

Université

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Positive surface charge: a predictive factor for nanoparticle toxicity ?

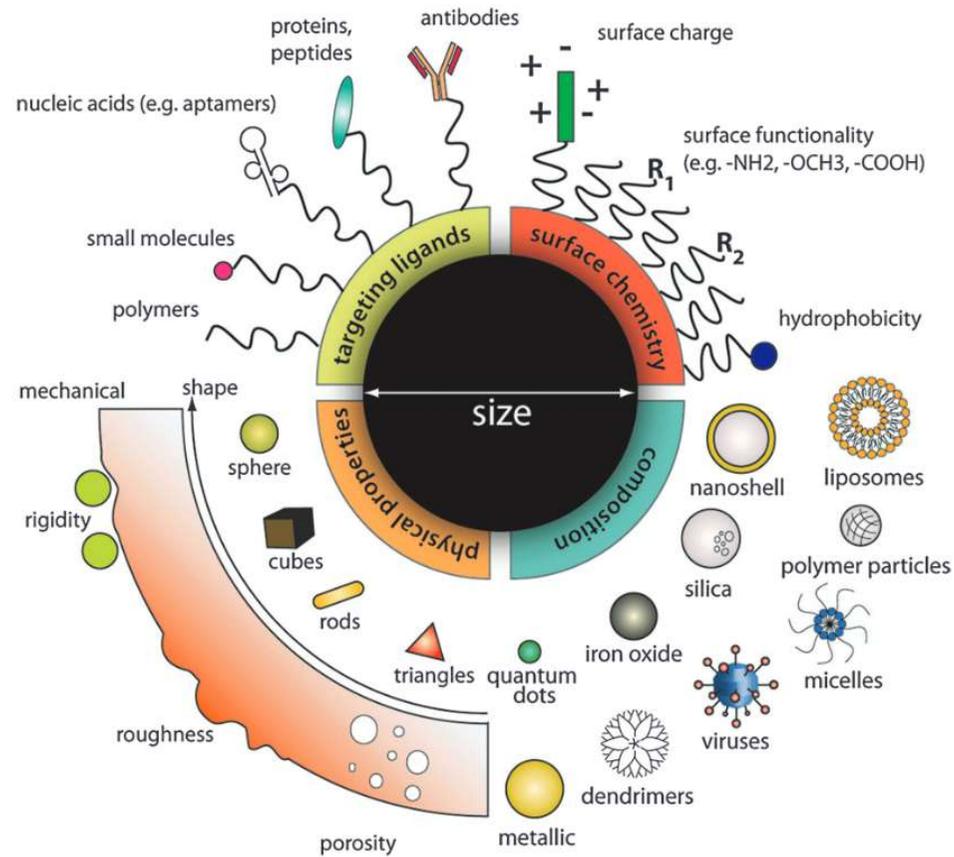
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Background

Physicochemical characteristics of manufactured nanoparticles



Background

Role of NP physicochemical characteristics on their safety : *In vitro* screening of a library of carbon dots (CD)

CD properties

Carbon-based NPs
Spherical
Few nm size
Easy to synthesize
Tunable surface chemistry
Intrinsic fluorescence

CD synthesis

Carbon source
(Citric acid, Glucose...)

+

Passivation agent
(EA, PEHA, DMEDA,
PEI25k, PEI600, PEG)

+

Catalysts
(HCl, H₃PO₄, H₂SO₄, ...)

