THE OXIDATIVE POTENTIAL OF NANOMATERIALS AS A PREDICTIVE INDICATOR OF THEIR TOXICITY

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Characterization of the oxidative potential of nanomaterials

nanOxiMet

Oxidant generating capacity as a metric to allow grouping of nanomaterials and prediction of human health effects

Project partners:

Coordinator:
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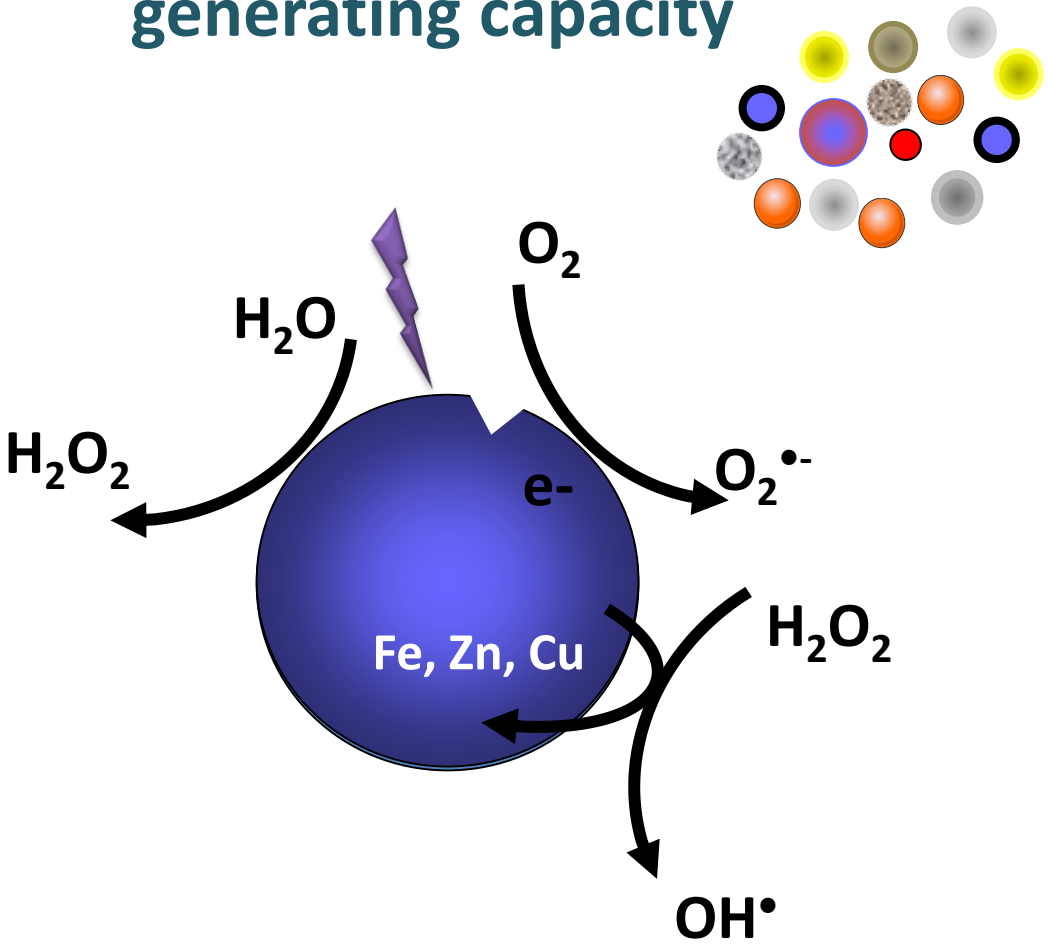
Sponsored by:

