Input of proteomics in nanoparticles toxicology: the example of macrophages responses to mineral nanoparticles

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The challenge of transposing lab. toxicology results into real life

Gold standard: lab animals (healthy life)

Combinatorial explosion of interfering factors (lifestyle, prof. etc...)

Necessity of obtaining molecular mechanisms

Role of high content in vitro approaches

The different toxicities of nanoparticles

(besides the shape/size/agglomeration issues)

Intrinsic toxicity

- examples: asbestos, crystalline silica
- targeted approaches
- high throughput screening

Helper toxicity

- adsorption As on Fe oxides (Auffan et al. Langmuir 2008)
- adsorption Cd on amorphous silica (Guo et al. J. Hazard. Mat. 2013)
- case of diesel exhaust particles

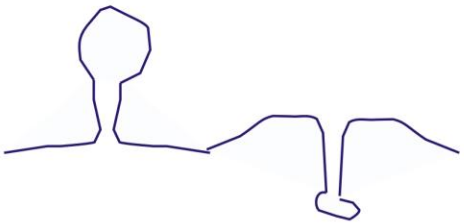
Cross-toxicities

- synergitic toxicities without direct interactions
- **response mechanisms** => vulnerability points => sorting cross toxicities

The price for life: complexity

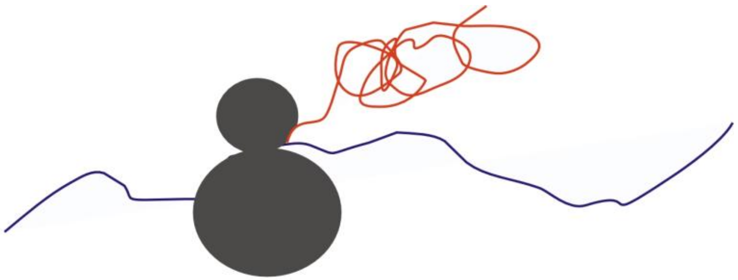
DNA

1500



mRNA

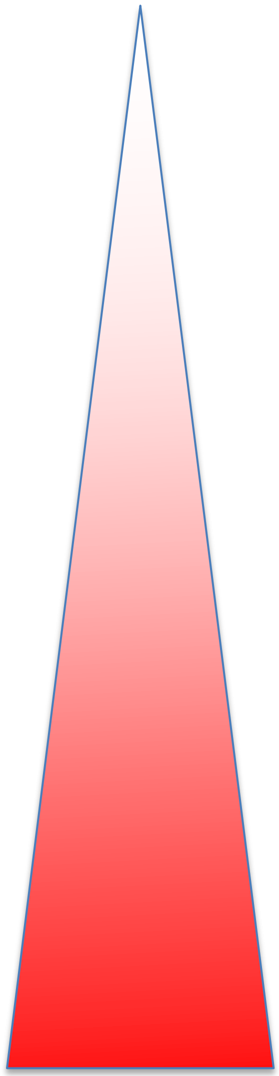
2000



Translation

Protein

2500



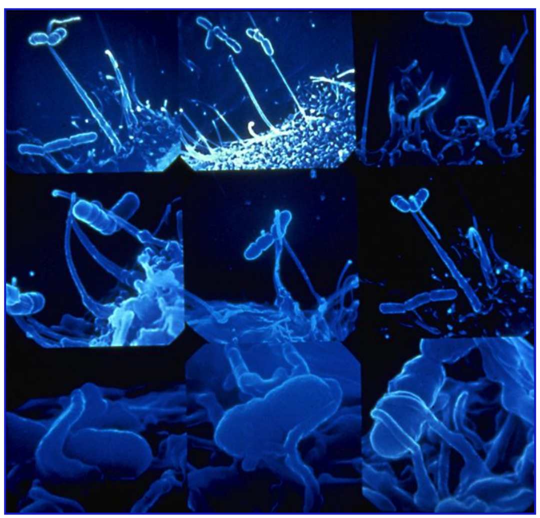
Chemical diversity
Dynamic range

Genomics	The world of possibles
Transcriptomics	What is going to happen (maybe)
Proteomics	What is happening now
Metabolomics	What has happened

Macrophages: first line sentinels, immunity effectors and final scavengers

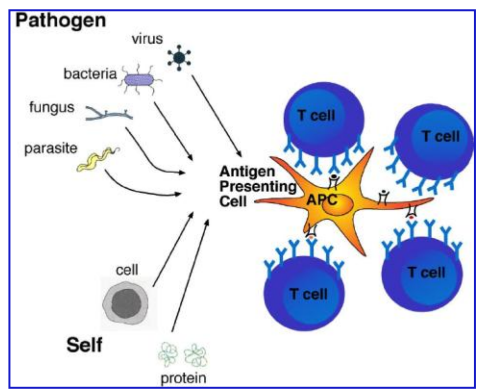
Macrophages

Phagocytosis, destruction of pathogens and abnormal cells

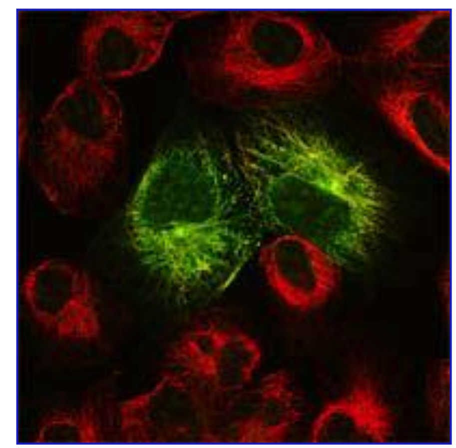


Scavenging of toxic particles (e.g. altered LDL)

