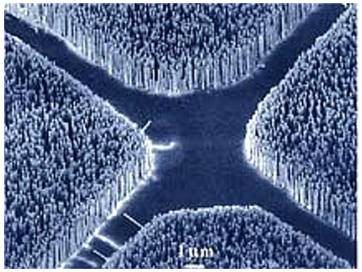

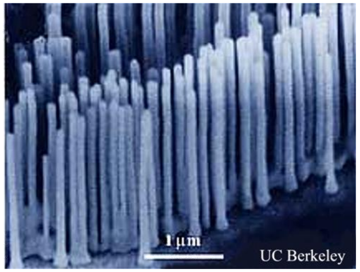

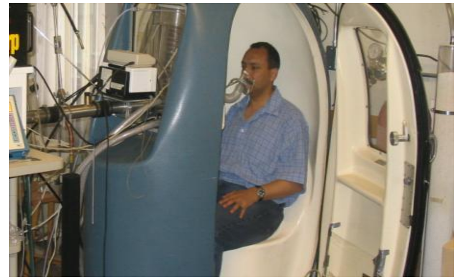
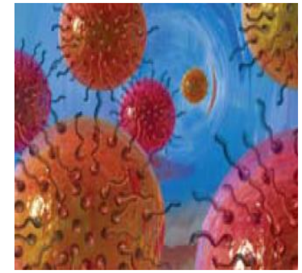
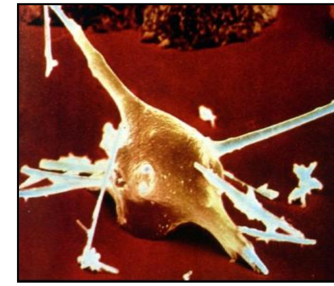

A "Safe by Design" Concept for Flame-generated Nanomaterials

Philip Demokritou[1], Samuel Gass[1,2], Georgios Pyrgiotakis[1], Joel M. Cohen[1], Georgios Sotiriou[2], Sotiris Pratsinis[2]

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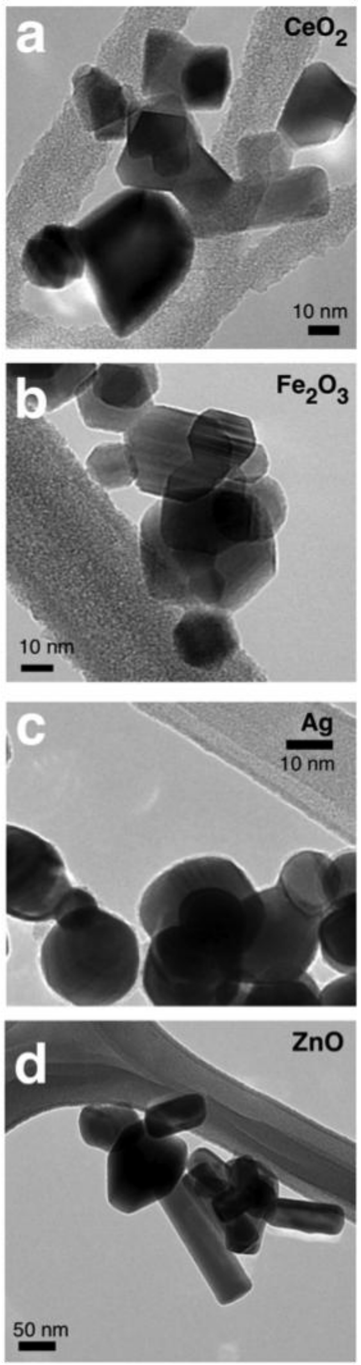
Swiss Federal Institute of Technology Zurich

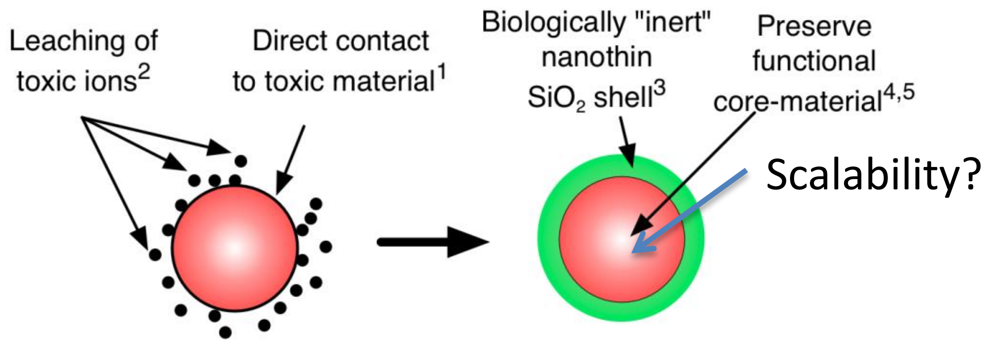
NIOSH

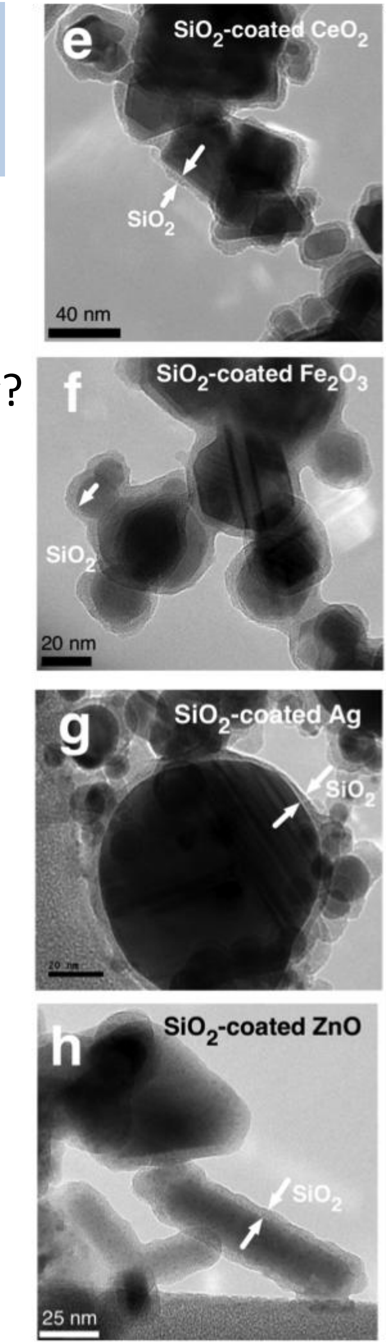
Background Information

- The likely success or failure of NT industry depends on nano-EHS matters.
- Research on "safe by design" approaches is lacking behind but is considered crucial to the sustainability of the nanotech industry.
- Flame generated Me and MeO ENMs are currently the most widely used by volume, family of ENMs (90% by volume).
- Me/MeO: Many applications in cosmetics, building materials, antimicrobial applications, etc



A Safer Formulation Concept for flame-generated ENMs





Study Objectives:

Objective 1: Develop and Validate the concept for a comprehensive nanopanel of industry-relevant ENMs (CeO2, ZnO, Fe2O3, Ag).

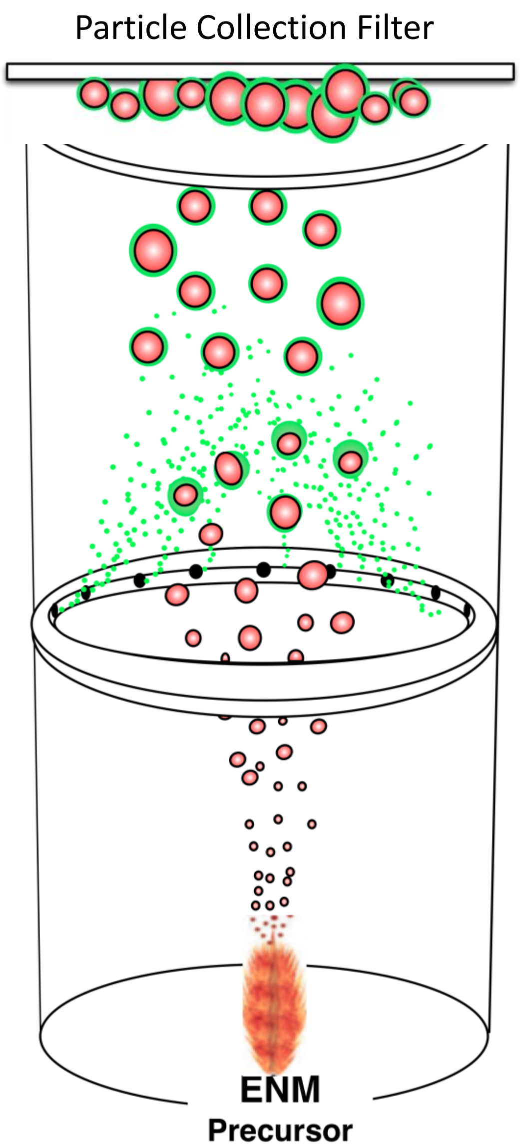
Objective 2: Understand the coating fundamentals and assess coating efficiency. Characterize Physicochemical and morphological properties of SiO2 coated and uncoated ENMs