Federal Ministry of Education and Research
Germany

FP7 ERA-NET

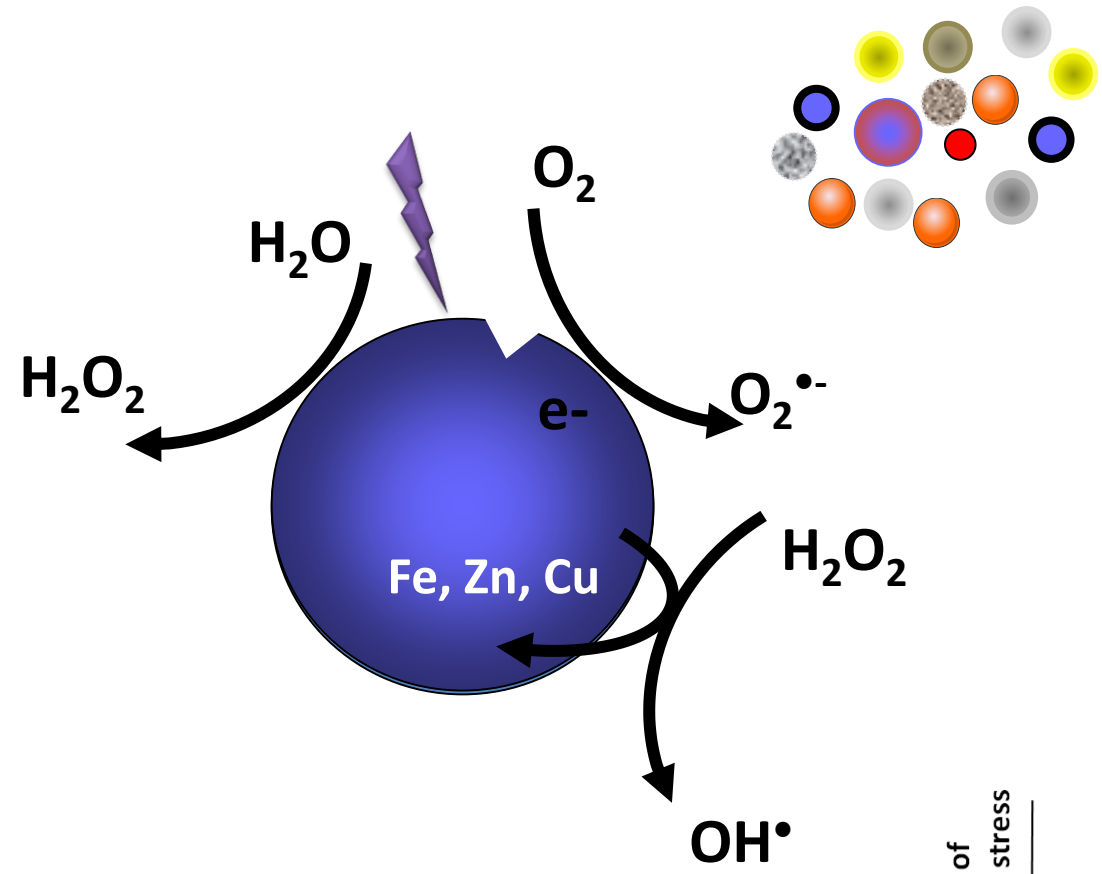
Reactivity of nanomaterials

Intrinsic oxidant generating capacity

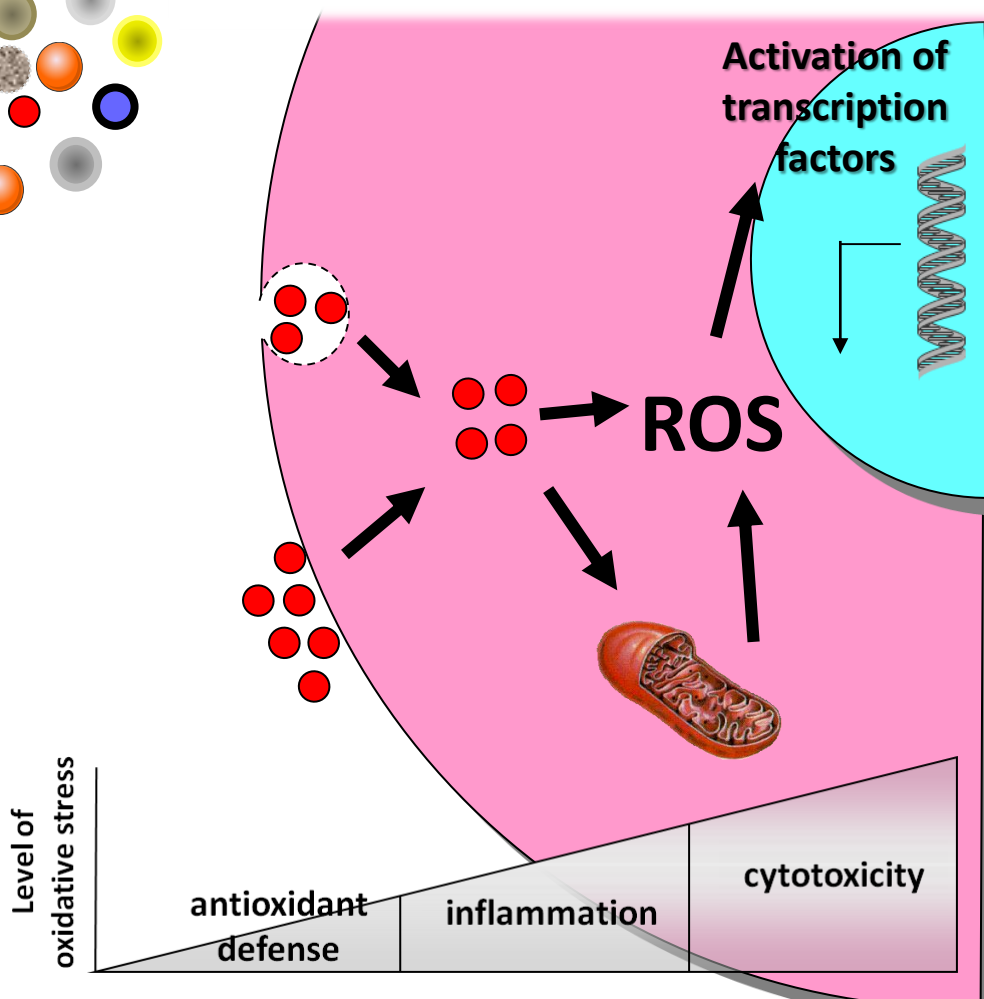


Reactivity of nanomaterials

Intrinsic oxidant generating capacity

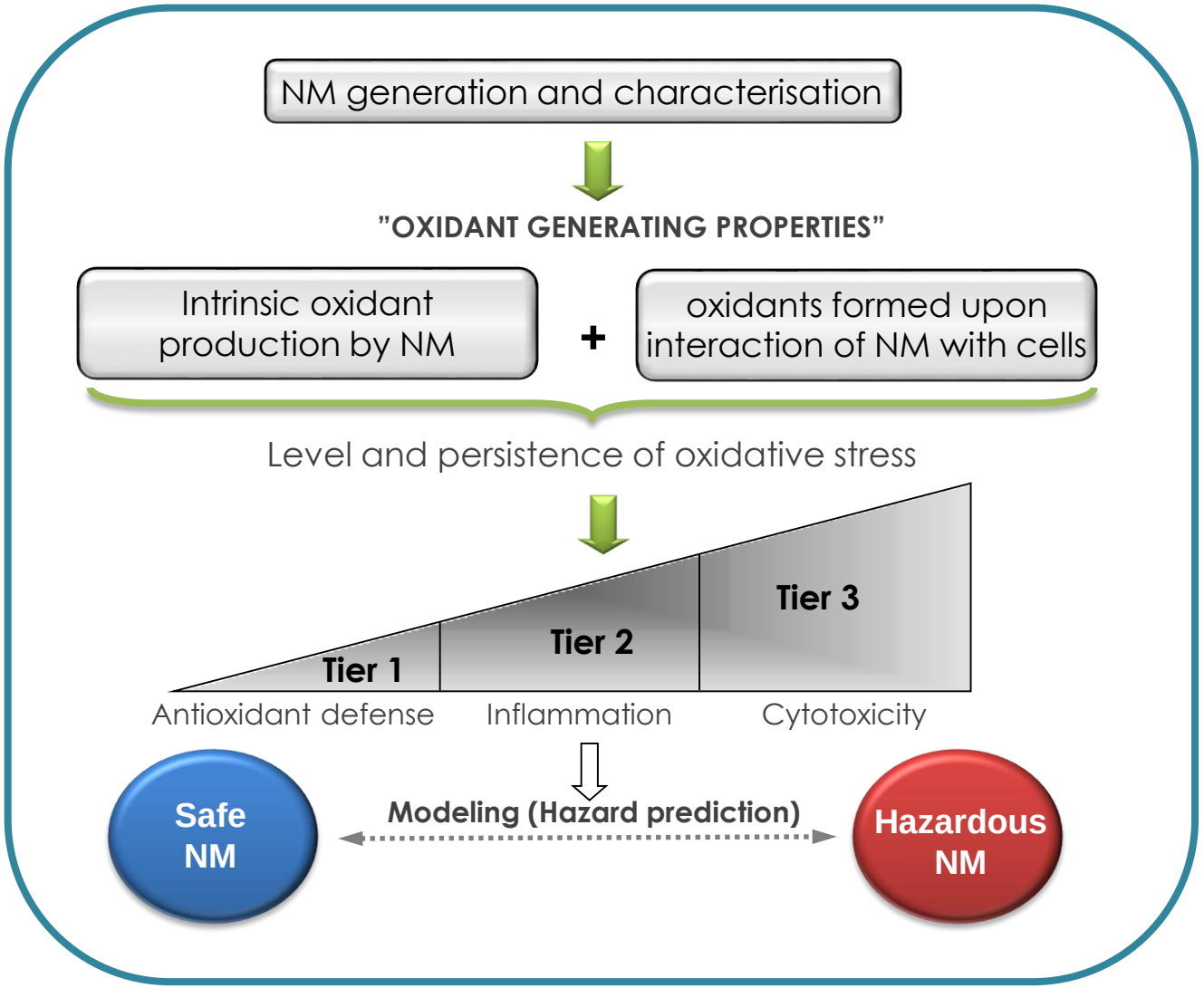


Cell-mediated oxidant generating capacity



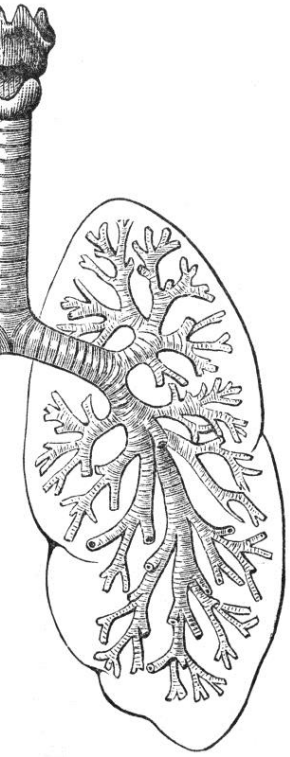
Project structure

nanOxiMet



Cell models

Nanoparticles



NCI-H292
Human bronchial epithelial cells

THP-1 macrophages
Differentiated from human monocytes
3h 500 nM 12-O-tetradecanoylphorbol-13-acetate (PMA)