↓
Microwave irradiation
Solvothermal
Pyrolysis
Post-functionalisation

Library of 34 NPs

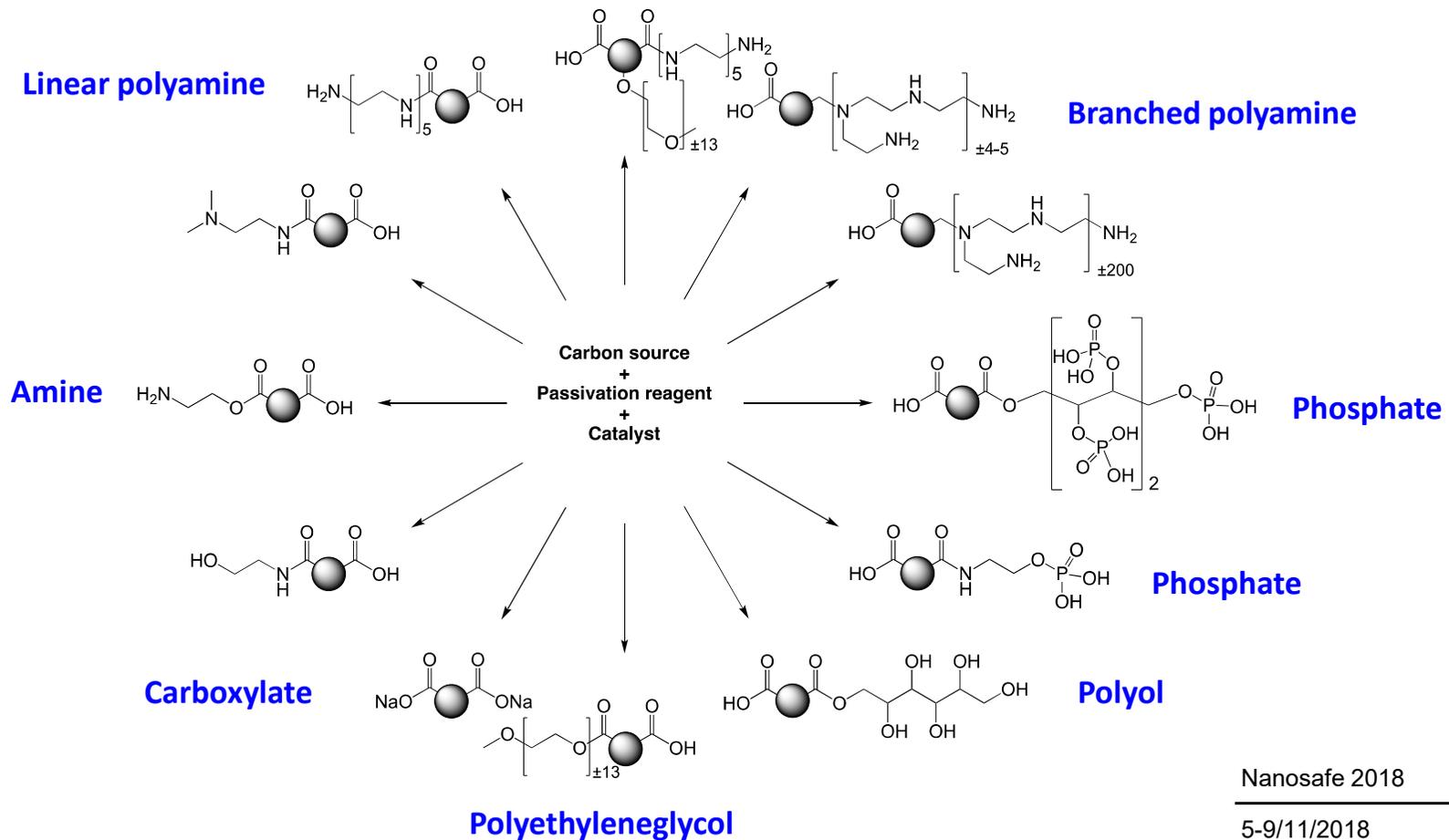
CD physicochemical characteristics

Zeta potential (DLS)	-44 mV to +41 mV
Size (DLS)	7 nm to 284 nm
Chemical composition	N (%) = 0-15 C (%) = 20-54 H (%) = 2-9

Background

Role of NP physicochemical characteristics on their safety :
In vitro screening of a library of carbon dots (CD)