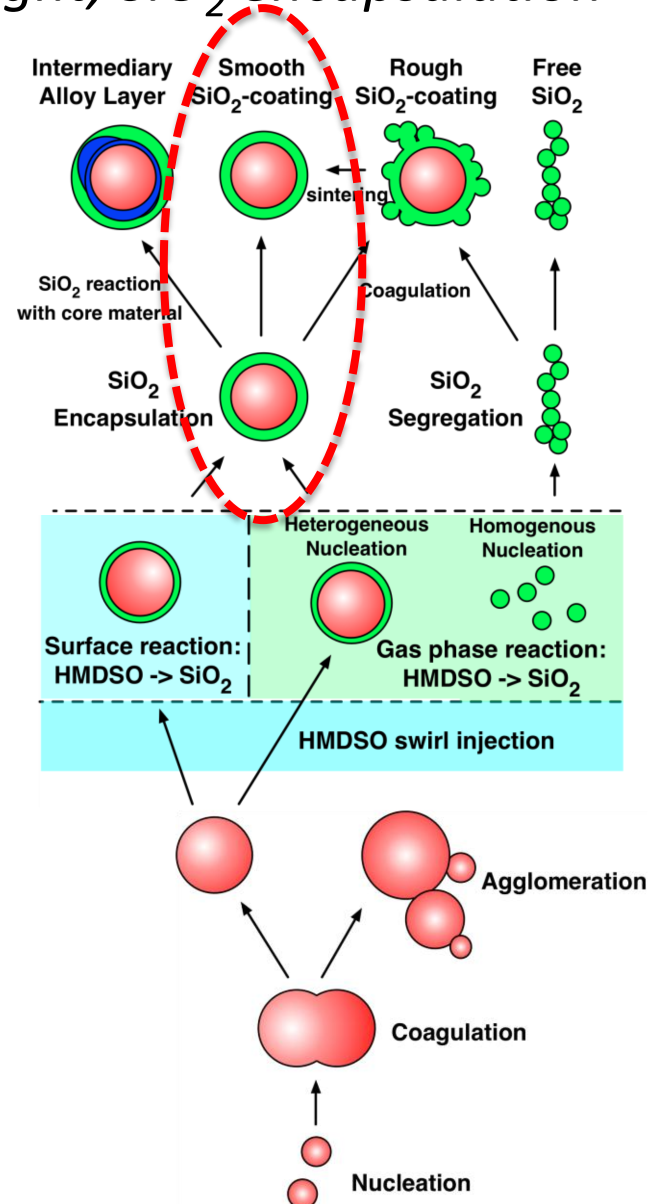
Objective 3: Assess the effect of SiO2 coatings on nano-bio interactions using state of the art in-vitro cellular and in-vivo bio-assays

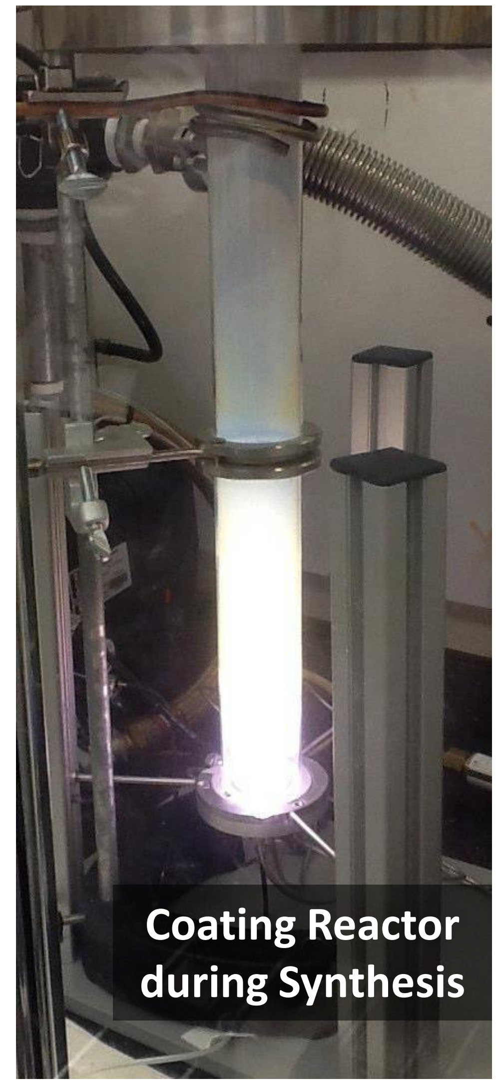
(1) Sotiriou et al., Curr Opin Chem Eng **2011**, 1, 3 – 10
(2) Xia et al., ACS Nano **2011**, 5, 1223 – 1235
(3) Napierska et al., *Particle and Fibre Toxicology* **2010**, *7*,39
(4) Teleki et al., *Chem. Mater.* **2009,** *21,* 2094–2100
(5) Sotiriou et al., *Adv. Funct. Mater.* **2010,** *20,* 4250–4257

Methods (1/5) : Synthesis using the VENGES platform

Scalable, One Step, In-flight, SiO_2 encapsulation







(1) Demokritou et al. Inh. Toxicology, 2010

(2) Buesser et al., Chem Eng Sci **2011**, 65, 5471-5481

Methods (2/5): State of the art P-c-m Characterization
(powder, liquid, aerosol)

As *nanopowder*:

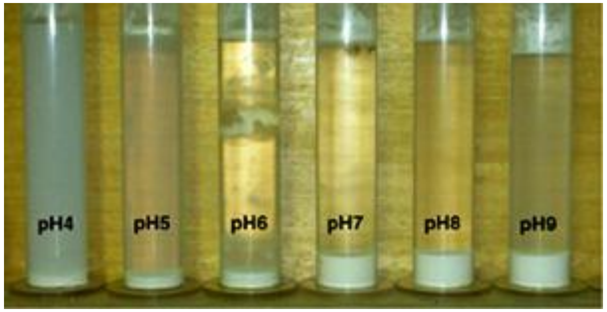
X-ray Diffraction XRD (Crystal Structure):
- Crystal phase and composition
- Crystal Size

BET N_2 adsorption:
- Specific surface area (m^2/g)

X-ray Photoelectron Spectroscopy (XPS):
- Quantifiable elemental surface composition & coating efficiency

Isopropanol Chemisorption was also used to validate coating efficiency

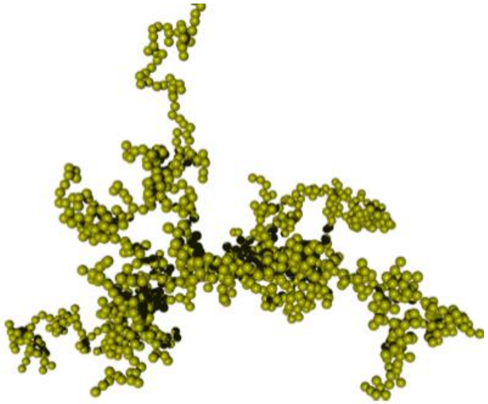
TEM/SEM for morphology



In *liquids* (DI, bio media):

Dynamic Light Scattering:
- Hydrodynamic diameter
- Zeta-potential

Agglomeration potential-Effective density (DeLoid et al, in preparation)



As *aerosol*:

Scanning Mobility Particle Sizer (SMPS):
- Mobility diameter (distribution)
- Particle concentration

MOUDI Cascade impactor:
- Mass size distriibution

Methods (3/5): Nano-cellular interactions

Assessing SiO_2 coating influence on ENM-bio interactions in-vitro.