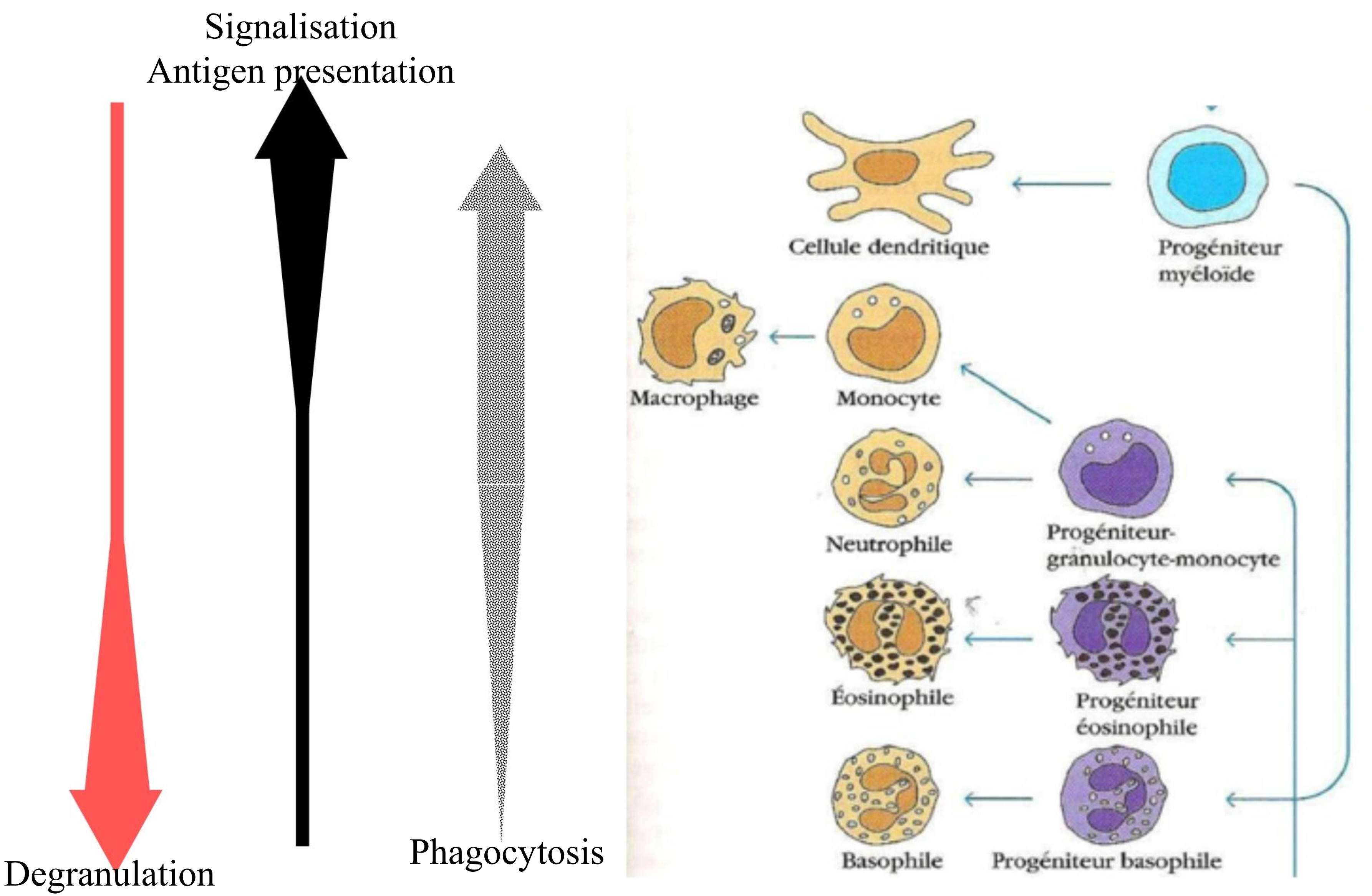
Antigen presentation



Cytokinic signalling



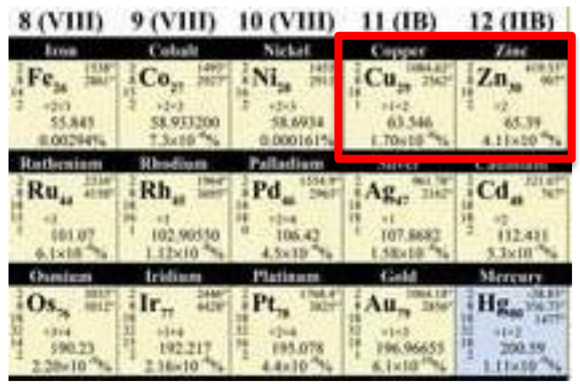
Inflammation
Tissue healing


Signalisation
Antigen presentation
Degranulation
Phagocytosis
Cellule dendritique
Progéniteur myéloïde
Macrophage
Monocyte
Neutrophile
Progéniteur-granulocyte-monocyte
Éosinophile
Progéniteur éosinophile
Basophile
Progéniteur basophile

The nanoparticles investigated: ZnO and CuO

ZnO (30,000 tons/year ww) used in sunscreens, biocidal, UV protection

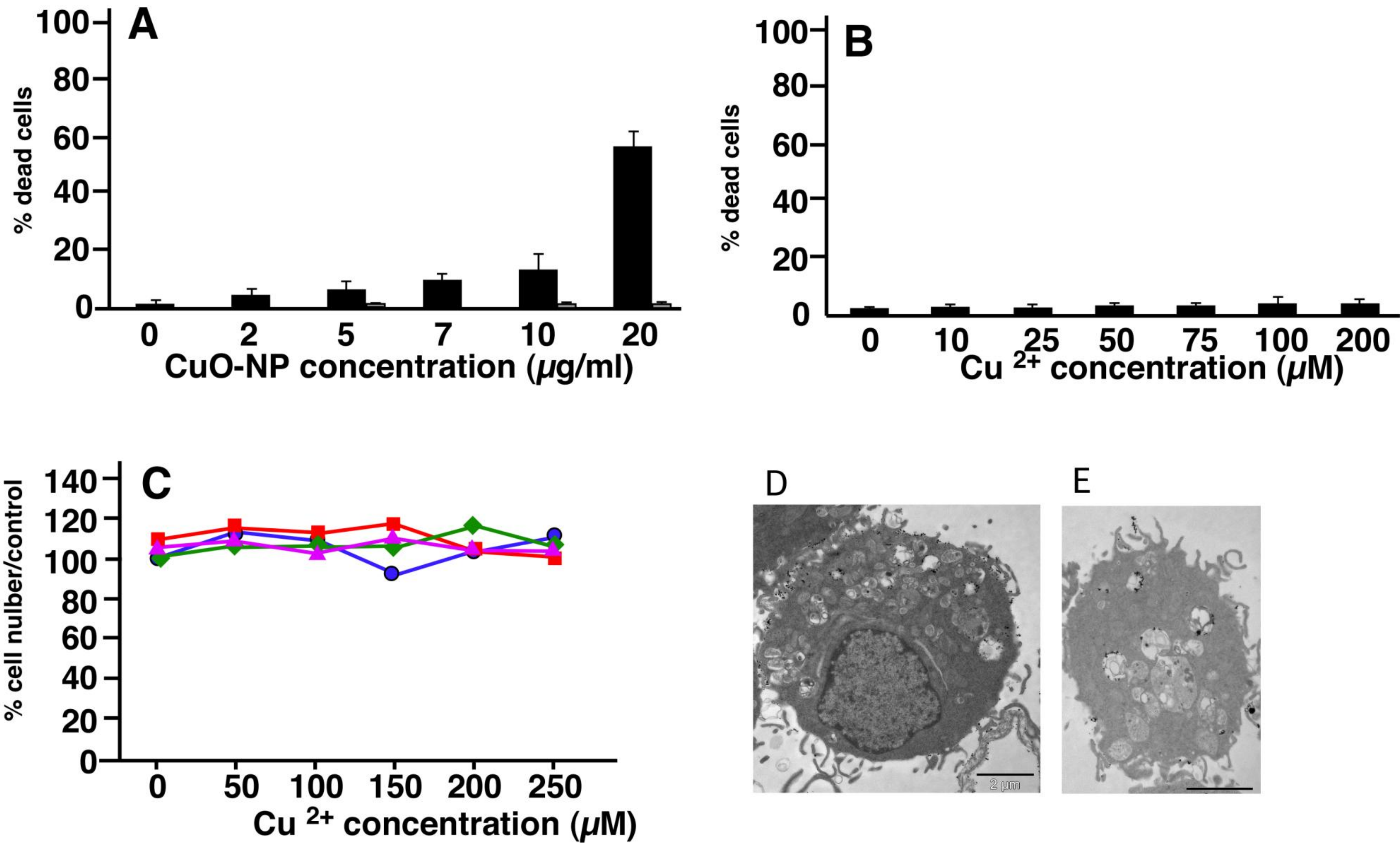
CuO used in water depollution, biocidal, conductive inks, wood treatment