Concentrations
0.15 – 80 µg/cm²
(0.5 – 256 µg/ml)

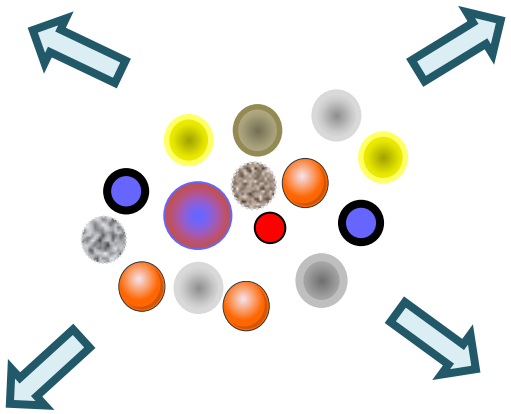
	Primary particle diameter (nm)	Specific surface area (m^2/g)	Hydrodynamic ∅ DLS (H_2O)
TiO_2 NM100 (A)	42-90	13	326
TiO_2 NM101 (A)	6	416	654
TiO_2 NM103 (R)	20	55	461
TiO_2 NM104 (R)	20	71	265
$Fe(III)_2O_3$	<50		673
Al_2O_3	13	99	337
CeO_2 NM212	28	23	369
$BaSO_4$NM220	10-150	50	150
ZnO	42		
Cu	40-60	n.m.	413
Ag 100 nm	100	6	787
Ag 20 – 30 nm	20 - 30	25	751
Au	5	14	1712
Ni	<100		Nf
Printex 90	14	367	288
Lamp black	95	20	1297

Intrinsic oxidant generation potential

Induction of DNA strand breaks (plasmid assay)

Depletion of antioxidants in synthetic lung surfactant

ascorbic acid
uric acid
glutathione



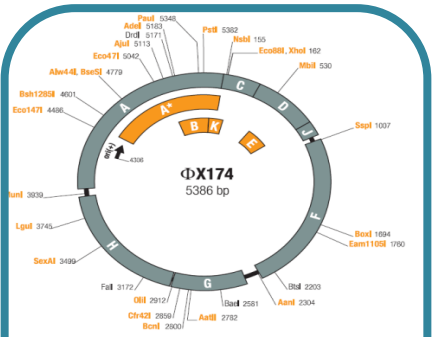
Oxidation of dichlorofluorescein (DCFH assay)

Depletion of dithiothreitol (DTT assay)

IUF ESR

Intrinsic oxidative potential of MNM

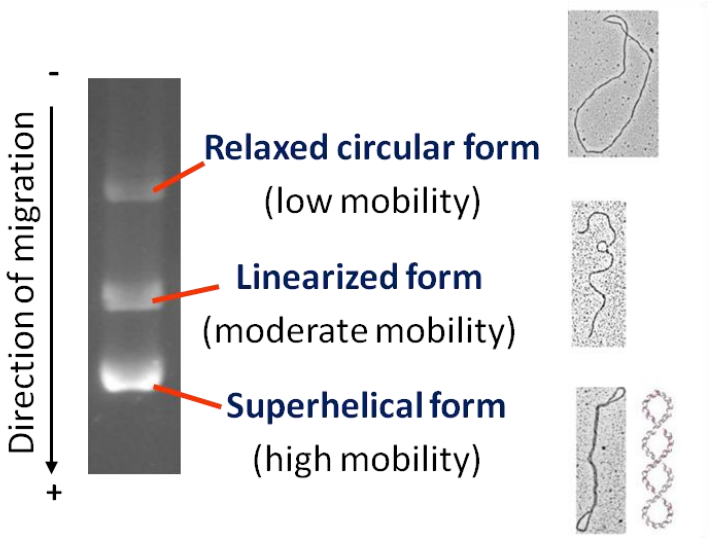
Induction of DNA strand break, NP surface-associated free radicals

