is mainly bound to intracellular GSH in macrophages and also to MeT in hepatocytes (HepG2)

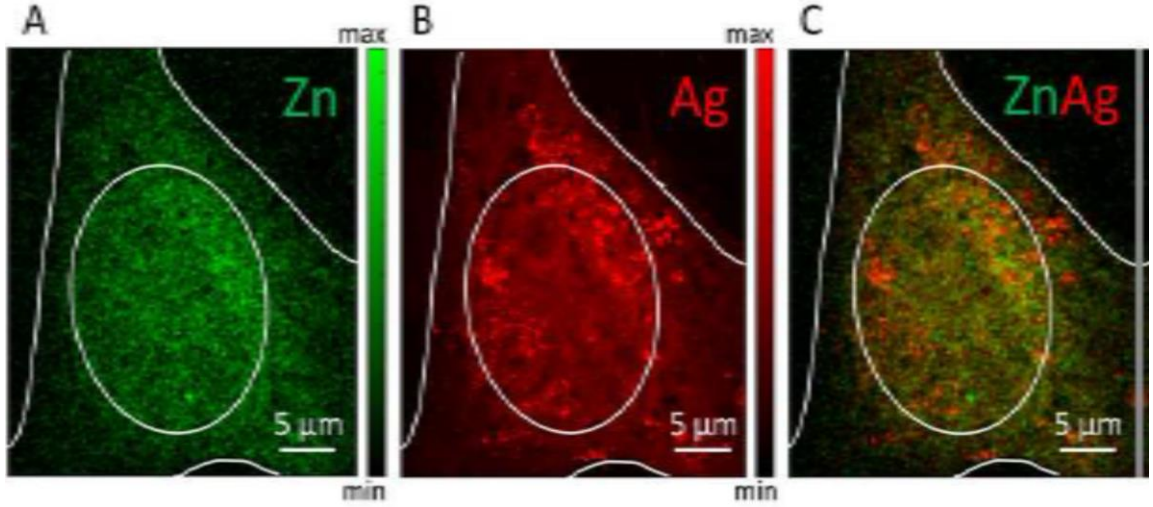
3) Detection of dissolved Ag(I) in a unique cell thanks to the µXRF on ID16B (exceptional sensitivity of **few attograms /pixel of 70x70nm²**) as a function of the coating