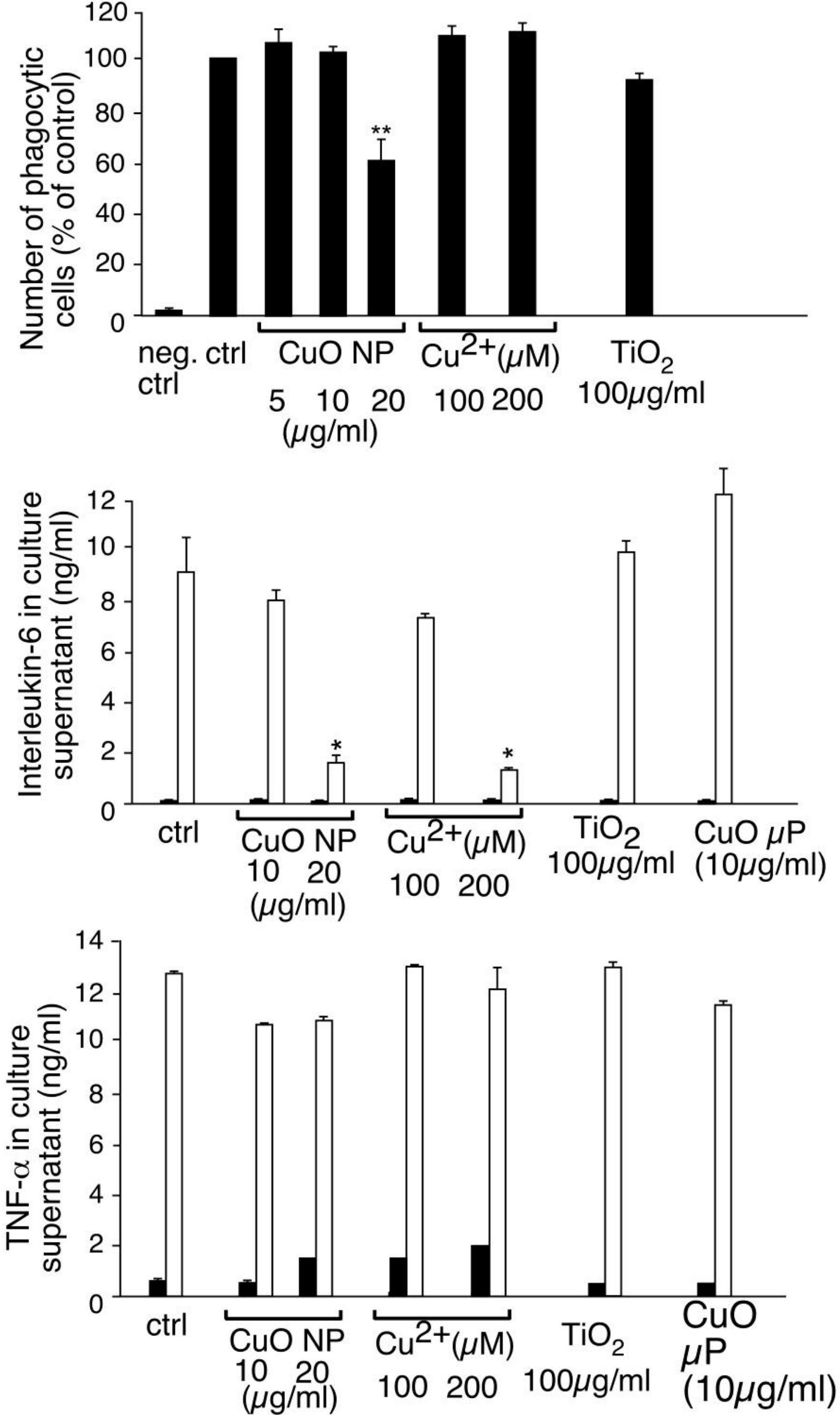


Very similar parameters: -primary particle size <50nm
-agglomerate size in culture medium ca. 200-250 nm
-similar toxicity (LD20 ca. 10 µg/ml)

Fairly different proteomic responses for ZnO and CuO

Effect of copper on cell survival

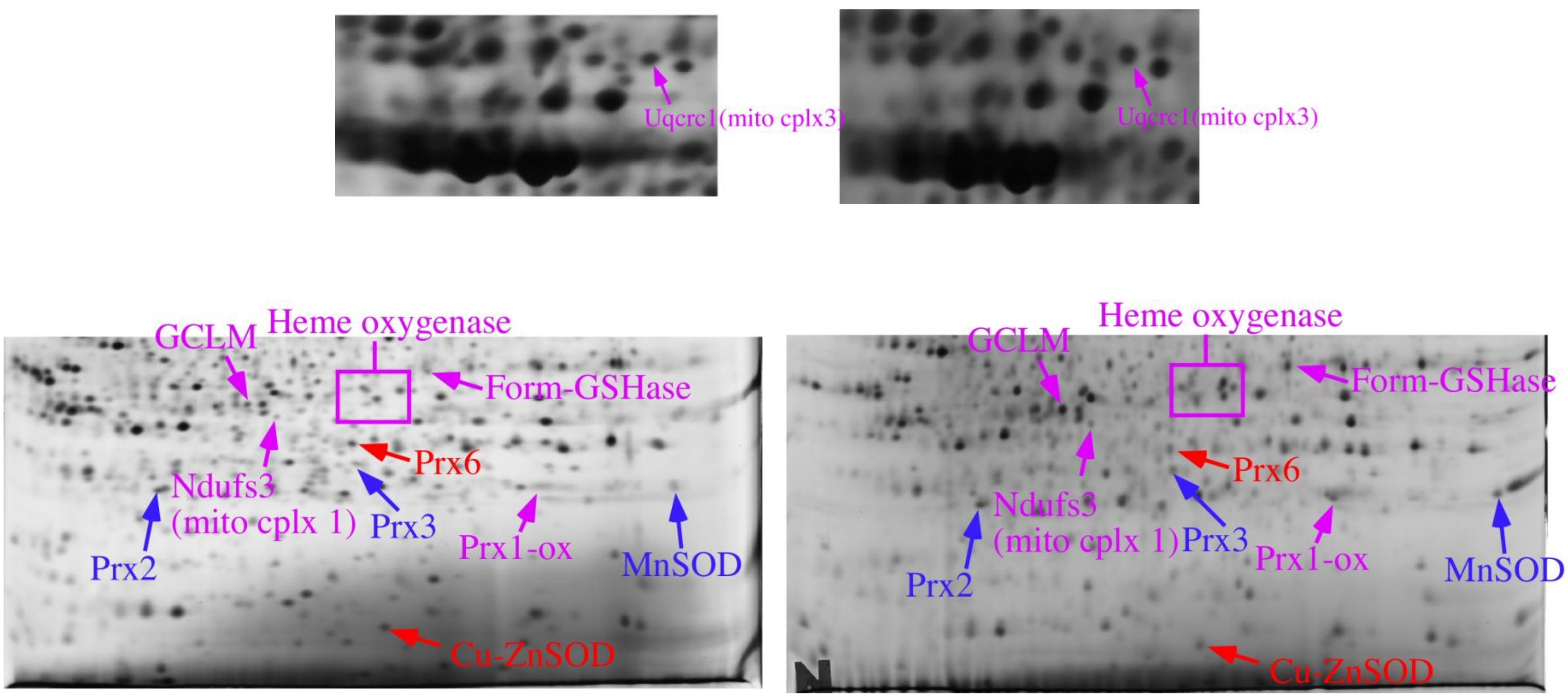




Functional effects of Cu on macrophages

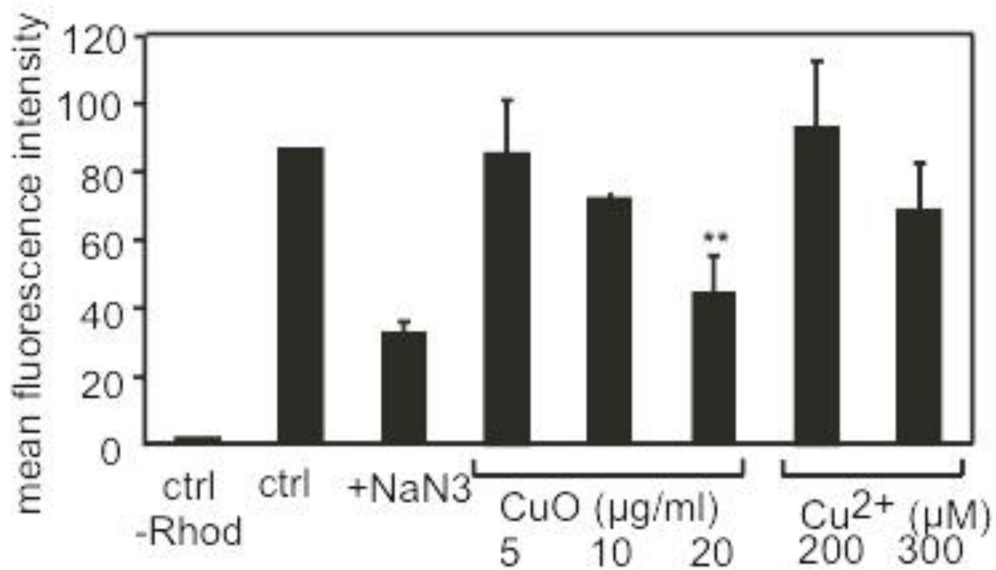
Oxidative and mitochondrial stress response to CuO (1)