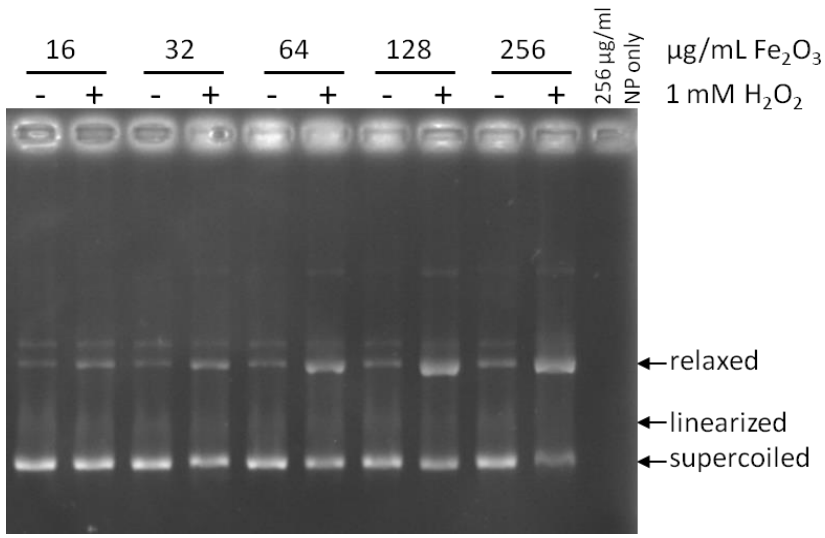


Bacteriophage ϕX174, 290 ng
+
NM 16 - 256 µg/ml

4h incubation at RT

Agarose gel electrophoresis

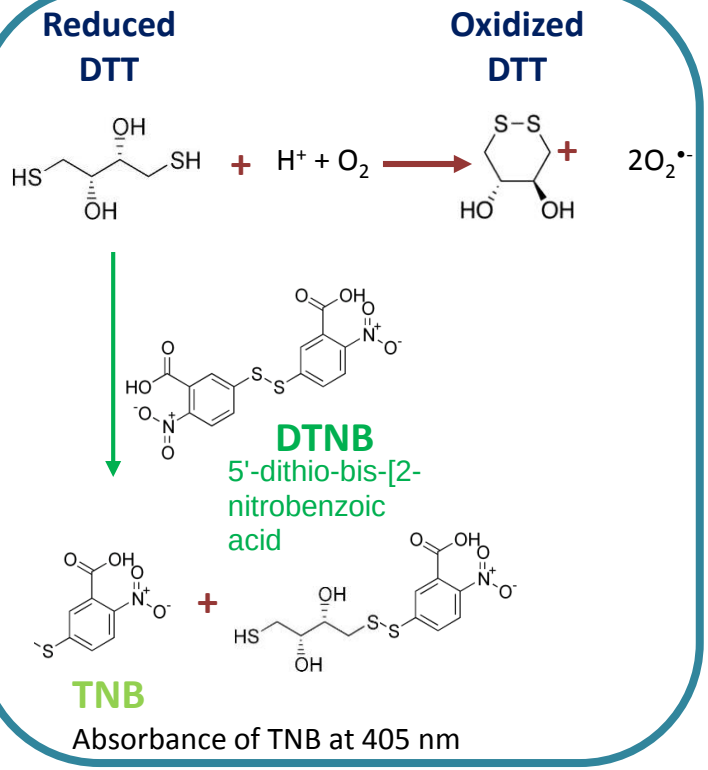


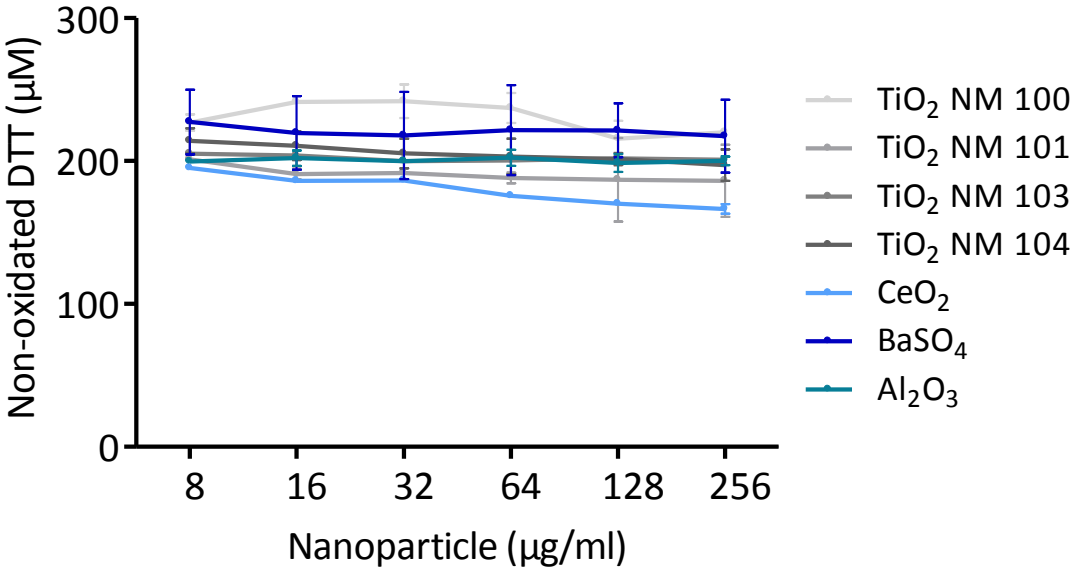


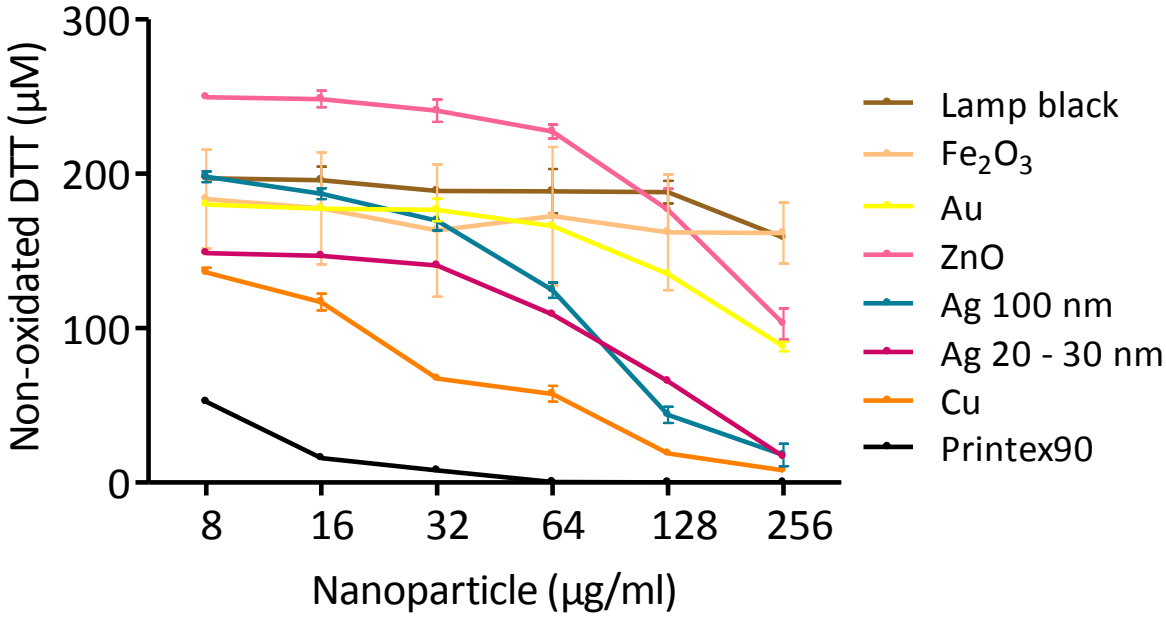
	Induction of DNA strand breaks
TiO_2 NM100	-
TiO_2 NM101	- / +(H_2O_2)
TiO_2 NM103	-
TiO_2 NM104	- / +(H_2O_2)
Printex 90	-
Lamp black	-
$BaSO_4$	-
CeO_2	-
ZnO	+/++ (H_2O_2)
Al_2O_3	-
Fe_2O_3	+/++ (H_2O_2)
Cu	++++
Ni	nd
Au	-
Ag 100 nm	-
Ag 20 – 30 nm	-

Depletion of DTT

NM with DTT
30 min at 37°C





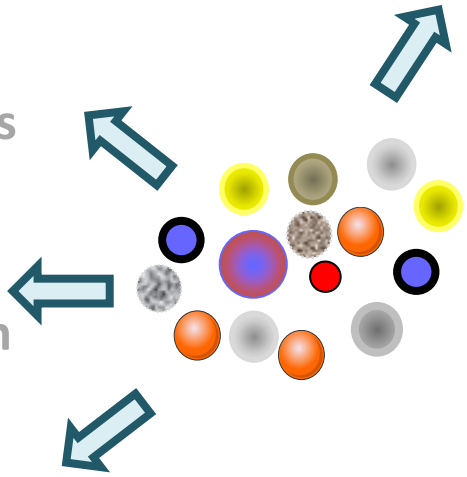


Intrinsic oxidant generation potential

Induction of
DNA strand breaks

Oxidation of
dichlorofluorescein

Depletion of
dithiothreitol (DTT)



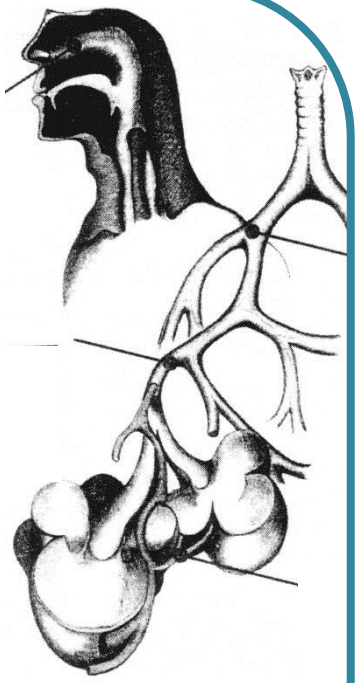
Depletion of antioxidants in synthetic respiratory tract lining fluid