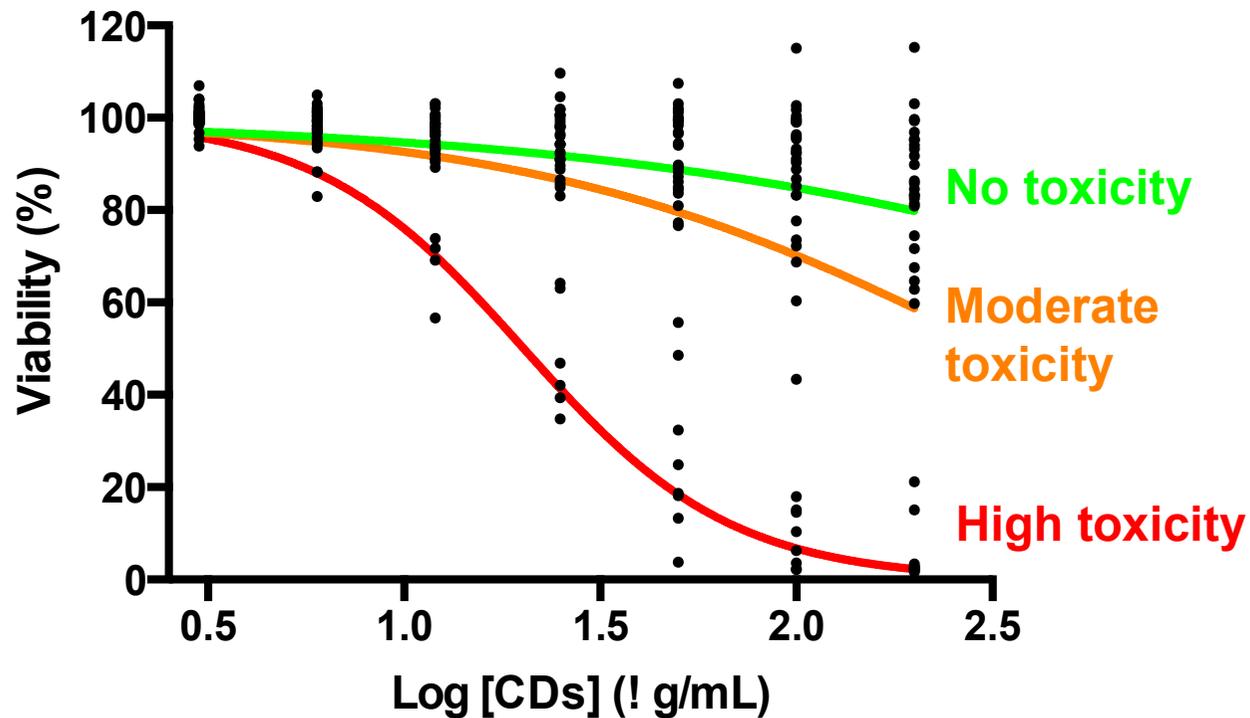
Diversity of surface chemistry of the library



Background

Role of NP physicochemical characteristics on their safety :
In vitro screening of a library of carbon dots (CD)