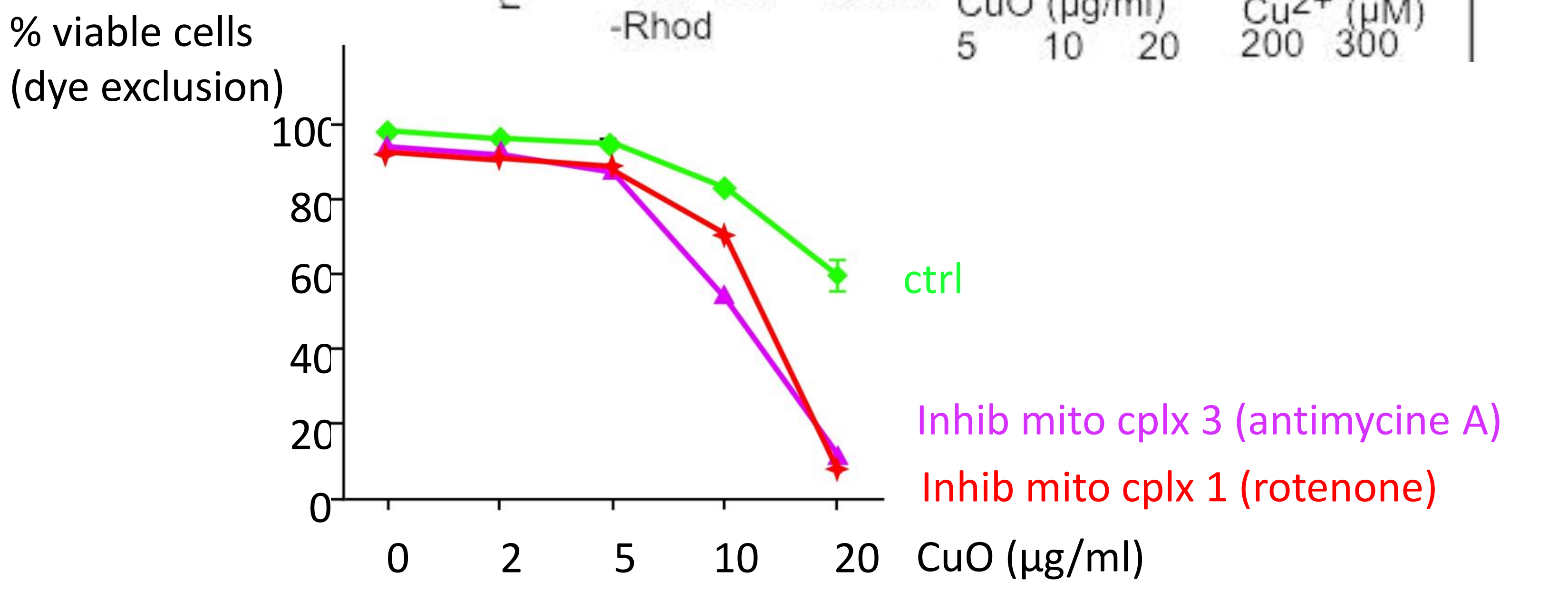
Control | CuO-treated



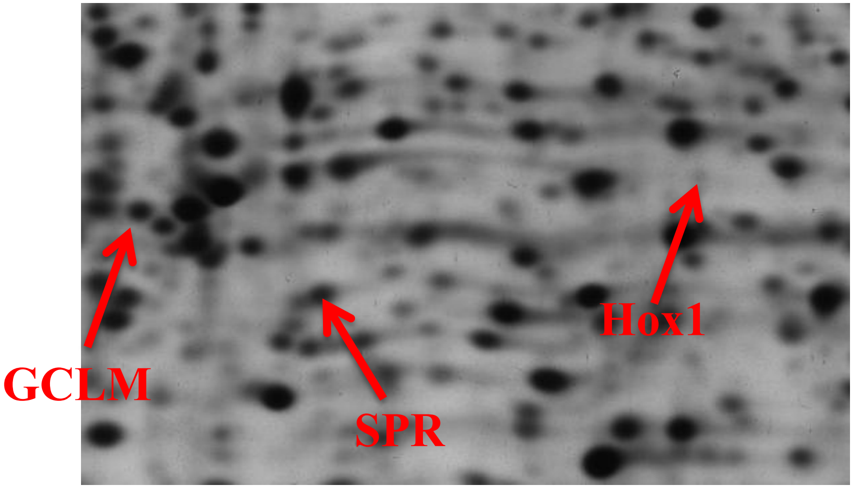
Oxidative and mitochondrial stress response to CuO (2)

Induction of mitochondrial respiratory complexes

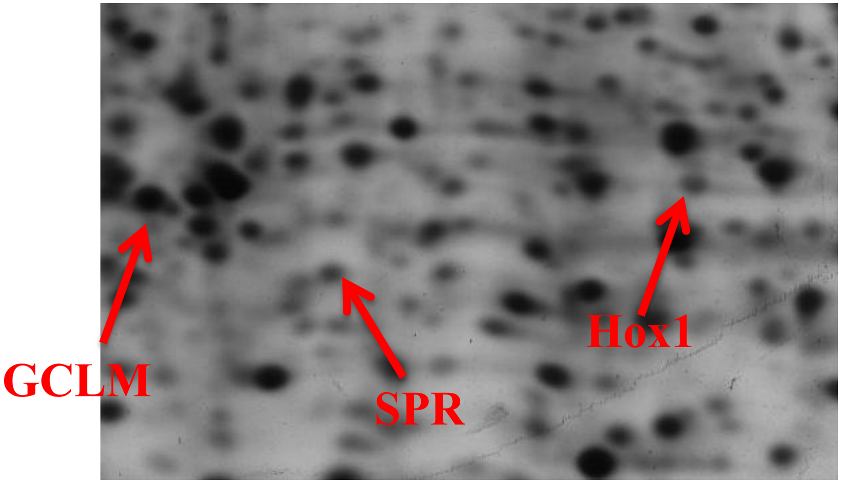




Further validation of proteomic findings

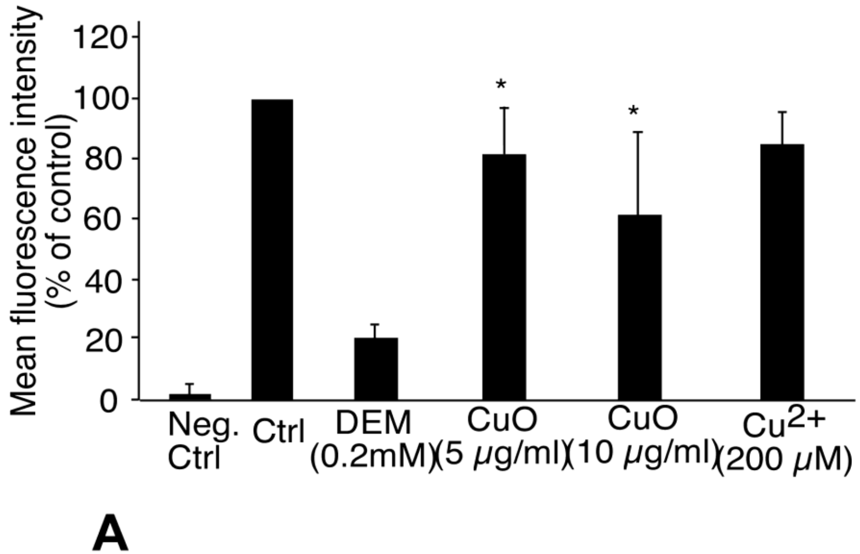


Control

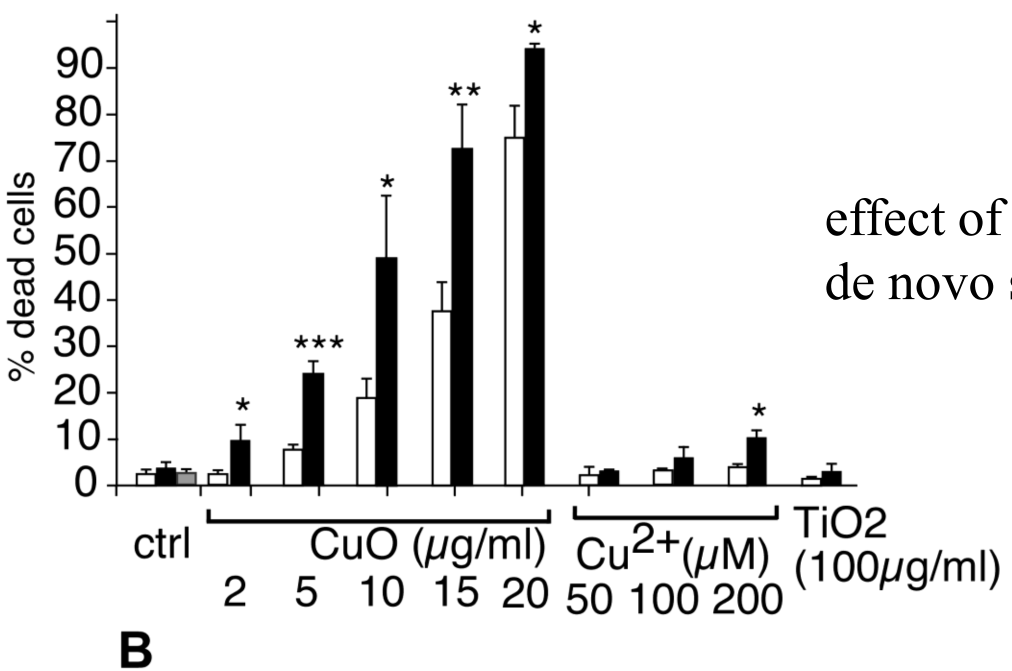


CuO-treated

Role of glutathione in survival to copper oxide nanoparticles (GCLM)