200 µM ascorbic acid
200 µM uric acid
200 µM glutathione
pH7.4, in 0.9% NaCl
450 µl

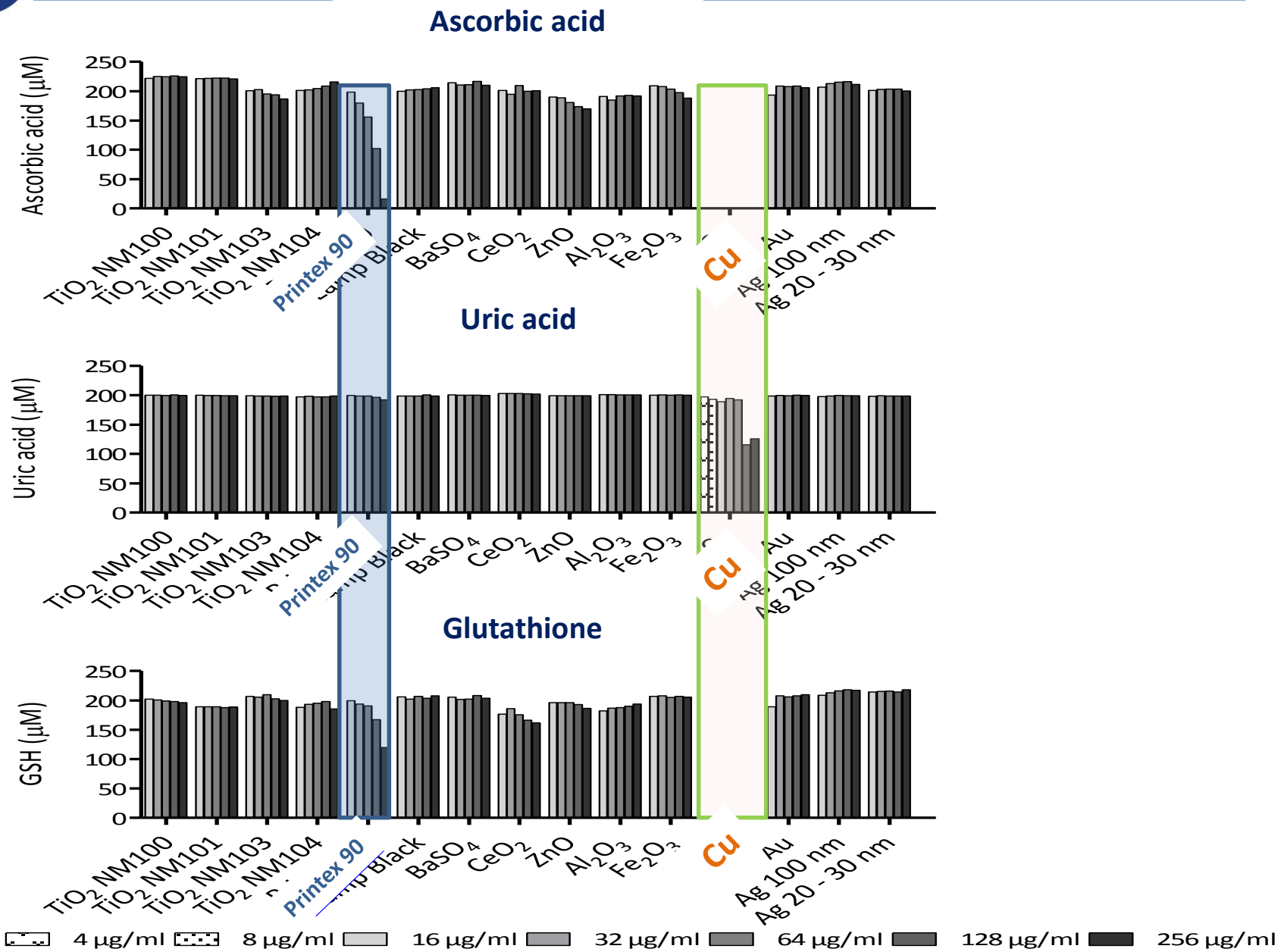
Nanoparticle suspension
50 µl

4 hours incubation at 37°C

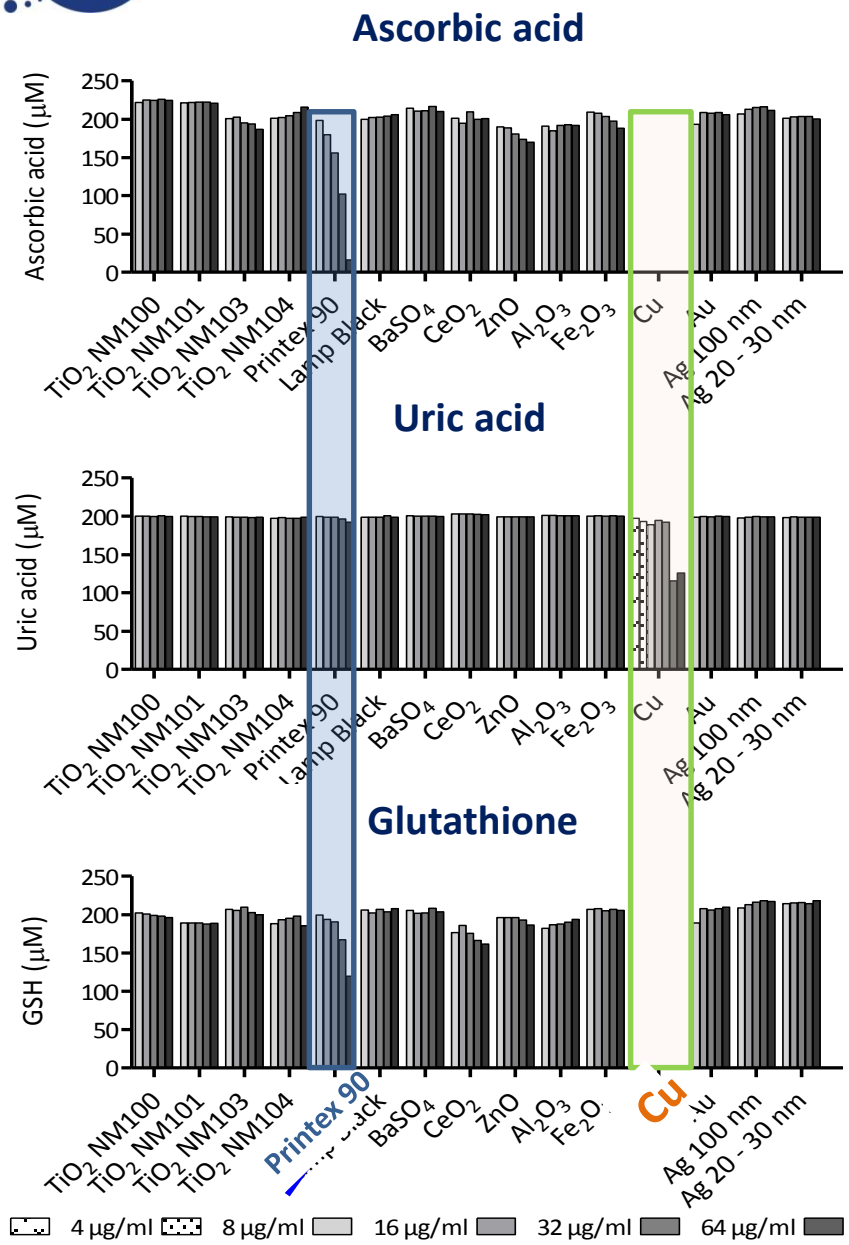
Quantification of antioxidants by HPLC

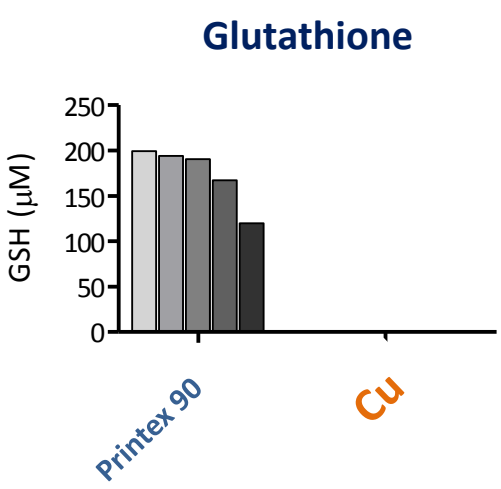


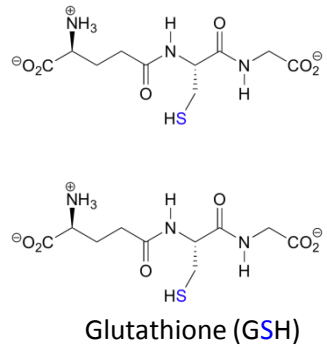
Antioxidants depletion

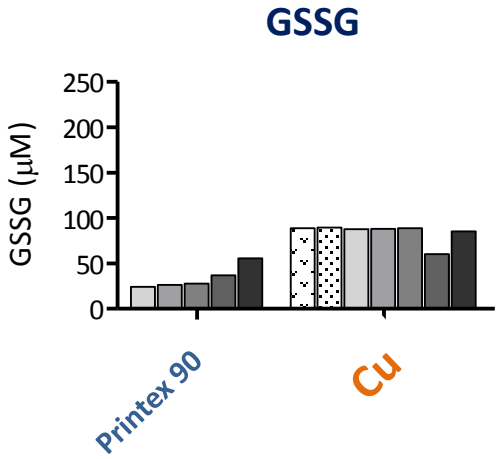


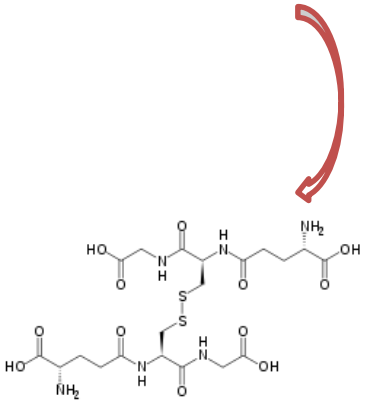
Antioxidants depletion









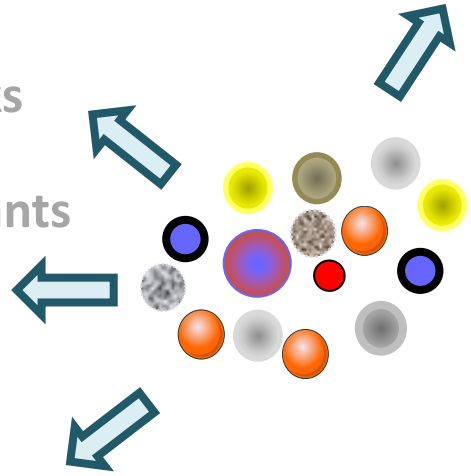


Intrinsic oxidant generation potential

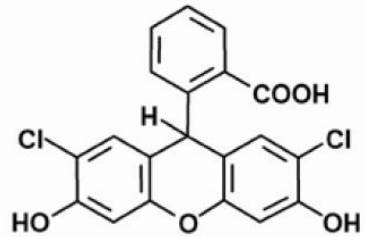
Induction of
DNA strand breaks

Depletion of antioxidants
in synthetic RTLF

Depletion of
dithiothreitol (DTT)