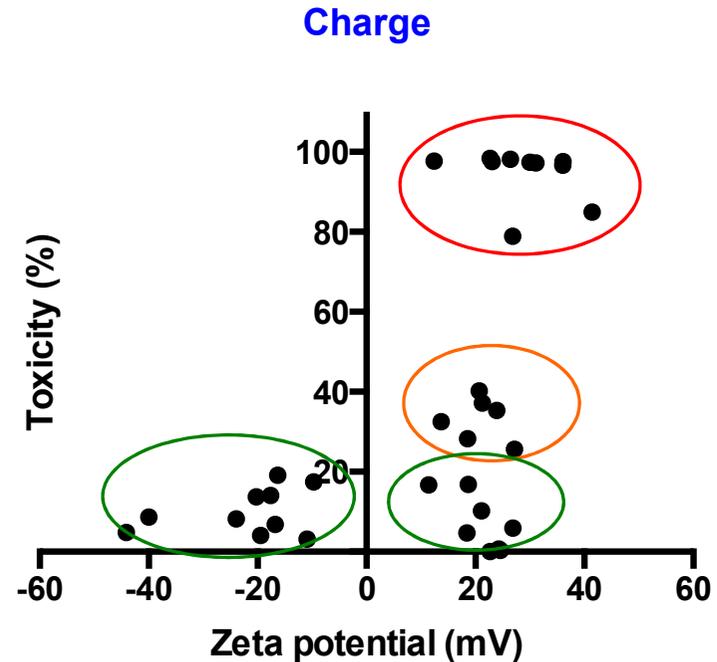
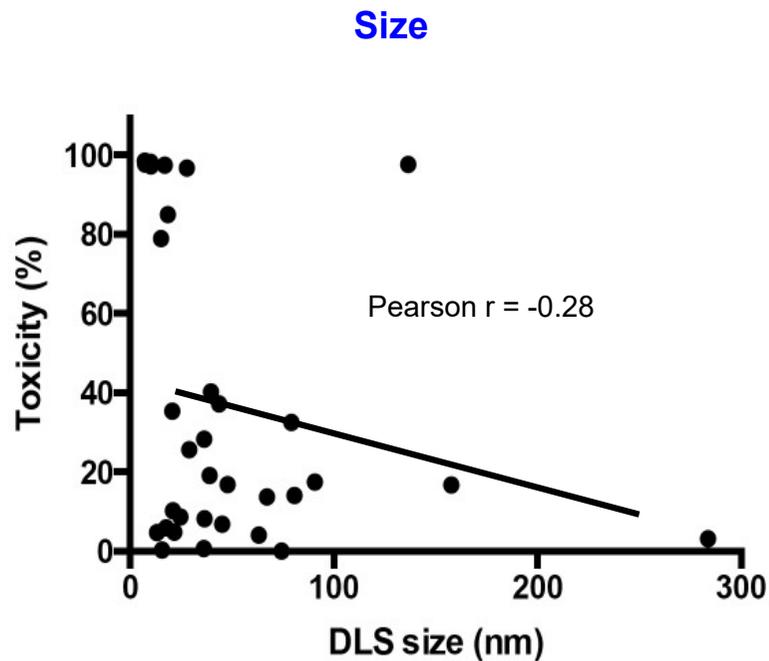
Toxicity of the library on THP-1-derived macrophages



Background

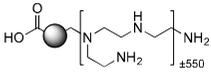
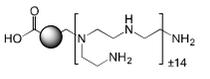
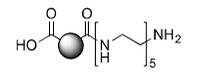
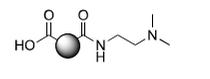
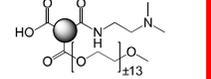
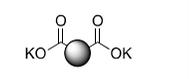
Role of NP physicochemical characteristics on their safety : *In vitro* screening of a library of carbon dots (CD)

Role of size and charge on the NP cytotoxicity



Aim of the study

To get further insight into the link between NP cationic charge and toxicity :
in-depth toxicity study on a focused NP library

	NP1	NP2	NP3	NP4	NP5	NP6
Reactants	Citric acid bPEI 25K	Citric acid bPEI 600	Citric acid PEHA	Citric acid DMEDA	Citric acid DMEDA mPEG 550	Citrate NH ₄ ⁺ KH ₂ PO ₄
Surface chemistry						
Size (nm) <i>(pH 7.4, saline solution)</i>	13 ± 3	10 ± 2	19 ± 4	44 ± 10	18 ± 4	25 ± 5
Charge (mV) <i>(pH 7.4, saline solution)</i>	+43 ± 9	+25 ± 3	+30 ± 2	+21 ± 2	+27 ± 2	-40 ± 1