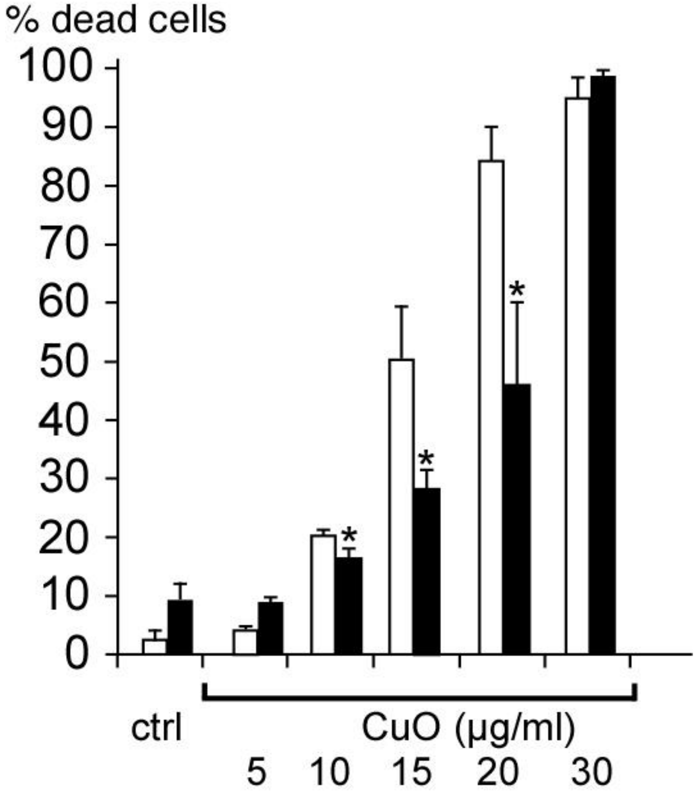
glutathione levels



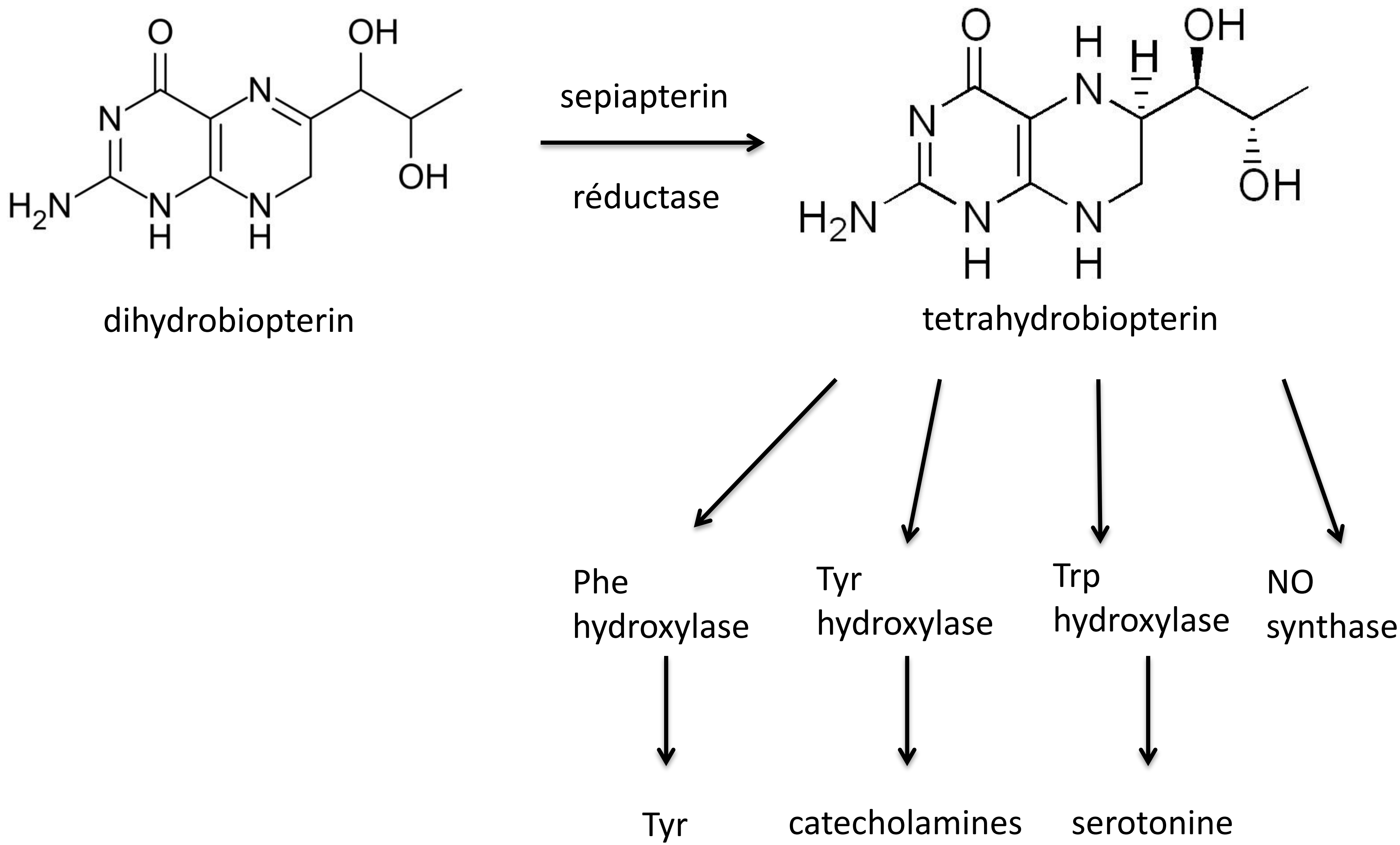
effect of glutathione
de novo synthesis inhibition

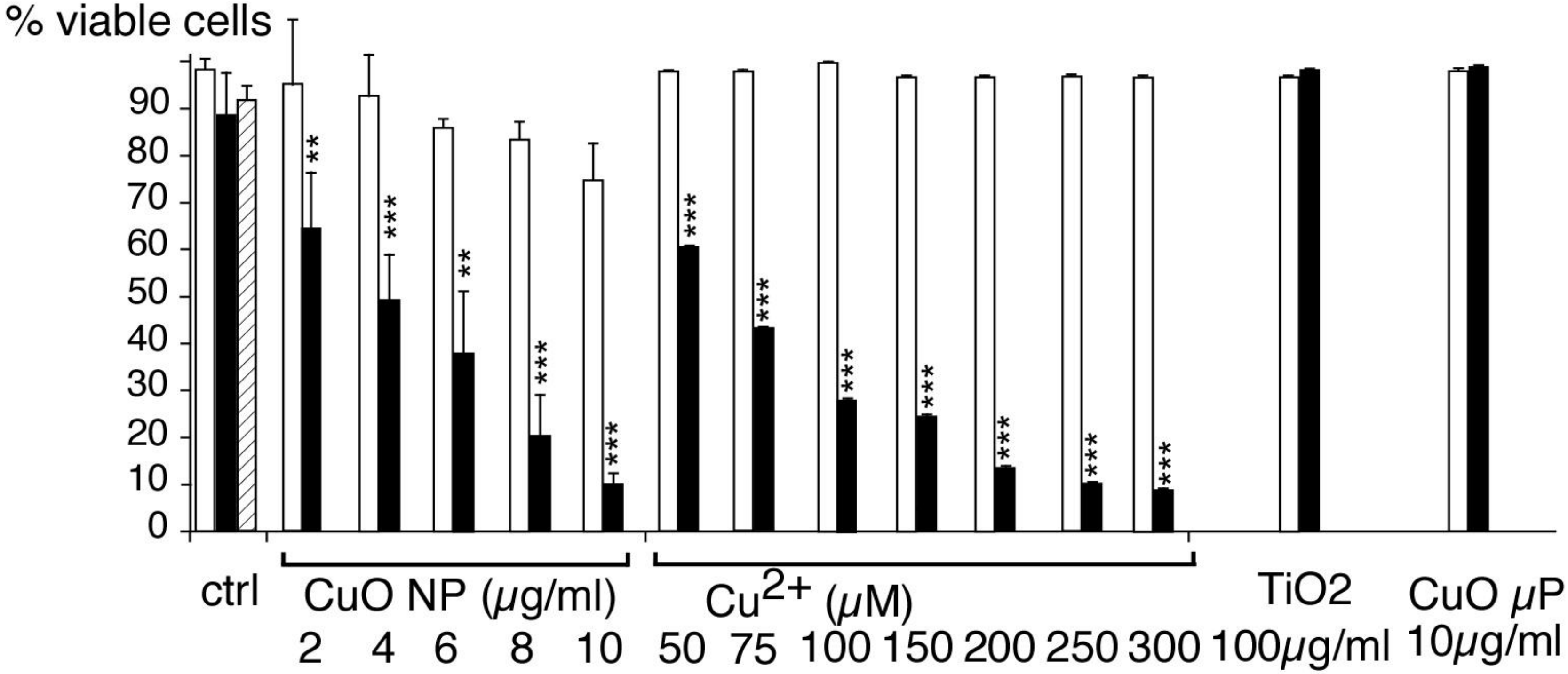
Role of heme oxygenase (HOX1) in copper resistance