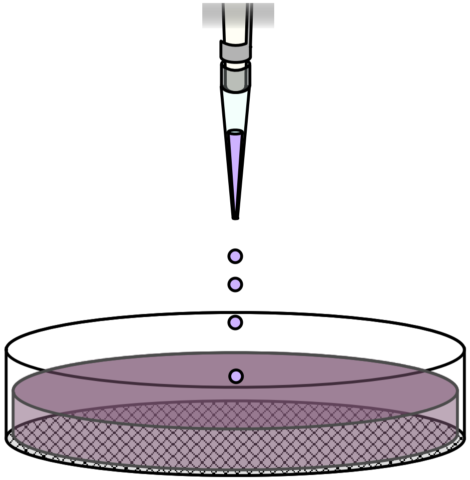
Multiple Cell lines : *A549, CALu3, Primary AM*

ENM nanopowder dispersion and dosimetry according to Cohen et al., 2012

Multiple Assays: *MTT, WST* (metabolic activity)*, LDH* (membrane integrity)*, etc*

Exposure Period: 24 h

Administered doses: 6.25, 12.5, 25.0, 50.0, 100.0 mg/ml

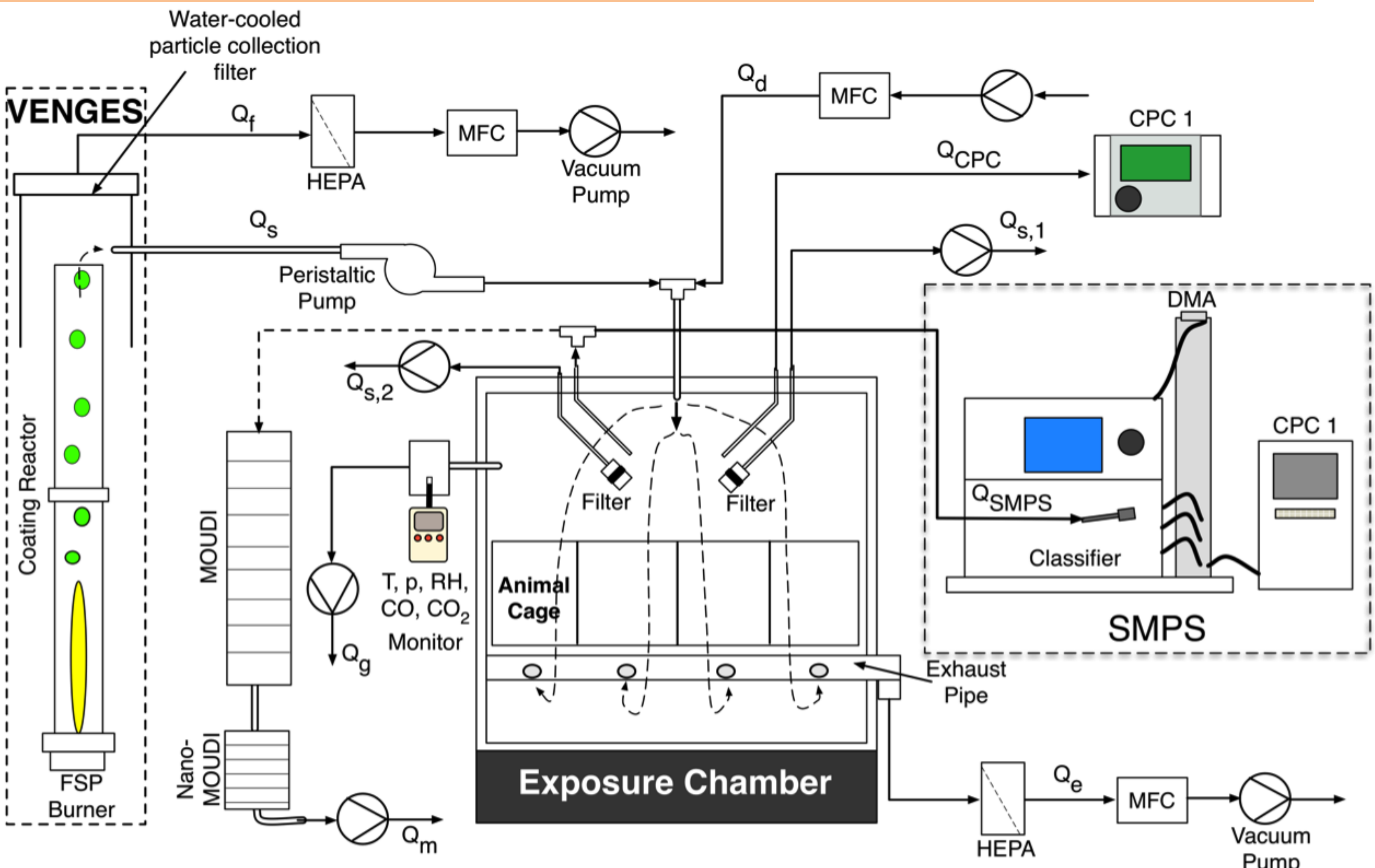


(1) Cohen et al. Nanotoxicology **2012**, doi:10.3109/17435390.2012.666576

Methods (4/5): In-vivo validation for CeO2

Assessing SiO_2 coating influence on ENM-bio interactions <u>in-vivo</u>.

Inhalation Study Exposure system (CeO2 case study):



(1) Demokritou et al., Nanotoxicology, 2012

Methods (5/5): In-vivo validation for CeO2

Assessing SiO_2 coating influence on ENM-bio interactions <u>in-vivo</u>.

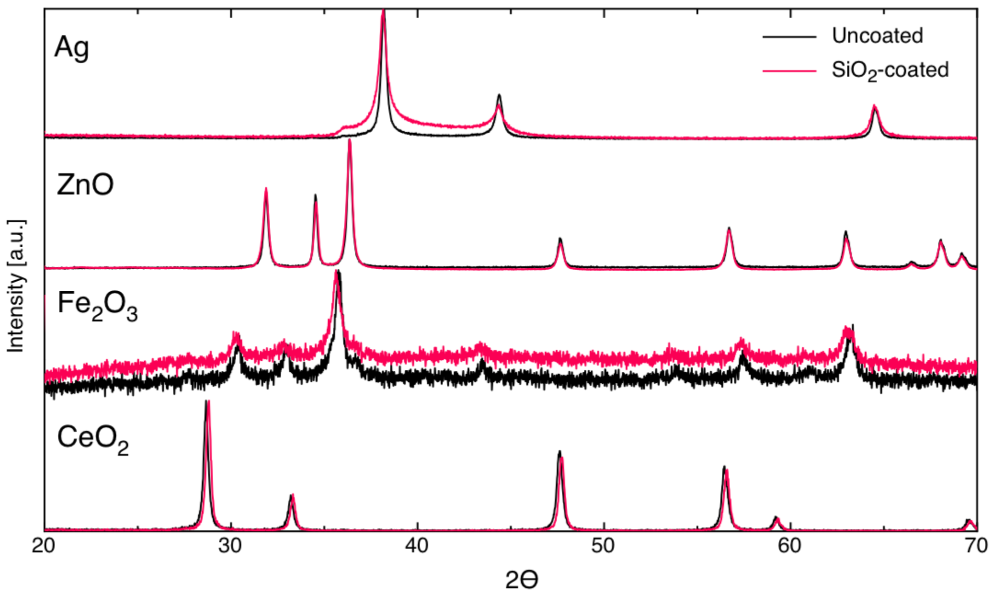
Study Design:

- Animal Model: Male Sprague-Dawley rats
- The animals (n = 6/group) were exposed to either SiO_2-coated CeO_2, uncoated CeO_2 or particle-free environments (controls)
- Exposure : 2 h/day, 4 days.
- BAL biochemical analysis at 24 h post exposure.
- Inflammatory and cytotoxicity biomarkers: PMNs, LDH alveolar macrophages (AM), and albumin)
- **Special considerations:** Match deposited to lungs dose for both coated and uncoated aerosol s. (How? We matched both aerosol mass concentrations and size distribution for SiO_2-coated and uncoated CeO_2)

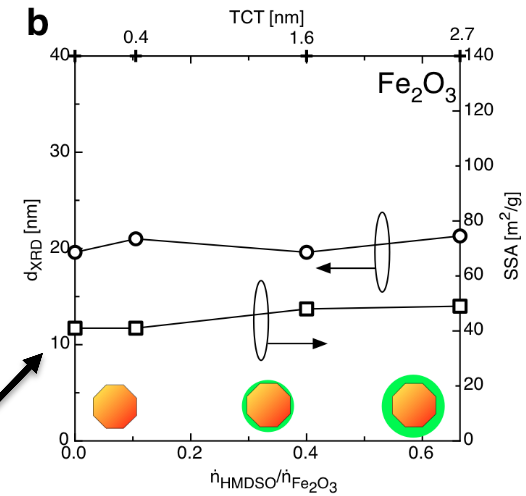
Results (1/5): Synthesis and Characterization

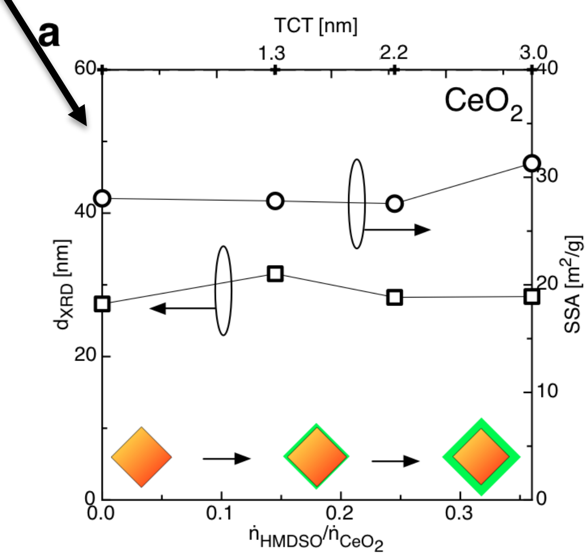
Assessing SiO_2 coating influence on core material crystal structure.