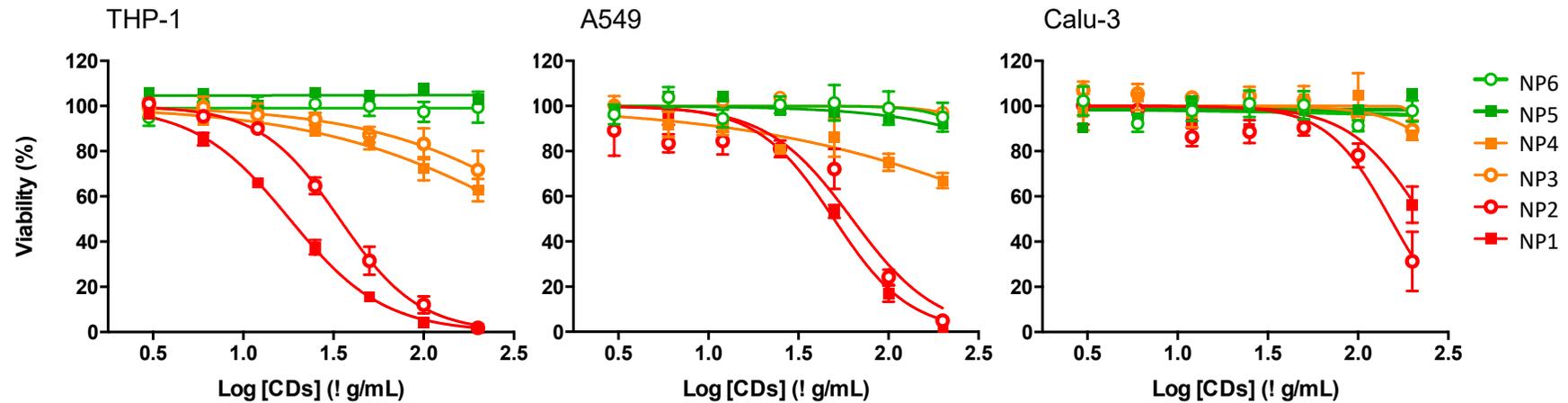
Macrophages / Airway epithelial cells
(THP-1 / A549, Calu-3)

Cell viability (*MTT assay*)
NP internalization (*FACS, CLSM*)
Inflammation (*Mutli-Cytokine Array and ELISA*)
Oxidative stress (*GSH assay*)
Mitochondrial perturbation (*JC-10 probe*)

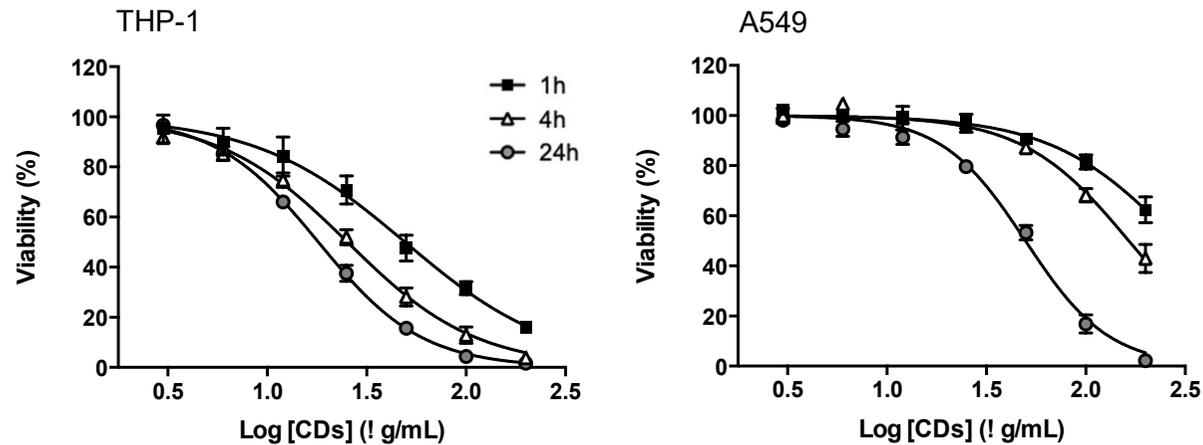
Results

Effect of the NP on cell viability

Dose-dependent effect of the six NPs (24h)