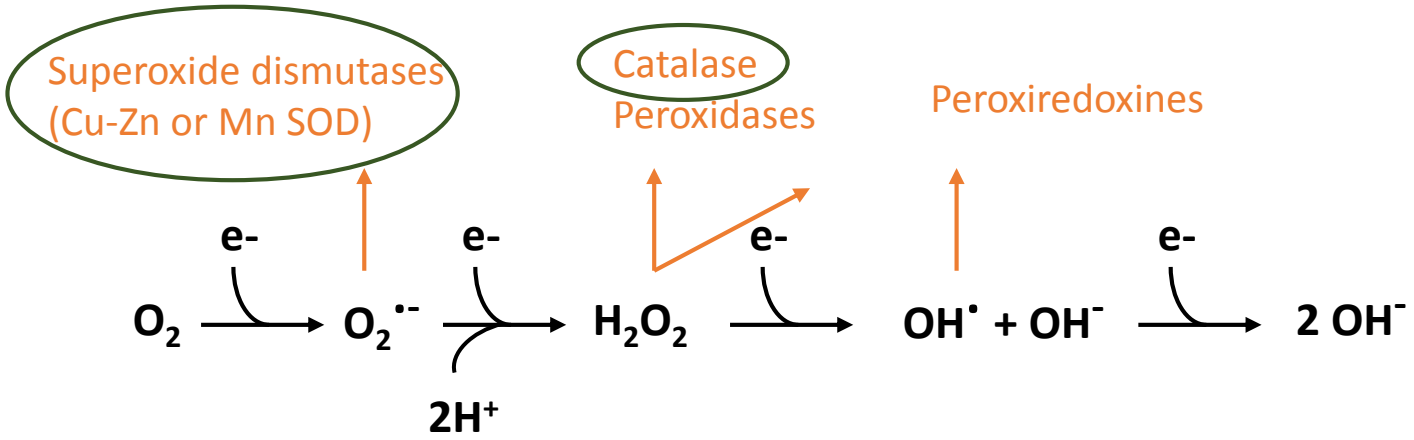


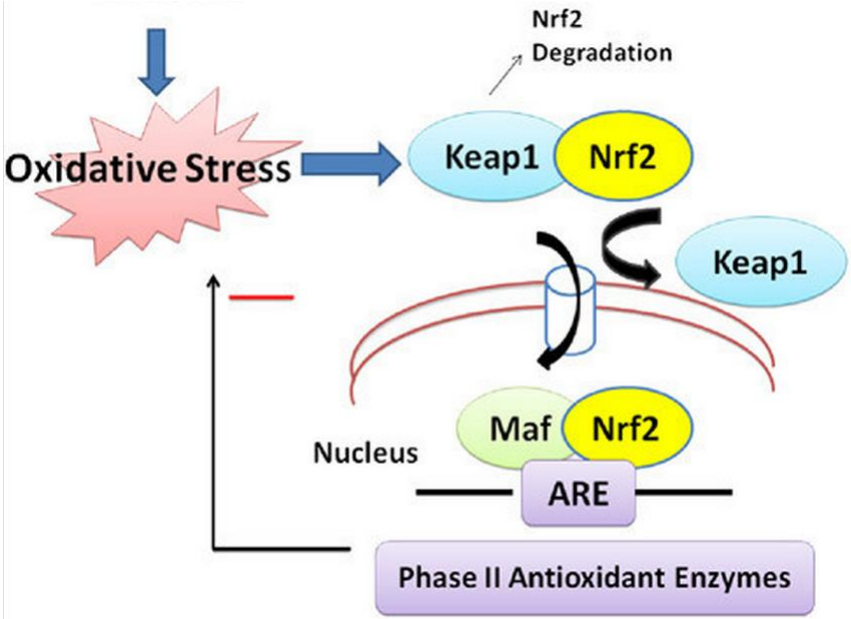
Veronesi et al. *Inorg. Chem.* (**2015**), 54, 11688.
Veronesi et al. *Nanoscale* (**2015**), 7,7323
Veronesi , Deniaud et al, **(2016)** *Nanoscale*

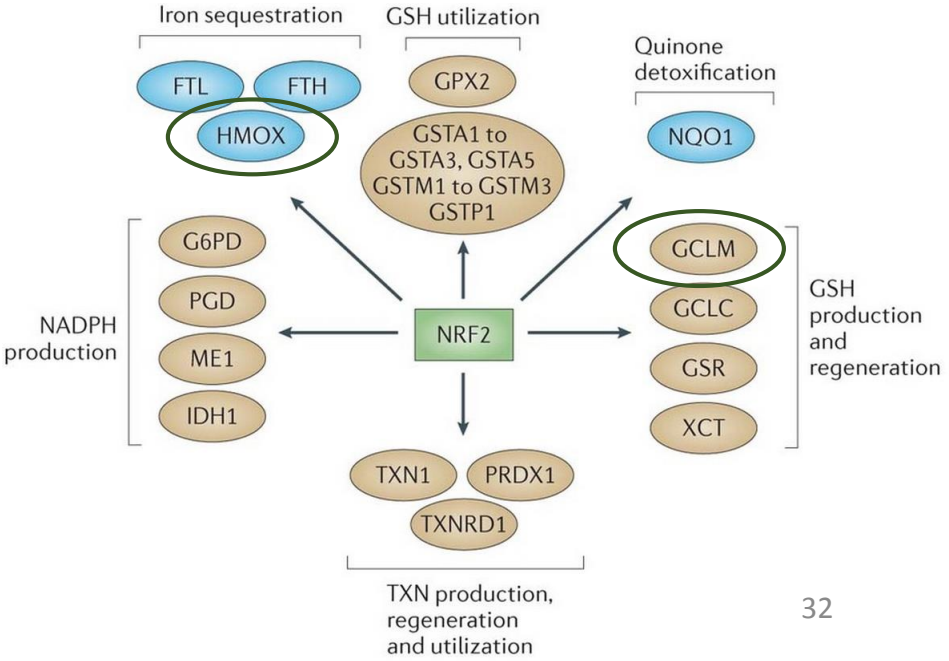


X Ray Fluorescence elemental images highlighting the distribution of
(A) Zn and (B) Ag, and (C) their co-localization in a single hepatocyte (HepG2) exposed for 24 h to citrate-coated Ag-NP.
Images were acquired in the X-ray nanoprobe ID16B-NA of ESRF
Interestingly, both particulate (intense Ag hot spots) and ionic forms (diffuse signal) of silver could be visualized with XRF.