X-Ray Diffraction



Independent control over crystal size and coating thickness

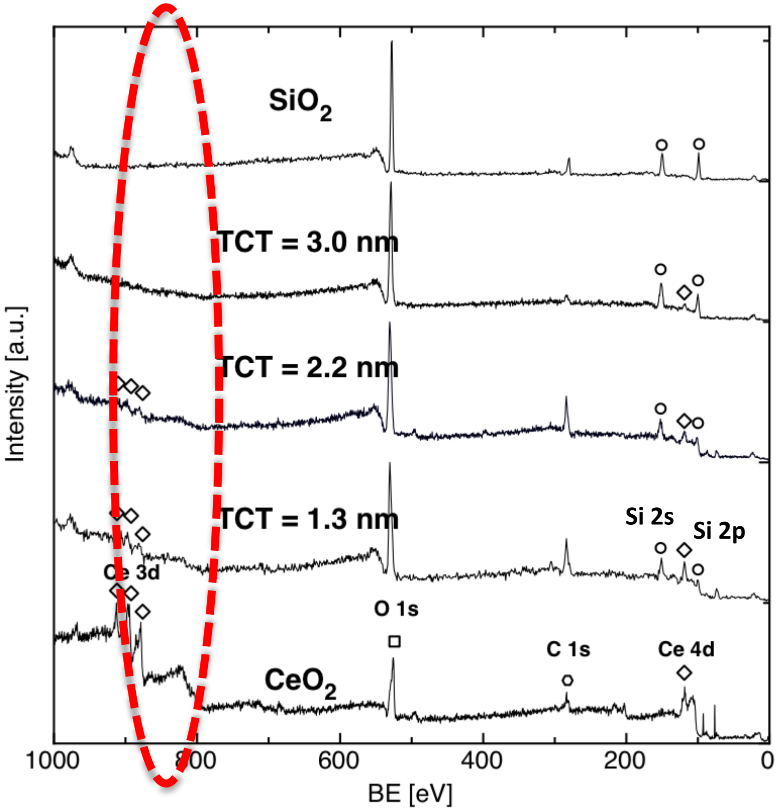




SiO_2-coating has **minimal effect on core crystal structure** (heterogenous nucleation) (Gass et al., Manuscript in submission)

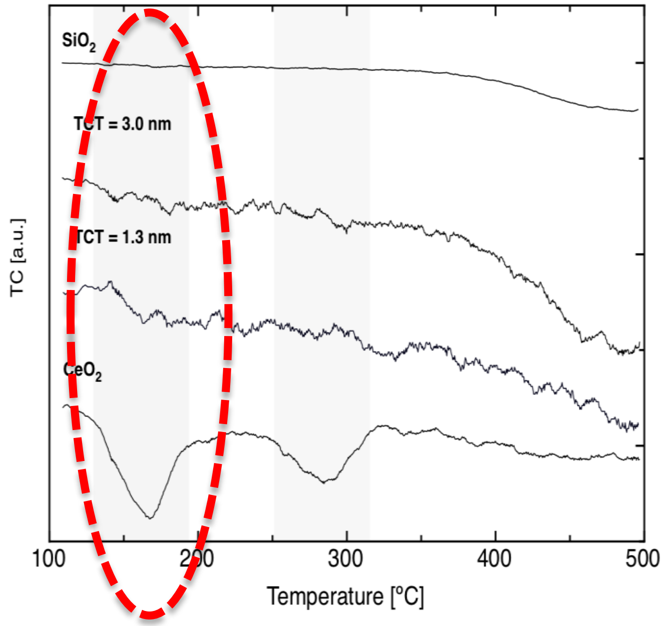
Results (3/5): Assessing SiO2 coating efficiency qualitatively and quntitatively

XPS (CeO2):

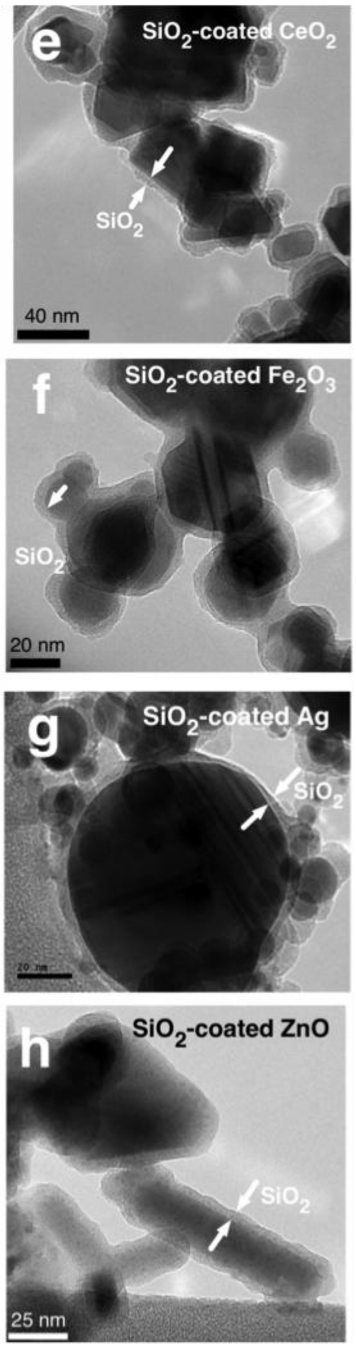


Core electron transitions (Ce 3d) **disappears** for TCT = 3 nm
-> full encapsulation

Isopropanol Chemisorption (CeO2)



Isopropanol desorption bands disappear for TCT = 3 nm
-> full encapsulation



Results: In-vitro Cellular data

Assessing SiO_2 coating influence on ENM-cellular interactions in-vitro (A549, THP1 human macrophages).

LDH:

MTT:

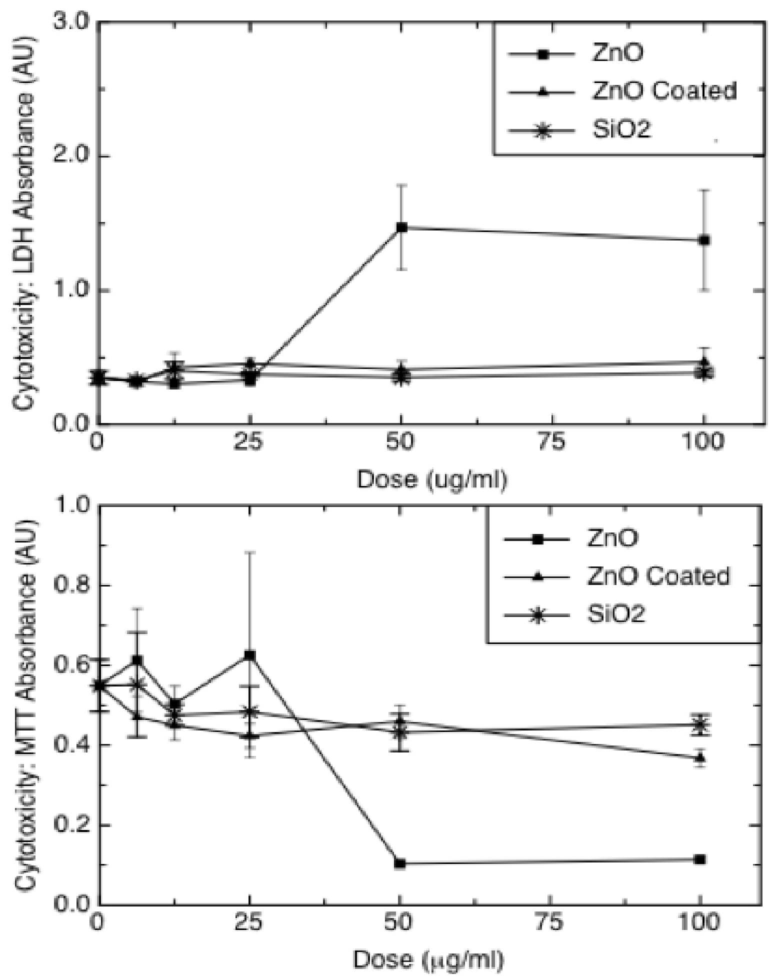
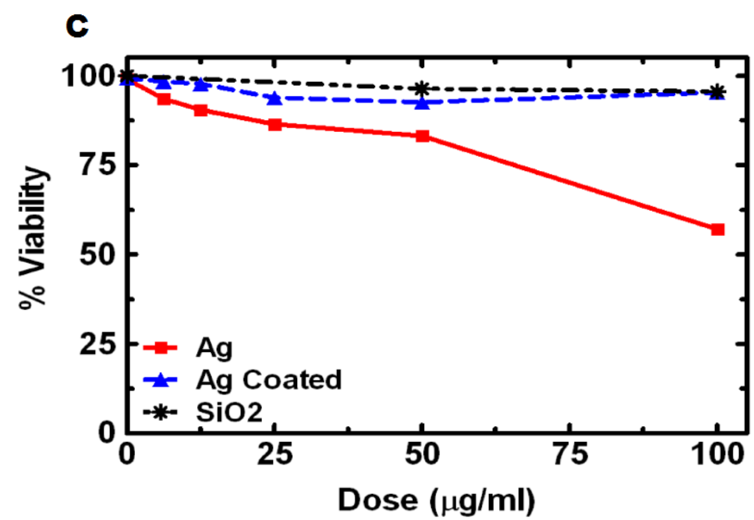


Figure 8: Cytoxicity results for ZnO and SiO_2 coated ZnO .