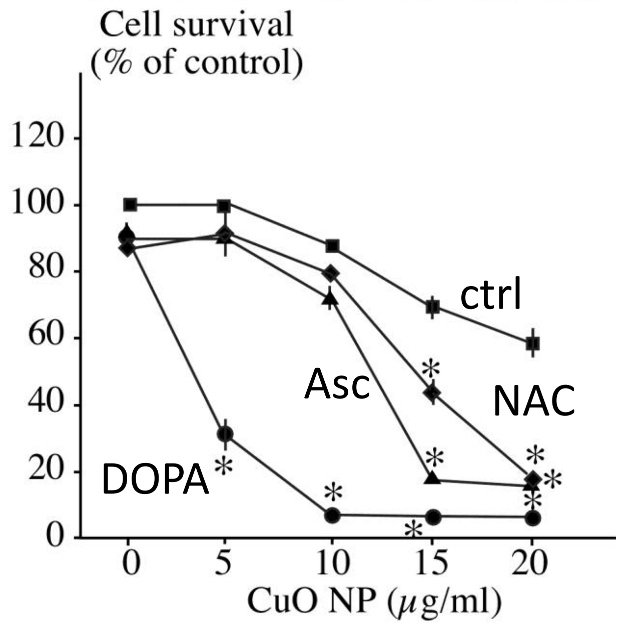
Pre induction of Hox with 1µM lovastatin for 6hrs prior to CuO challenge



Role of sepiapterin reductase (SPR)




% viable cells
90
80
70
60
50
40
30
20
10
0
ctrl
CuO NP (μg/ml)
2 4 6 8 10
Cu2+ (μM)
50 75 100 150 200 250 300
TiO2
100μg/ml
CuO μP
10μg/ml


Cell survival
(% of control)
120
100
80
60
40
20
0
0 5 10 15 20
CuO NP (μg/ml)
ctrl
Asc
NAC
DOPA

The second nanoparticle investigated: ZnO

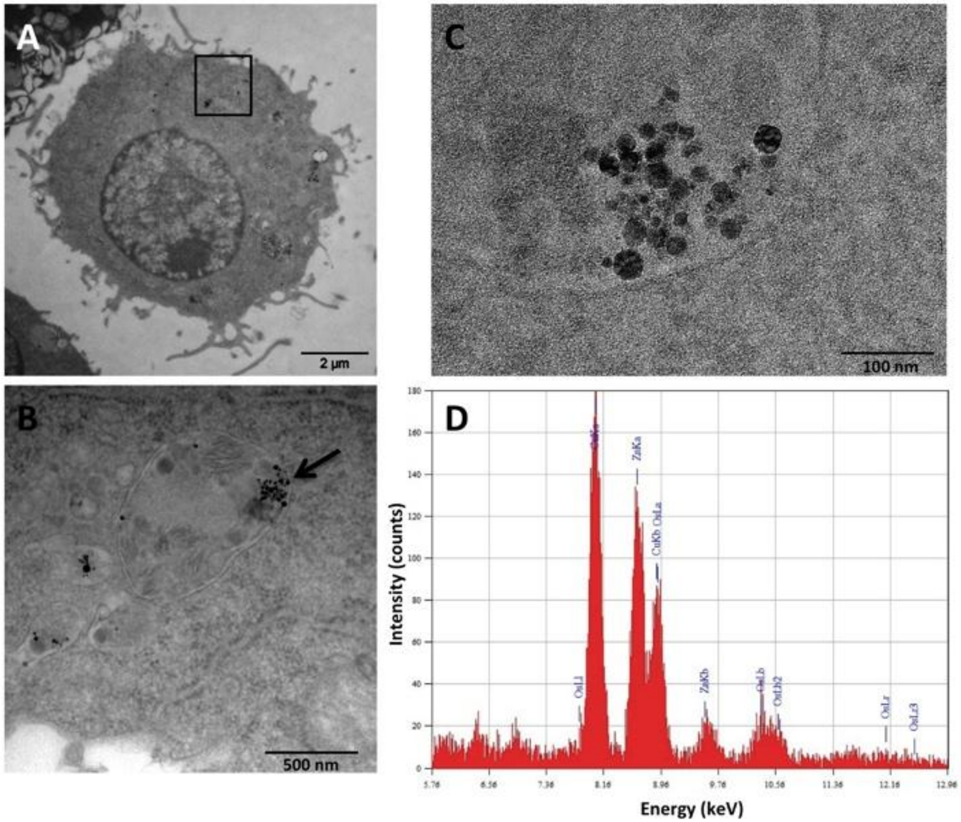
ZnO (30,000 tons/year ww) used in sunscreens, biocidal, UV protection

Parameters:
- **-primary particle size <50nm**
- **-agglomerate size in culture medium ca. 200-250 nm**
- **-moderate toxicity (LD20 ca. 10 µg/ml)**

ZnO: causative agent of the metal fume fever (at doses >50mg/m^3 air)

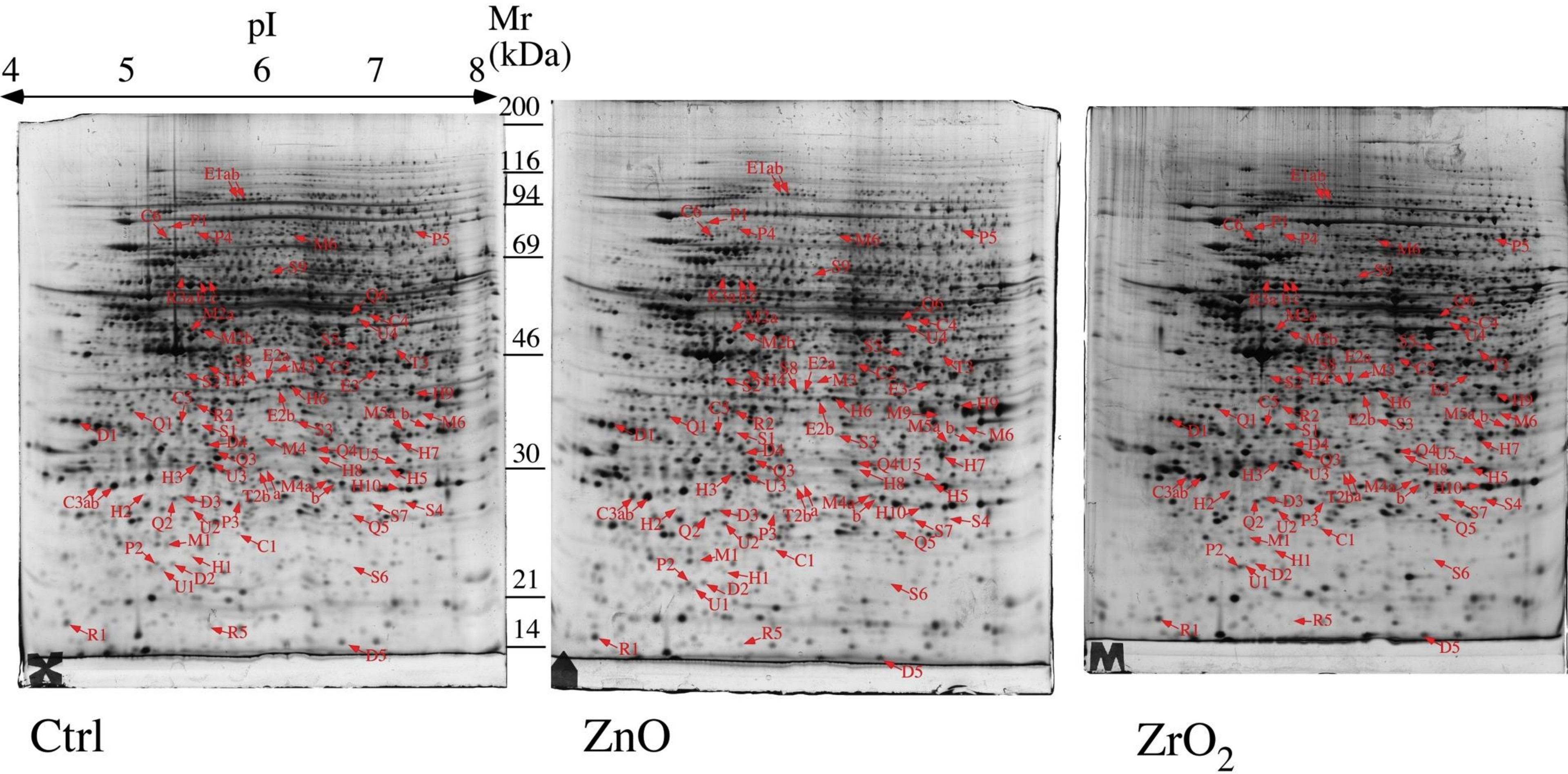
50mg/m^3 air => 10 ppm in our culture system