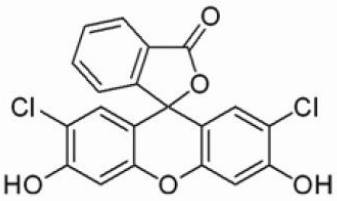


Oxidation of dichlorofluorescein

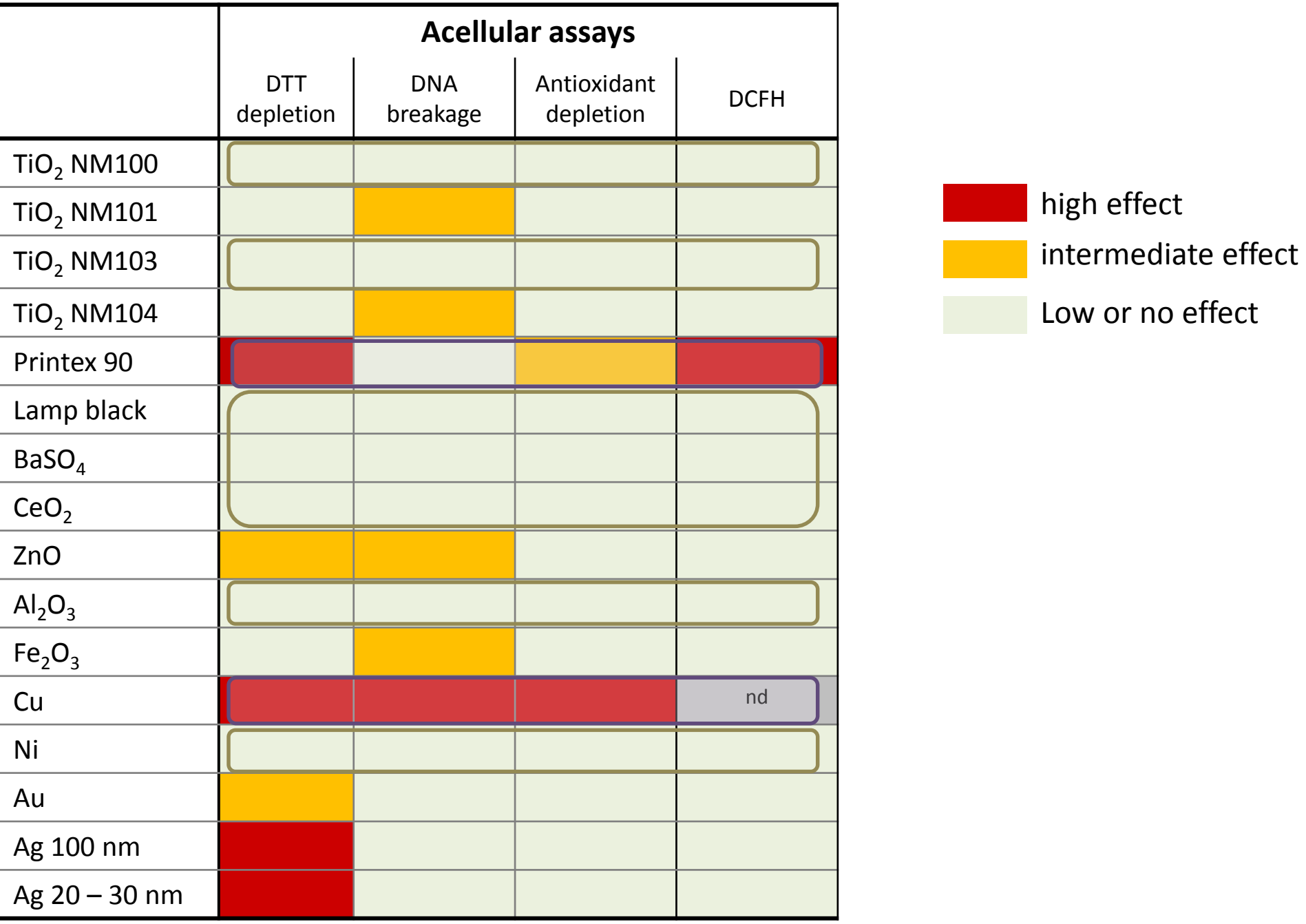


DCFH
(Non-Fluorescent)