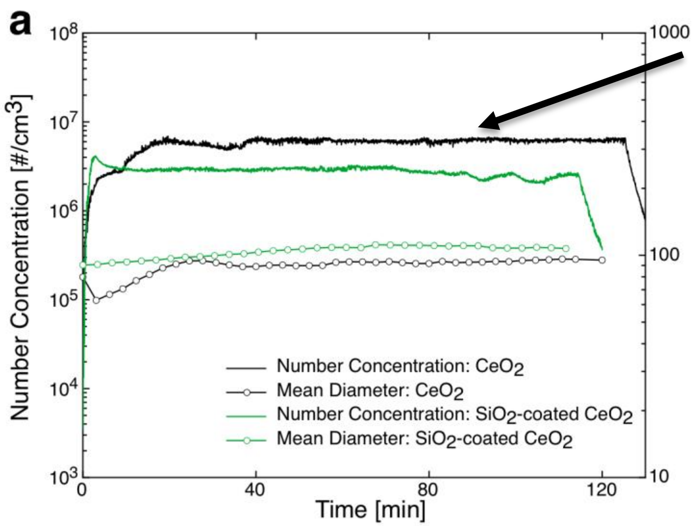


- ❑ SiO2 coated ENMs have lower toxicological properties as indicated in in-vitro cellular assays

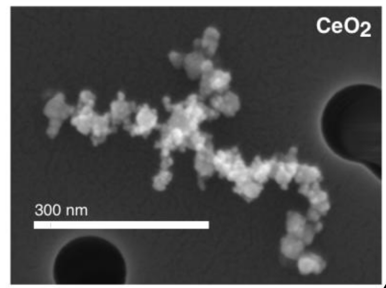
(1) Kroll et al., *Particle and Fibre Toxicology* **2011**, 8, 9

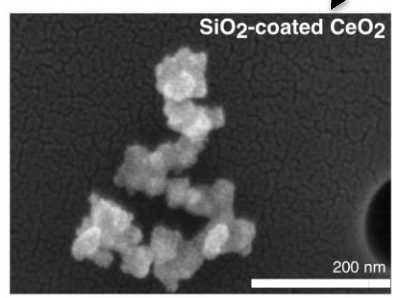
(2) Xia et al., ACS Nano **2011**, 5, 1223 – 1235

Results: In-vivo data-CeO_2 inhalation study

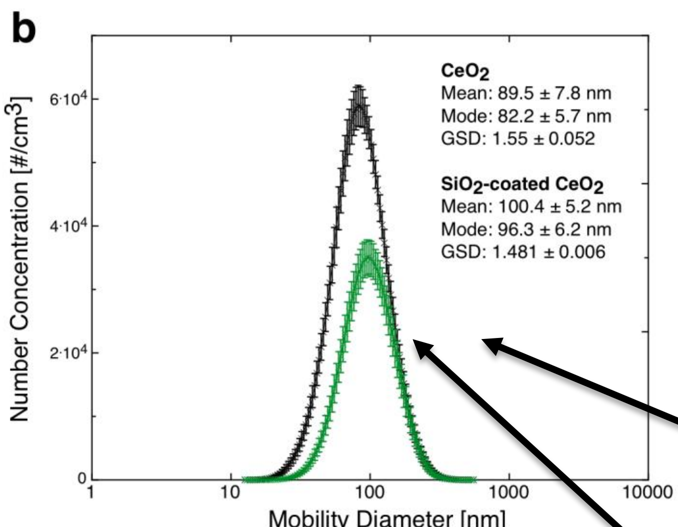


Very stable exposure atmospheres (VENGES)

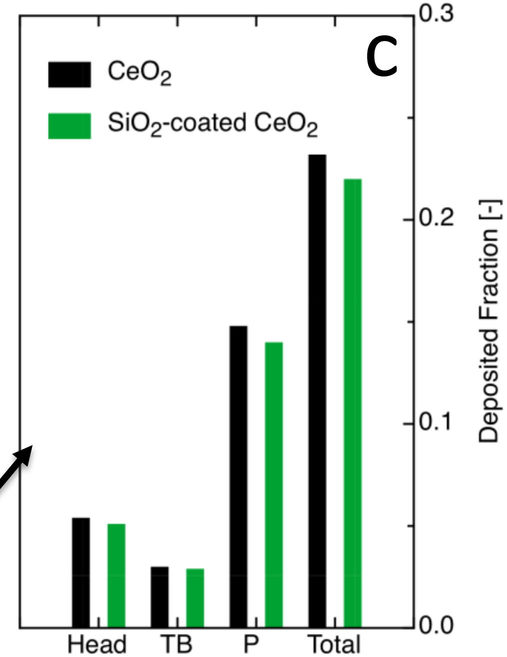




<u>SEM images</u> of *in-situ* sampled aerosol: **fractal structure** of agglomerates formed



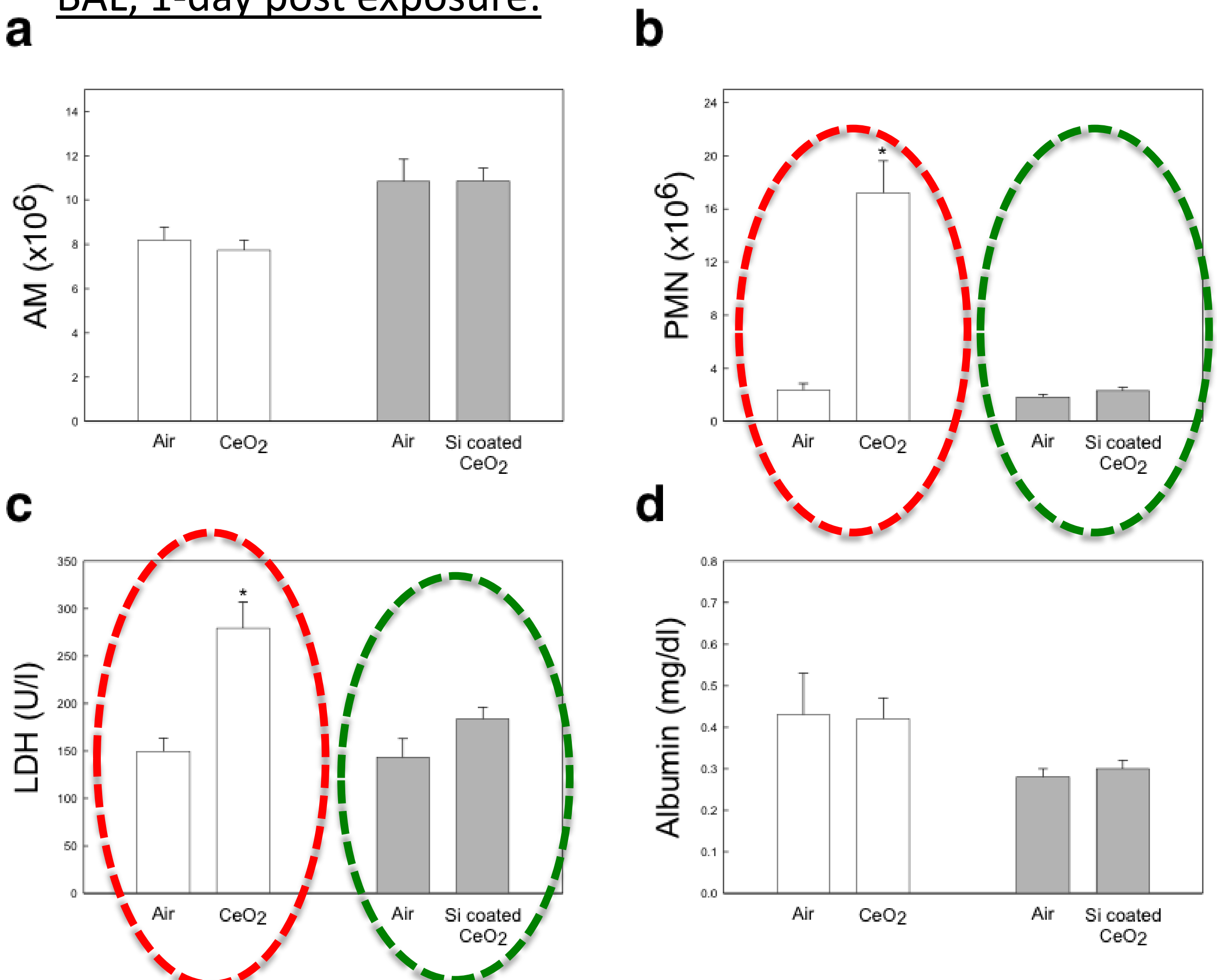
<u>Gravimetric Analysis</u>
Particle concentration
For both scenarios:
2.6 - 2.75 mg/m³