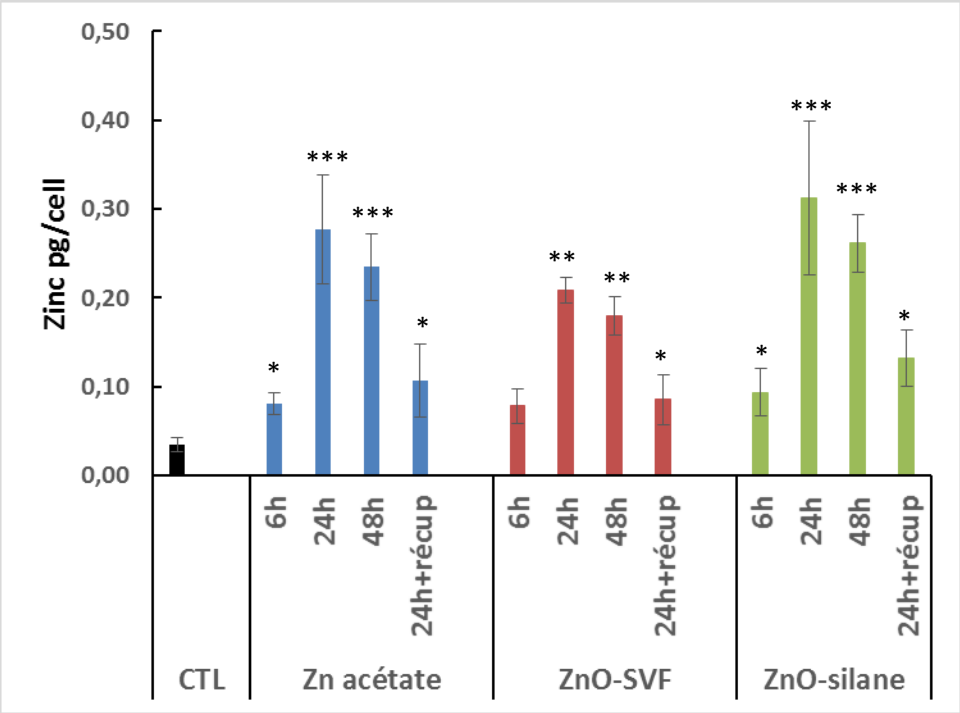
Does ZnO-NP interfere with redox equilibrium in Hepatocytes (HepG2) ?







Study of Zn content in cells



Measurement of the amount of Zn in HepG2 after incubation with 90 µM ZnO-NP or Zn acetate for 6 h and 24 h (ICP-OES)

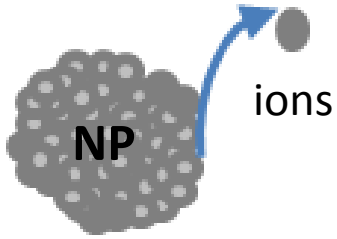
Very similar zinc accumulation between NPs and salt
The intra-cellular concentration of Zinc increased from 6h to 24h
No evidence of zinc release

≠

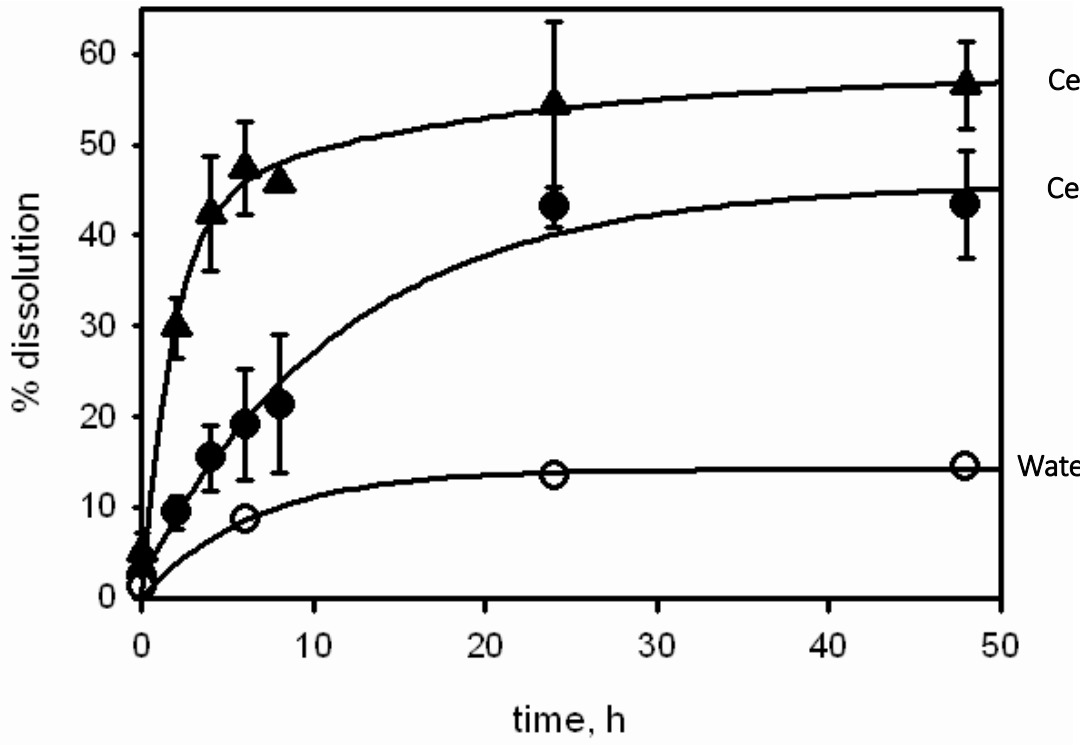
More copper is found in HepG2 exposed for 24h to CuO-NP than to $CuCl_2$

Studies of ZnO-NP, CuO-NP and Ag-NP

These particles can dissolve in water-based media and release ionic species



Cu ions release from CuO-NP



pH decrease favors ion release from NP → similar mechanism in endo- and lysosomal vesicles

Cuillel et al (2014) Nanoscale, 6: 1707