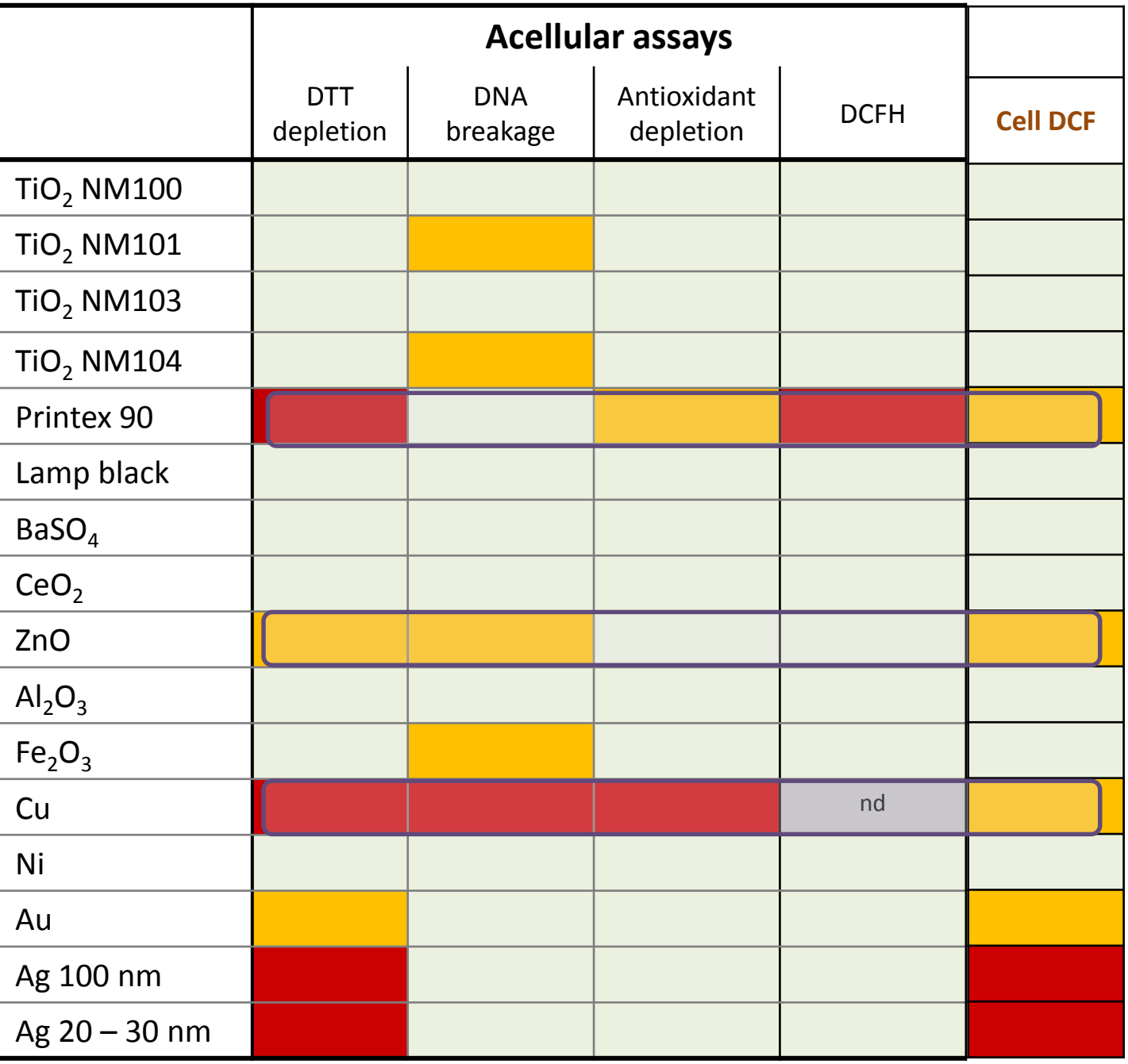
ROS

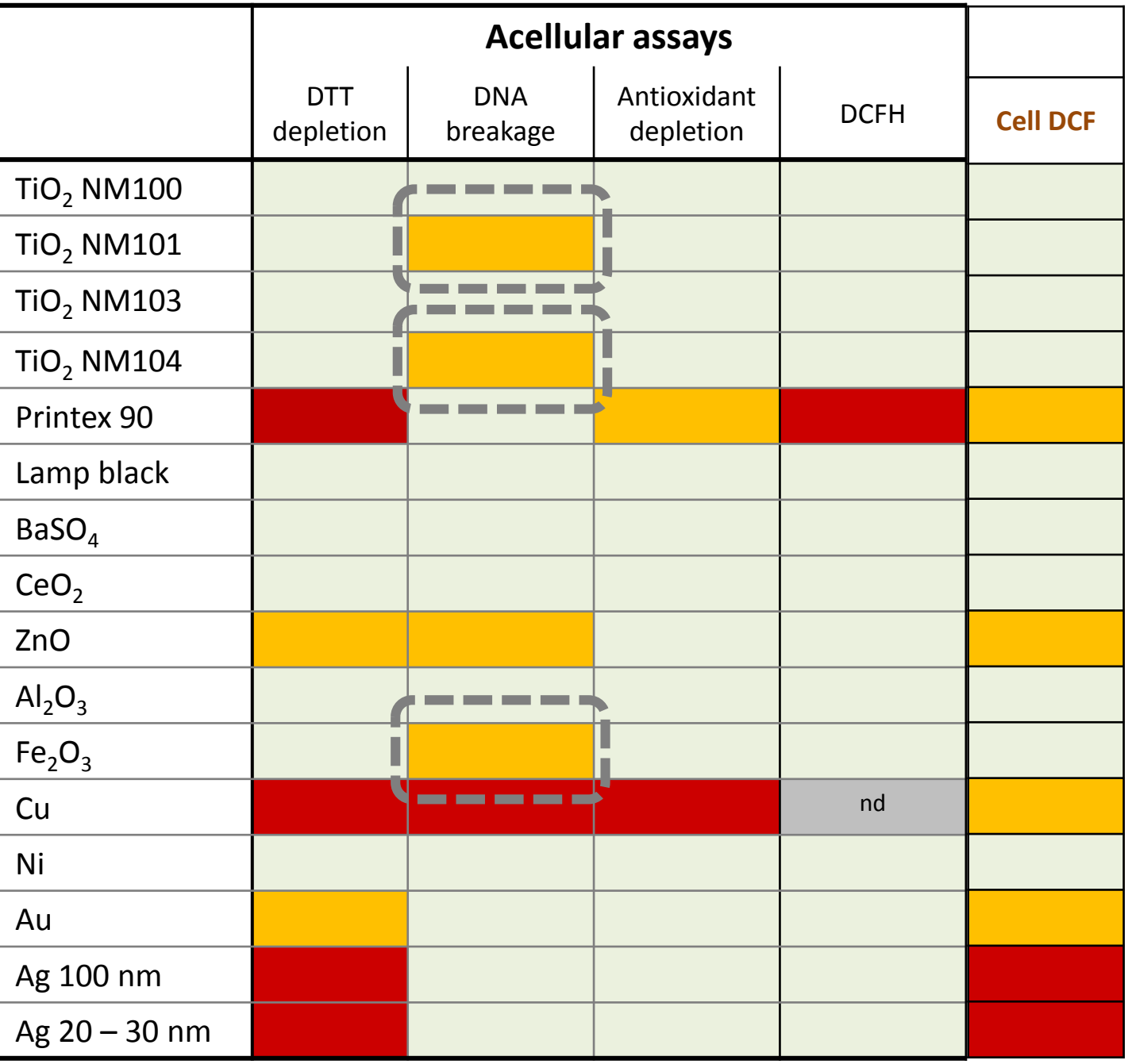


DCF
(Fluorescent)


Acellular assays
DTT depletion
DNA breakage
Antioxidant depletion
DCFH
TiO2 NM100
TiO2 NM101
TiO2 NM103
TiO2 NM104
Printex 90
Lamp black
BaSO4
CeO2
ZnO
Al2O3
Fe2O3
Cu
nd
Ni
Au
Ag 100 nm
Ag 20 – 30 nm
high effect
intermediate effect
Low or no effect

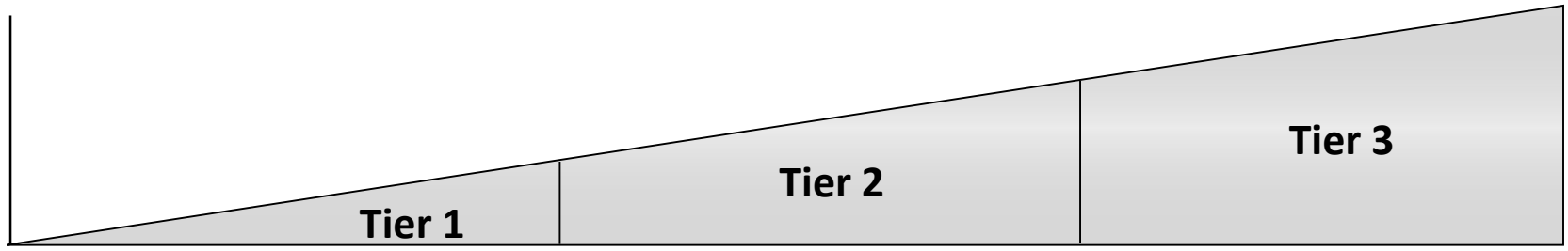
	Acellular assays				
	DTT depletion	DNA breakage	Antioxidant depletion	DCFH	**Cell DCF**
TiO_2 NM100					
TiO_2 NM101					
TiO_2 NM103					
TiO_2 NM104					
Printex 90					
Lamp black					
$BaSO_4$					
CeO_2					
ZnO					
Al_2O_3					
Fe_2O_3					
Cu				nd	
Ni					
Au					
Ag 100 nm					
Ag 20 – 30 nm					


Acellular assays
DTT depletion
DNA breakage
Antioxidant depletion
DCFH
Cell DCF
TiO2 NM100
TiO2 NM101
TiO2 NM103
TiO2 NM104
Printex 90
Lamp black
BaSO4
CeO2
ZnO
Al2O3
Fe2O3
Cu
nd
Ni
Au
Ag 100 nm
Ag 20 – 30 nm


Acellular assays
DTT depletion
DNA breakage
Antioxidant depletion
DCFH
Cell DCF
TiO2 NM100
TiO2 NM101
TiO2 NM103
TiO2 NM104
Printex 90
Lamp black
BaSO4
CeO2
ZnO
Al2O3
Fe2O3
Cu
nd
Ni
Au
Ag 100 nm
Ag 20 – 30 nm

	Acellular assays					LC 20 (µg/cm²)	
	DTT depletion	DNA breakage	Antioxidant depletion	DCFH	Cell DCF	NCI-H292	THP-1 macrophages
TiO_2 NM100						20	38
TiO_2 NM101						19	16
TiO_2 NM103						11	>40
TiO_2 NM104						8	>40
Printex 90						8	10
Lamp black						14	>40
$BaSO_4$						>40	>40
CeO_2						38	>40
ZnO						<1.25	<1.25
Al_2O_3						0.3	>40
Fe_2O_3						>40	>40
Cu				nd		8	6
Ni						37	>40
Au						>40	>40
Ag 100 nm						11	>40
Ag 20 – 30 nm						6	16

Cellular tiered response to nanomaterial exposure