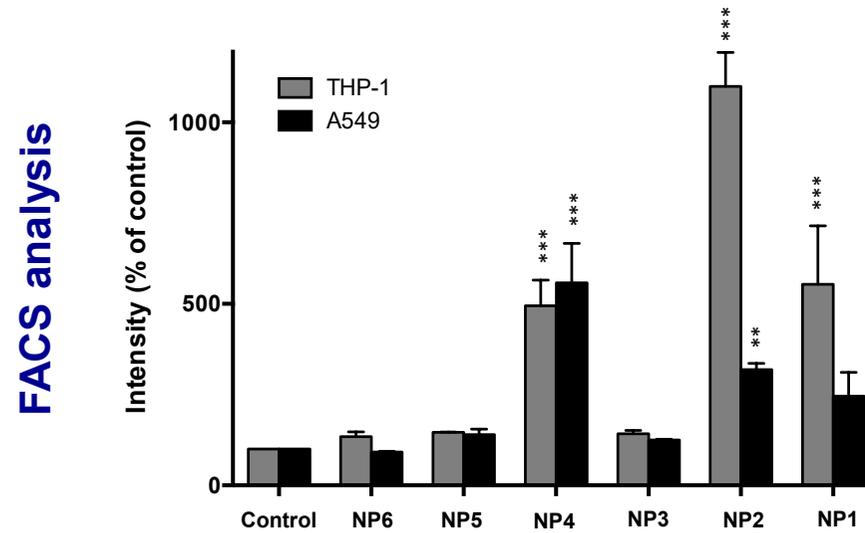


Time-dependent effect of NP1

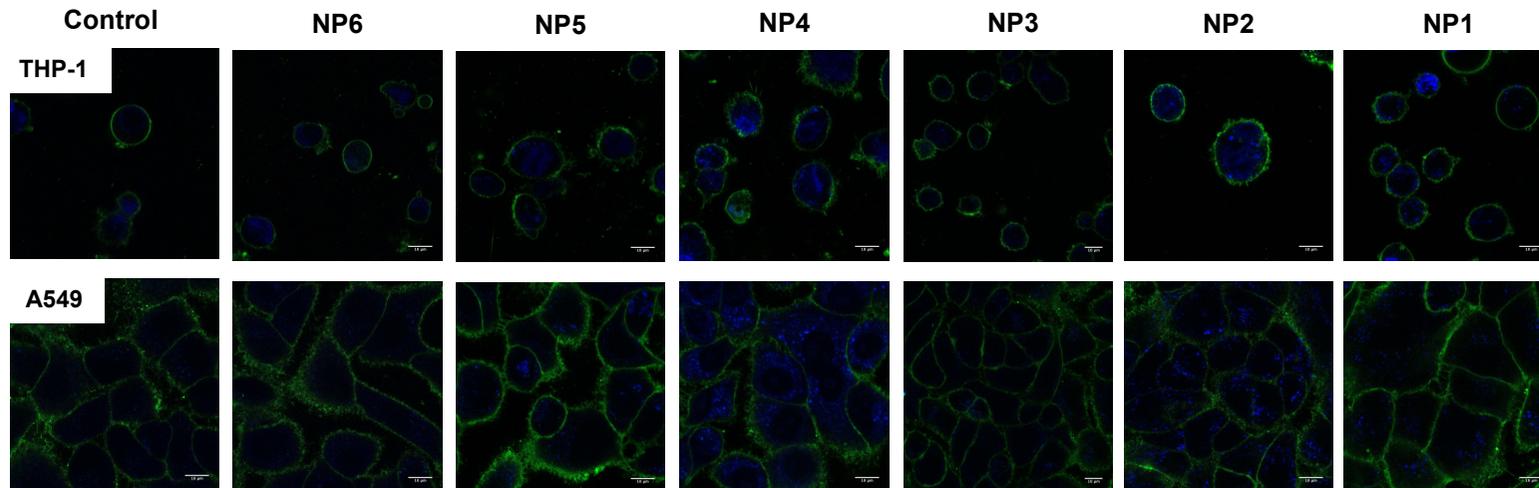


Results

Cell internalization of the NPs (4h)