Almost *identical* mobility size distributions -> **similar lung deposition (MPPD2 model)**

Results: : In-vivo data-CeO_2 inhalation study

<u>BAL, 1-day post exposure:</u>



<u>CeO_2</u> exposure induces **lung injury & inflammation**[1,2] -> increased PMN, LDH levels

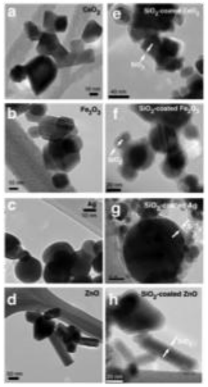
<u>SiO_2-encapsulation</u> inhibits **lung injury % inflammation** -> PMN, LDH levels comparable to particle-free controls

SiO_2-coated and uncoated CeO_2 did not cause **air/capillary damage** -> albumin levels as in control group

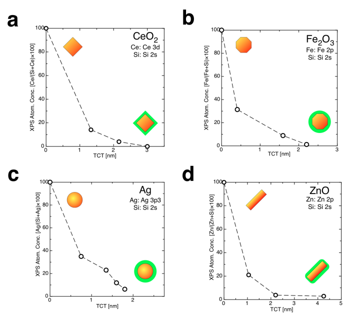
(1) Ma et al., *Nanotoxicology* **2011**, 5, 312 - 325
(2) Nalabotu et al., *International Journal of Nanomedicine* **2011**, 6, 2327-2335

Conclusions

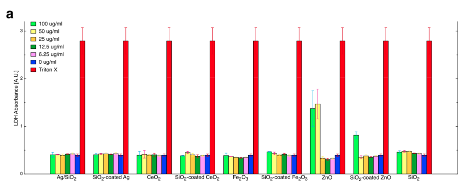
1) A scalable method for in-flight SiO_2 coating of Me an Me_xO_y ENMs was developed and **optimized** for a comprehensive nanopanel



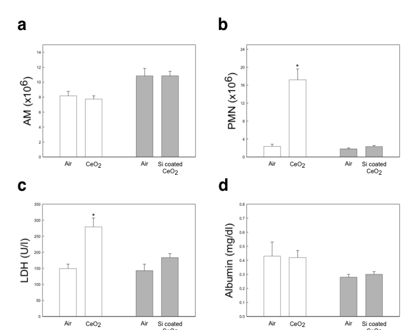
2) The method enabled the **hermetic coating** of all materials in the nanopanel (XPS, Chemisorption)



3) Once coated, nanomaterial exhibited agglomeration and surface charge **similar to pure SiO_2** when dispersed in **liquid media** (DLS).

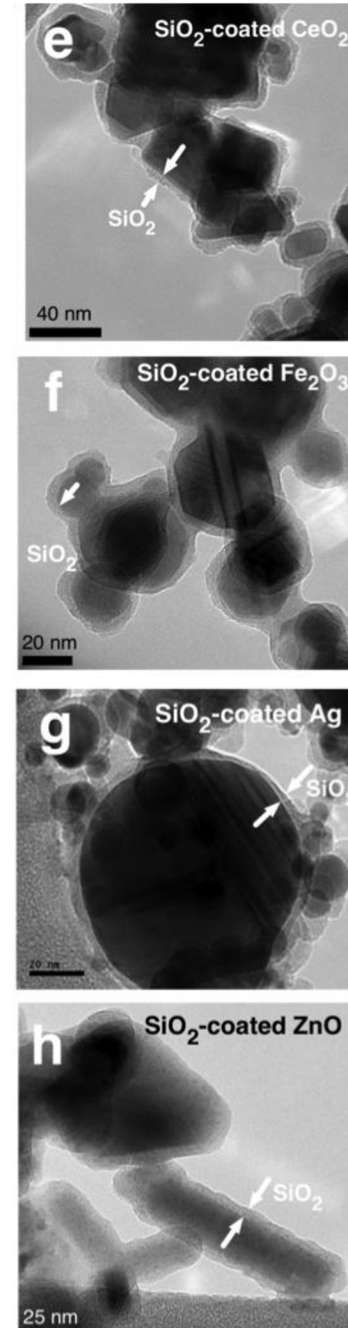


4) The SiO_2 coating was effectively **reduced toxicity in-vitro** (LDH, MTT on A549 for ZnO and Ag)



5) The Safer Formulation Concept was **validated in-vivo for CeO2**

Questions???


e
SiO_2-coated CeO_2
SiO_2
40 nm
f
SiO_2-coated Fe_2O_3
SiO_2
20 nm
g
SiO_2-coated Ag
SiO_2
h
SiO_2-coated ZnO
SiO_2
25 nm

List of <u>precursor solutions</u> used in *Nanopanel Synthesis*

ENM	**Precursor**	**Solvent**	**Precursor Molarity**
Ag	Silver acetate (Sigma Aldrich)	2-ethylhexanoic acid:acetonitril (1:1) (>99%, Sigma Aldrich)	0.5
ZnO	Zinc naphthenate, 65% in Mineral Spirits (10% Zn) (STREM)	ethanol (95%, Sigma Aldrich)	0.5
Fe_2O_3	Iron(III) acetylacetonate (Sigma Aldrich)	2-ethylhexanoic acid:acetonitril (1:1) (>99%, Sigma Aldrich)	0.5
CeO_2	Cerium(III) Ethylhexanoate, 49% in 2-ethylhexanoic acid (12 wt% Ce) (STREM)	xylene (>99%, Sigma Aldrich)	0.5
SiO_2	Hexamethyl disiloxane (HMDSO) (Sigma Aldrich)	ethanol (95%, Sigma Aldrich)	0.5

Summary of ENMs used to evaluate ENM-cellular interactions (in-vitro)

Material	Theoretical SiO_2 [wt%]	TCT [nm]	$d_{XRD,core}$	SSA [m^2/g]
SiO_2	100	N/A	19 (d_{BET})	147
CeO_2	0	0	27	28
SiO_2-coated CeO_2	20	3	28	31.3
Fe_2O_3	0	N/A	19.6	42
SiO_2-coated Fe_2O_3	33	2.7	21.3	49
Ag/SiO_2	50	N/A	20.4	57
SiO_2-coated Ag	10	1.8	28	29
ZnO	0	N/A	28	36
SiO_2-coated ZnO	23	2.5	27.0	66