Uptake of zinc oxide by macrophages



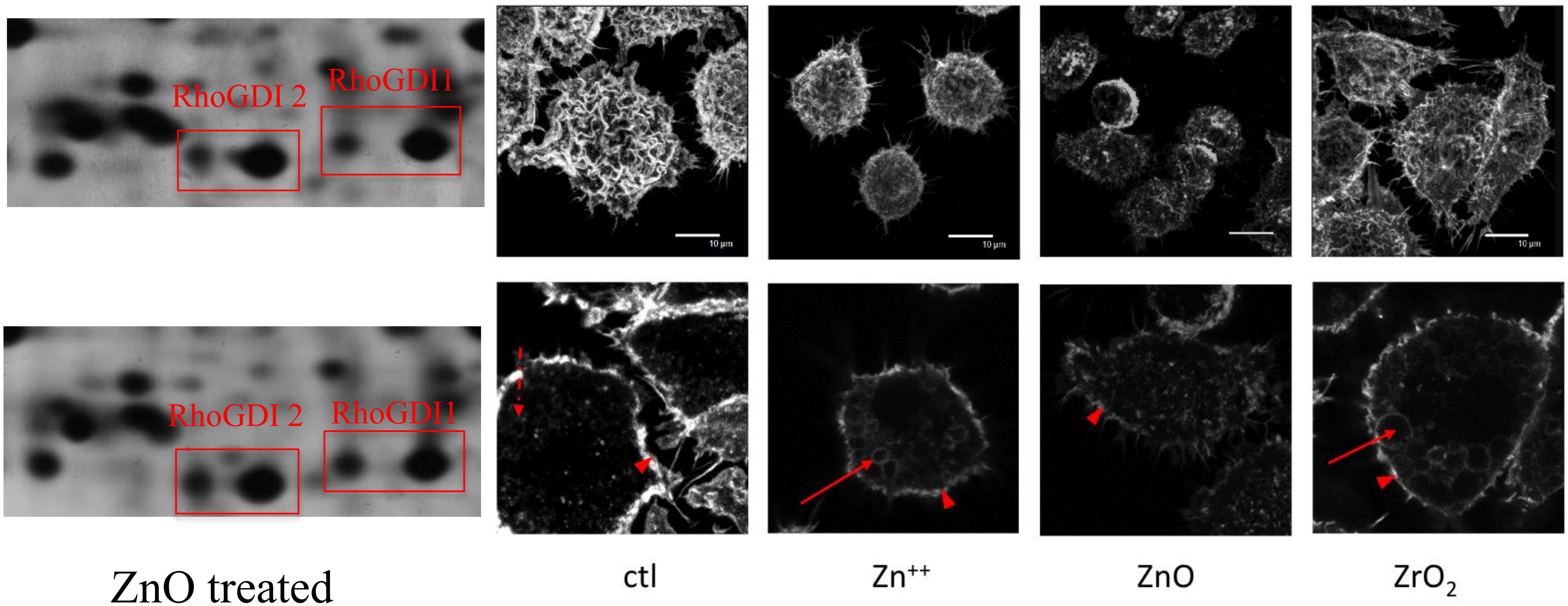
Input in cell culture	Concentration in cell extract	Concentration in cells
Cationic zinc oxide (90μM)	27±7μM	135±35 μM
Zinc acetate (100μM)	37.5±2.5 μM	187.5±12.5 μM
Zinc oxide (100μM)	30±1μM	150±5 μM