**Confocal laser scanning
microscopy**



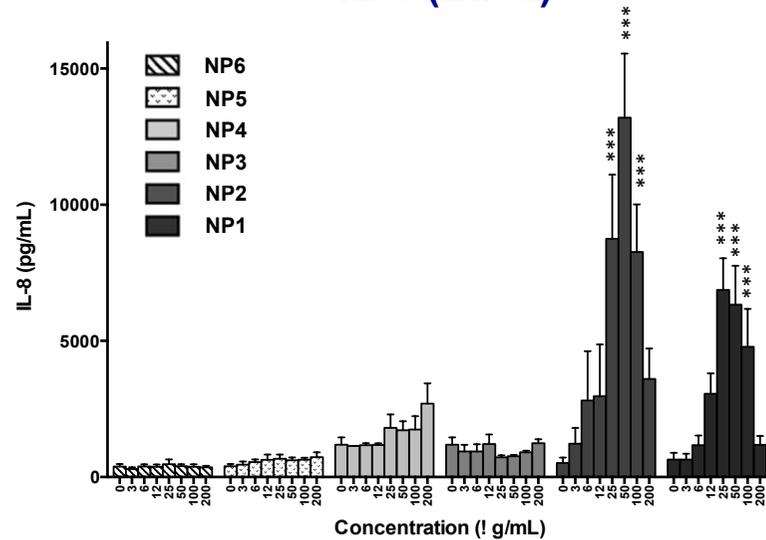
Results

Inflammation evoked by the NPs (THP-1)

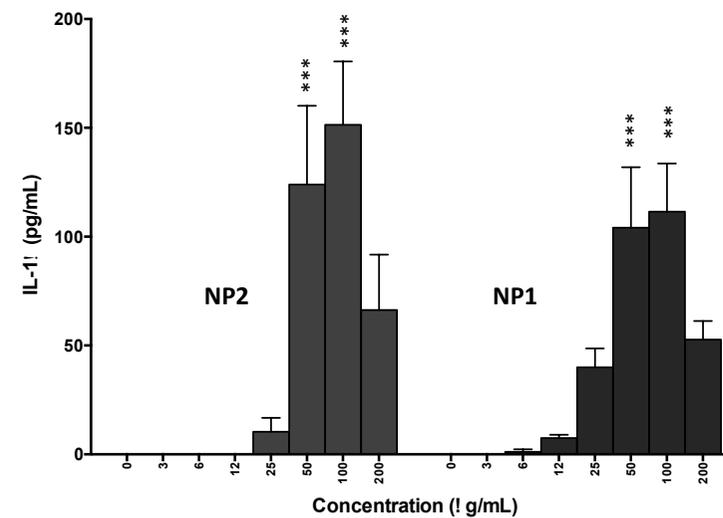
Multi-cytokine Array

	NP6	NP5	NP4	NP2	NP1
TNF- α	nd	nd	nd	nd	nd
IL-6	nd	nd	nd	nd	nd
IL-12	nd	nd	nd	nd	nd
IL-17	nd	nd	nd	nd	nd
Eotaxin	nd	nd	nd	nd	nd
MDC	-	-	-	-	-
IL-1 β	-	-	-	+++	++
MIP-1 α	-	-	+	+++	++
MIP-1 β	-	+	+	+++	++
MCP-1	-	-	+++	++	+++

IL-8 (Elisa)



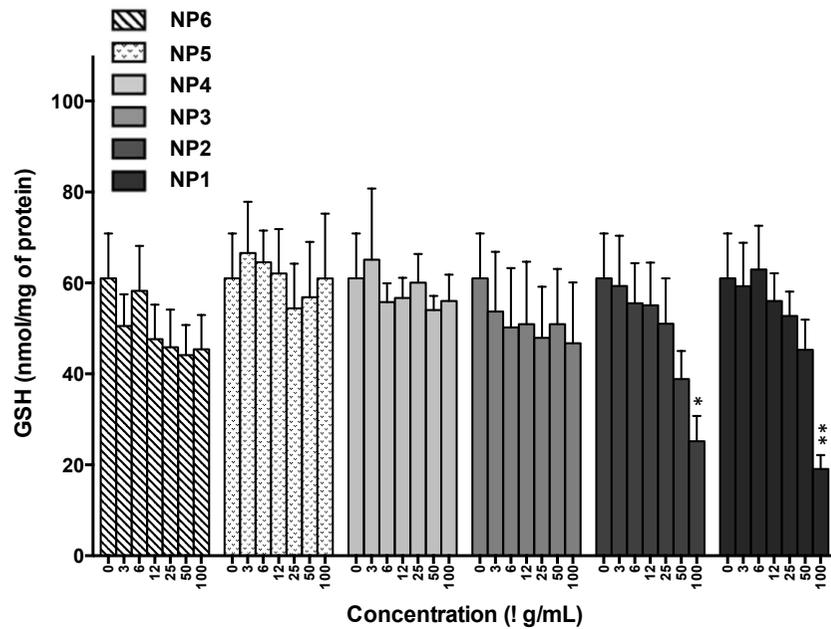
IL-1 β (Elisa)