Detailed schematic of VENGES reactor

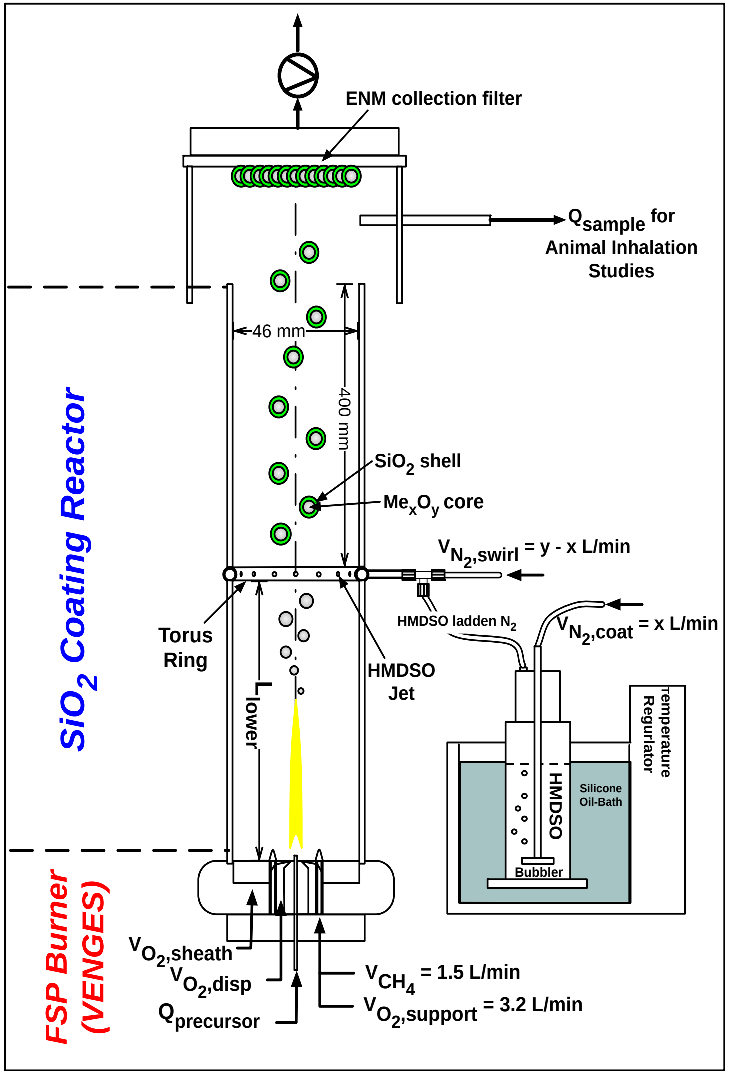
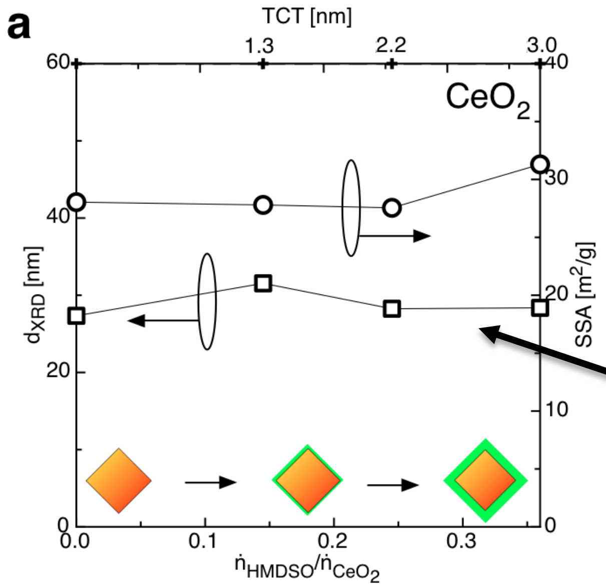
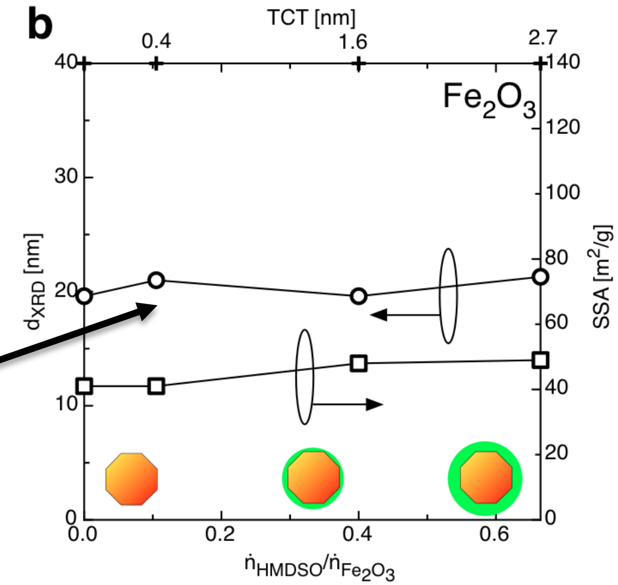


Figure 4: Detailed schematic of the SiO_2 coating reactor for synthesis of hermetically coated ENMs.

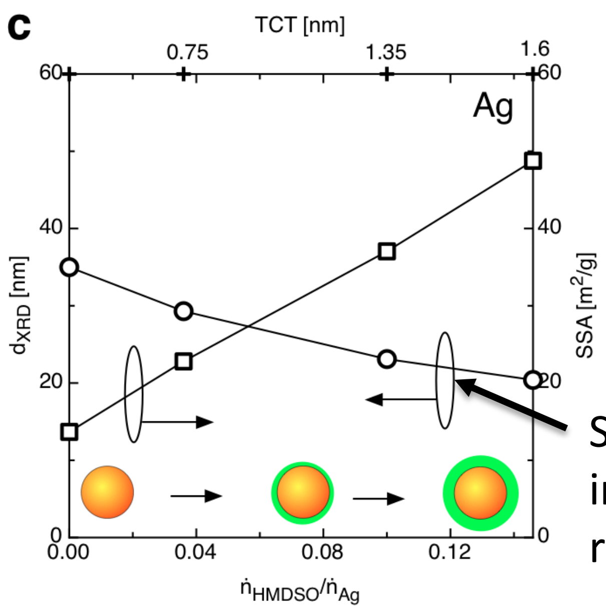
Results (2/5): *Synthesis and Characterization*

Assessing SiO_2 coating influence on Crystal Size and SSA.