Proteomic analysis of J774 cells in response to ZnO nanoparticles



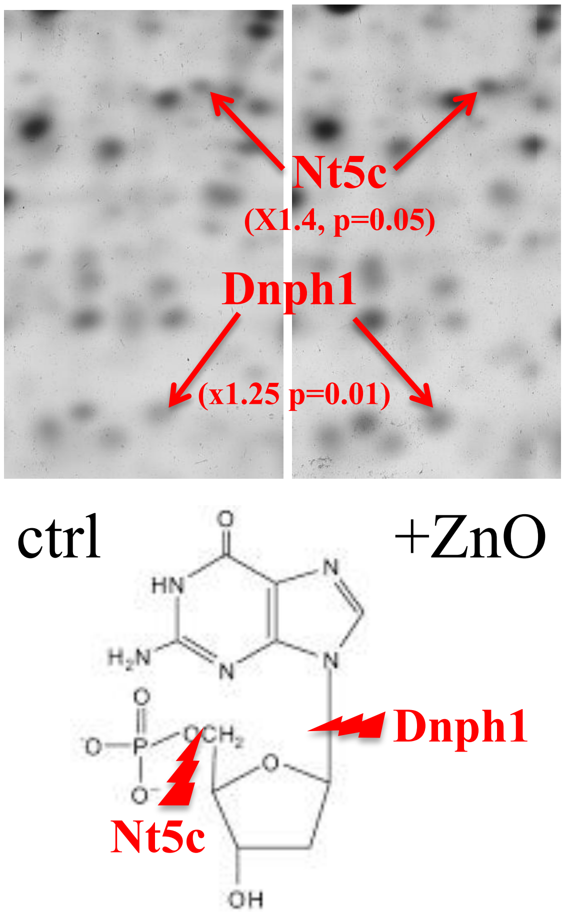
Changes in the actin cytoskeleton

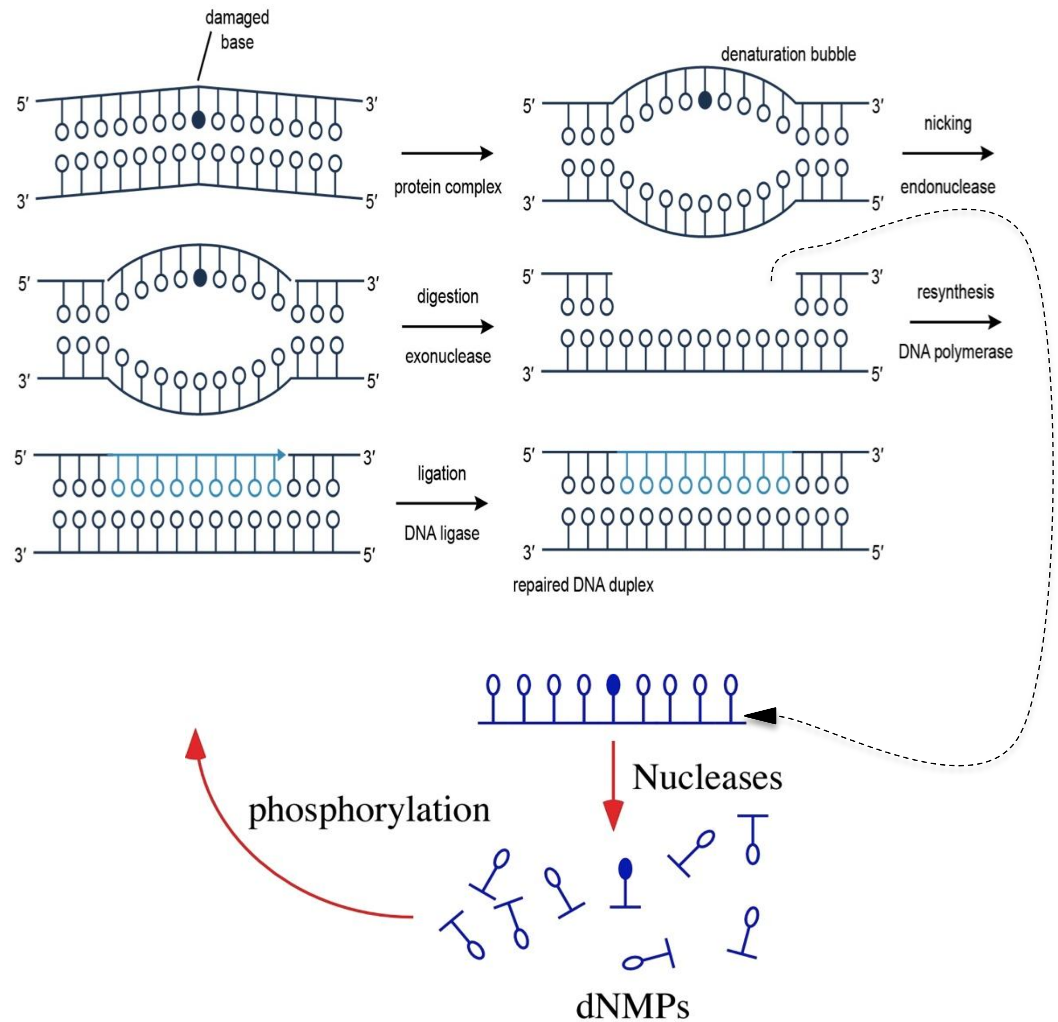
Control cells



ZnO treated

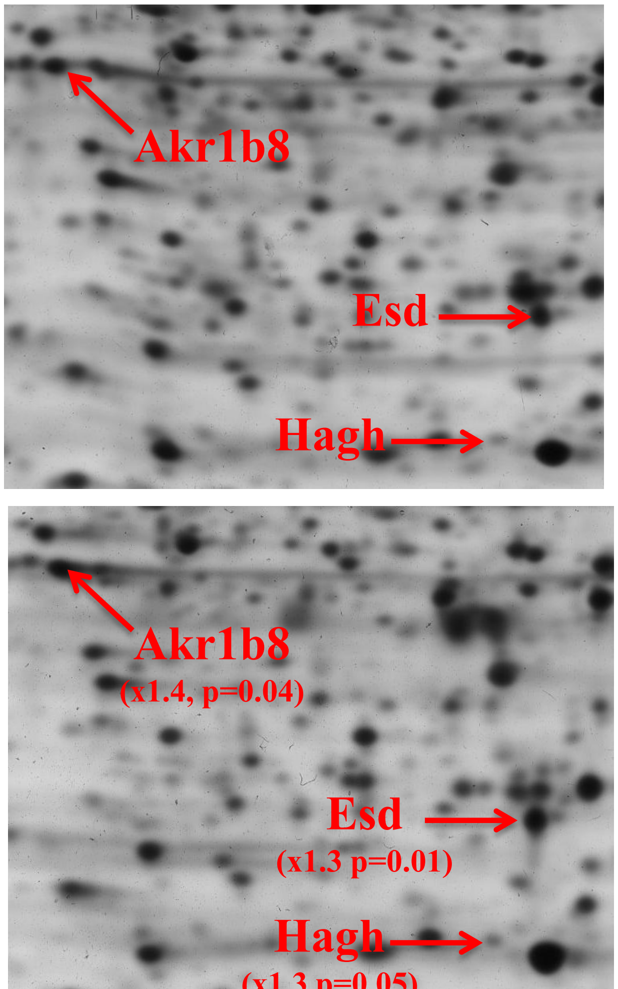
Zinc genotoxicity : the genotoxicity of a non-Fenton metal





ctrl

+ZnO

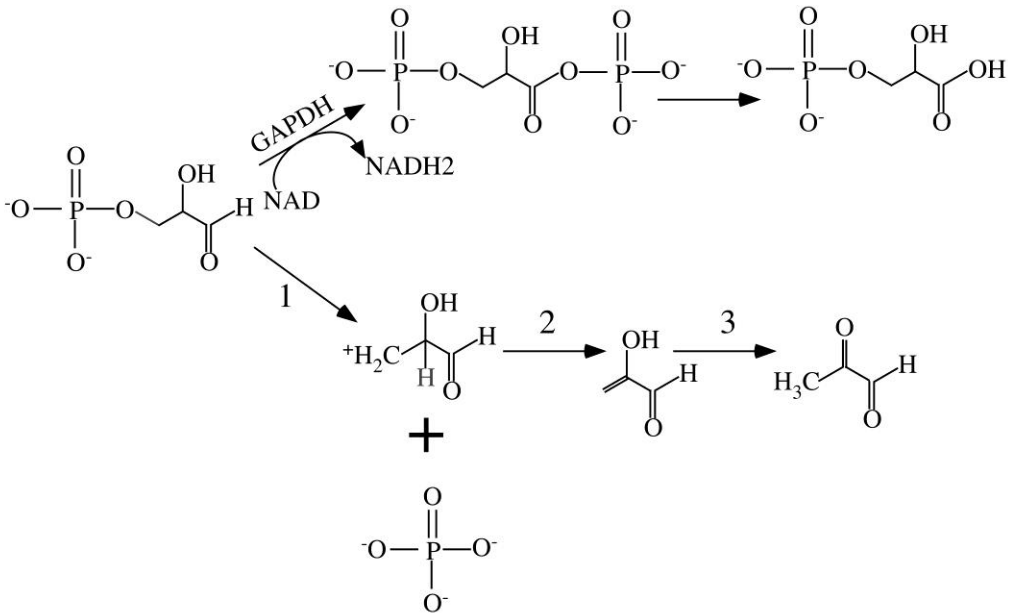


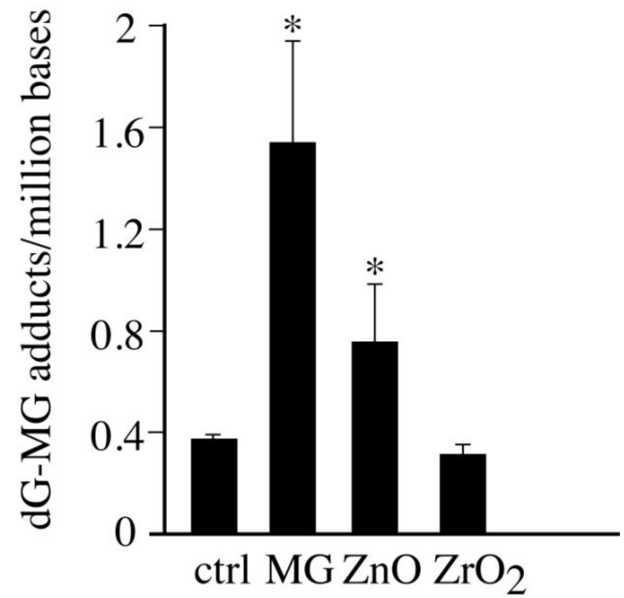
aldehydes detoxification

condition	GAPDH	condition	GAPDH
ctrl	16±2	0µM Zn	16.5±1
ZrO2	13.5±2.5	100µM Zn	11.5±0.6
ZnO	7.5±2.5	150µM Zn	7.2±0.8
		200µM Zn	4.9±1.5

The activities are expressed in units/mg protein, the unit being defined as 1µmole of substrate converted per minute

The methylglyoxal pathway in zinc toxicity





=> an indirect and composite genotoxic mechanism (DNA Pol ι and κ)

=> toward a proteomics-driven study of nanoparticles cross-toxic effects

Conclusion: proteomics can do the job

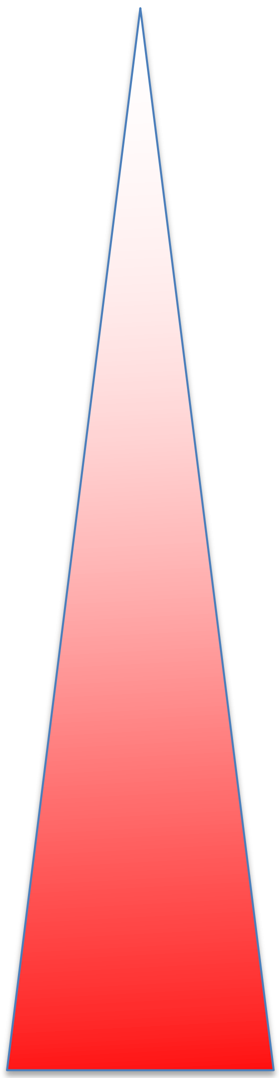
-Proteomics underscores biologically relevant responses at non toxic doses

(e.g. mitochondria, GSH biosynthesis, Hox, DOPA, methylglyoxal)

-Proteomics can sort different responses even if tox. parameters are similar

-Proteomics is able to underscore possible cross-toxicities
(e.g. Cu + rotenone, Cu+DOPA)

Full exploitation of proteomics data require functional validation



Chemical diversity
Dynamic range

Genomics — The world of possibles

Transcriptomics — What is going to happen (maybe)

Proteomics — What is happening now

Metabolomics — What has happened

Less is more, Less is bore, or mess in more ?

(Mies van der Rohe) (Robert Venturi)