Intensity of cellular response

Tier 1 **Antioxidant defense** *Nrf2 translocation* *HO-1 expression*	Tier 2 **Inflammation** *Cytokine expression* *(IL-8, IL-6, TNFα)*	Tier 3 **Cytotoxicity** *WST-1*
Cu Ag 20 - 30 nm ZnO = Ag 100 nm	Ag 20 - 30 nm Cu = Ag 100 nm Al_2O_3	Cu ZnO Ag 20 - 30 nm Al_2O_3 Printex 90
TiO_2 NM104 = Ni $Fe(III)_2O_3$ Printex 90 = Lamp black= Al_2O_3 = CeO_2	TiO_2 NM104 ZnO = Lamp black $Fe(III)_2O_3$ = CeO_2	Lamp black CeO_2 TiO_2 NM104
TiO_2 NM100/101/103 = Au $BaSO_4$	Printex 90 = TiO_2 NM101 $BaSO_4$ = TiO_2 NM100/103	TiO_2 NM100 /101 Ni TiO_2 NM103 = Ag 100 nm $Fe(III)_2O_3$ = $BaSO_4$

Conclusions

Excepting Cu NP, no NP is positive for all the acellular assays

↳ Each assay reveals specific properties of NP

DTT and DNA breakage assays allow discriminating a larger number of NP

↳ DTT: fast assay, highthrouput

↳ DNA breakage: semi-quantitative, time consuming

DCFH probe: poor correlation between acellular and cellular response

↳ Intracellular ROS production (tier response)

Cellular DCFH assay and cytotoxicity globally well correlated to DTT assay

Soluble group (Cu, ZnO, Ag) / Carbonaceous and insoluble group / Metal oxides /Ni and NM100 separate

Generation of ROS based NM data base

Steps towards harmonised measurement methods via public SOPs

nanOxiMet

http://www.nanoximet.eu/

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Safe Implementation of Innovative Nanoscience and Nanotechnology

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Institute for Energy and Environmental Technology, Duisburg, Germany

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