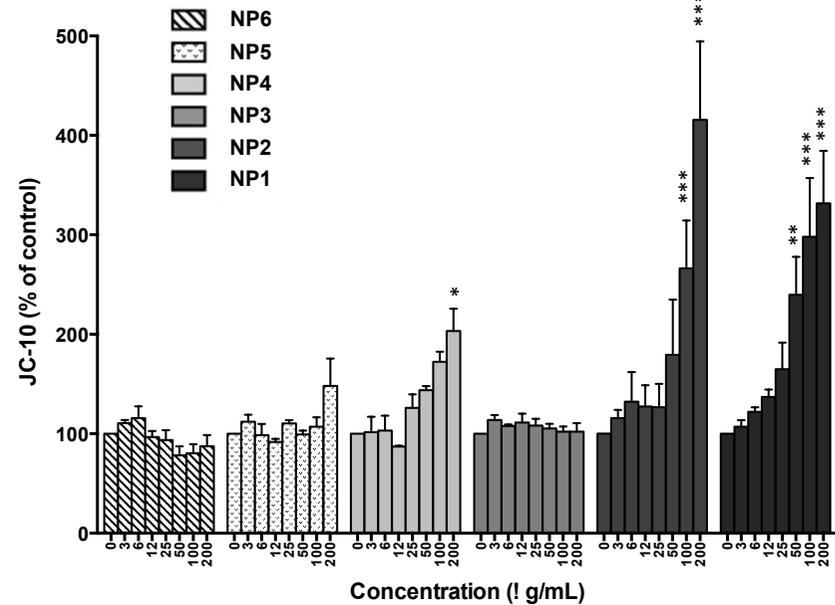
Results

Oxidative stress and mitochondrial perturbation (THP-1)

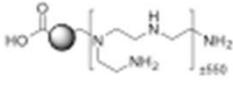
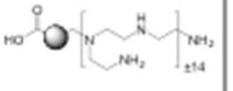
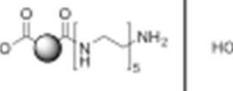
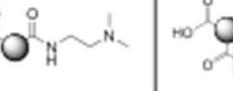
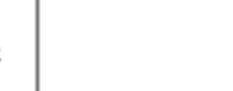
Oxidative stress (GSH)



Mitochondrial membrane potential (JC-10)



Summary

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Charge <i>(mV, saline solution)</i>	+43 ± 9	+25 ± 3	+30 ± 2	+21 ± 2	+27 ± 2	-40 ± 1
Cytotoxicity <i>(MTT assay)</i>	High	High	Moderate	Moderate	None	None
Oxidative stress <i>(reduced GSH)</i>	Yes	Yes	None	None	None	None
Inflammation <i>(IL-8)</i>	Yes	Yes	None	Moderate	None	None
Inflammasome <i>(IL-1 beta)</i>	Yes	Yes	-	None	None	None
Mitochondrial perturbation <i>(JC-10)</i>	Yes	Yes	None	Moderate	None	None
Cell uptake <i>(FACS, Imaging)</i>	Yes	Yes	No	Yes	No	No

Conclusion

Using a library of carbon nanoparticles, 3 cell types and multiple toxicity endpoints, we found that :

- The charge is not sufficient to predict cytotoxicity of NPs
- But, the charge together with the NP surface chemistry are predictive factors.



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