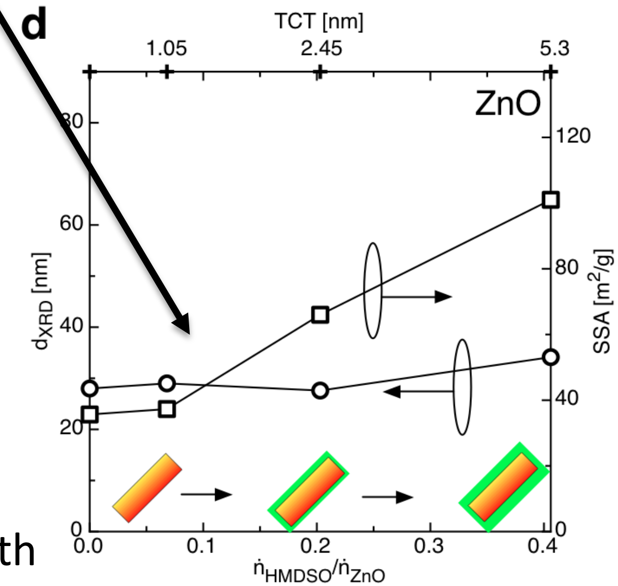




Independent control over crystal size and coating thickness

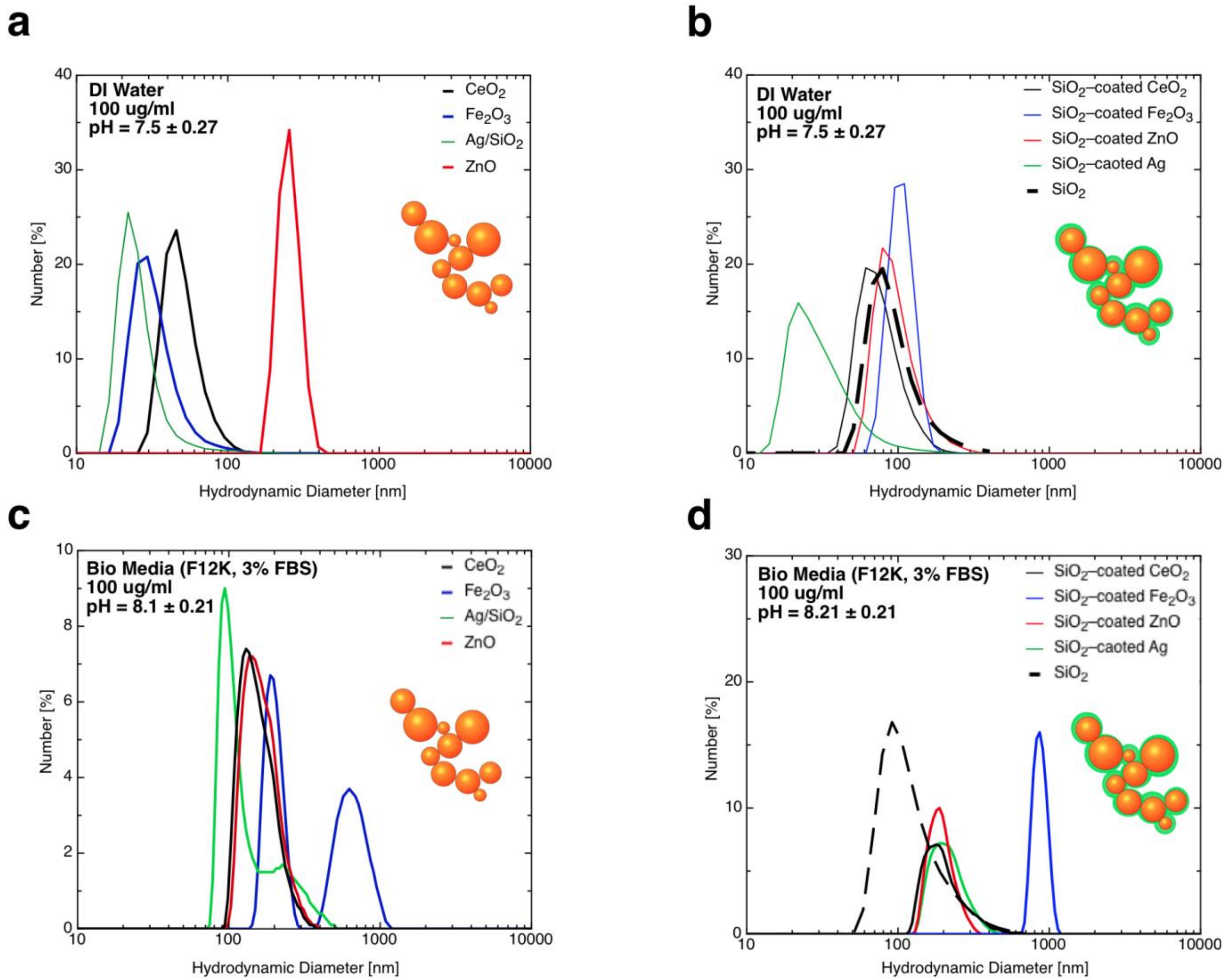


SiO_2 encapsulation inhibits post-torus ring Ag crystal growth



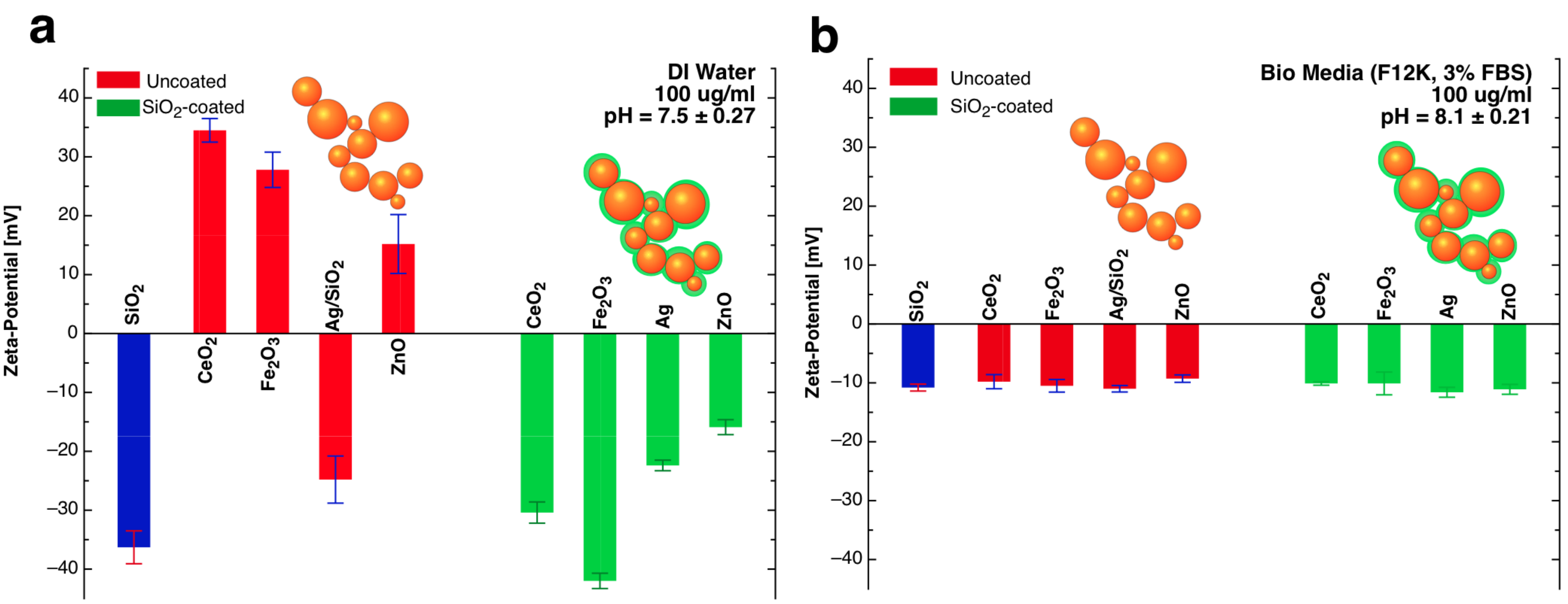
Results: ENM-media Interactions

Assessing SiO_2 coating influence on ENM agglomeration in liquid media.



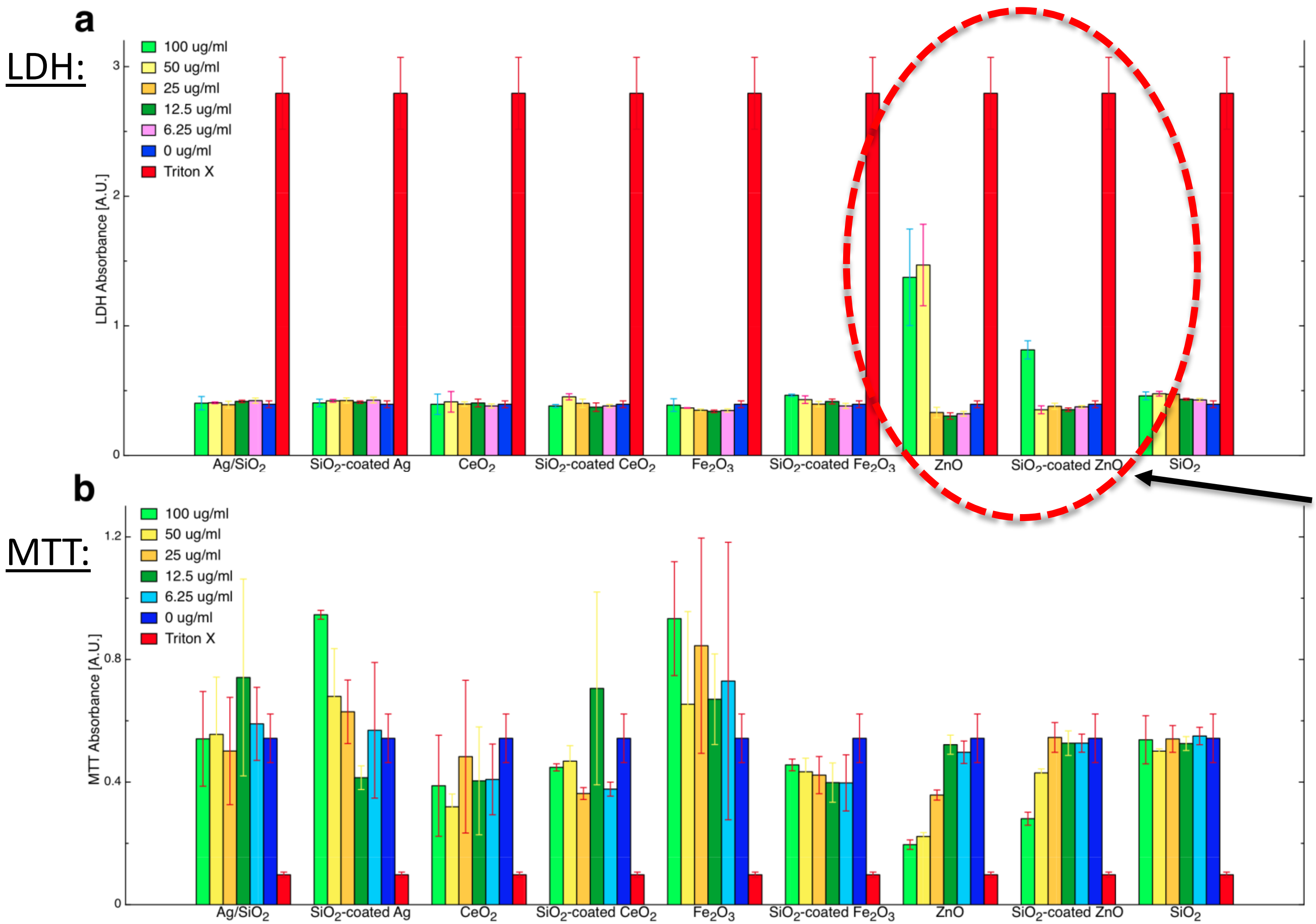
Results: ENM-media Interactions

Assessing SiO_2 coating influence on ENM surface charge in liquid media.



Results: ENM-Bio Interactions

Assessing SiO_2 coating influence on ENM-cellular interactions in-vitro (A549).



Most materials exhibit *minimal toxicity* for cells and endpoints investigated (in-vitro)[1]

SiO_2-coating effectively reduces the toxicity of ZnO -> inhibition of ion leaching[2]

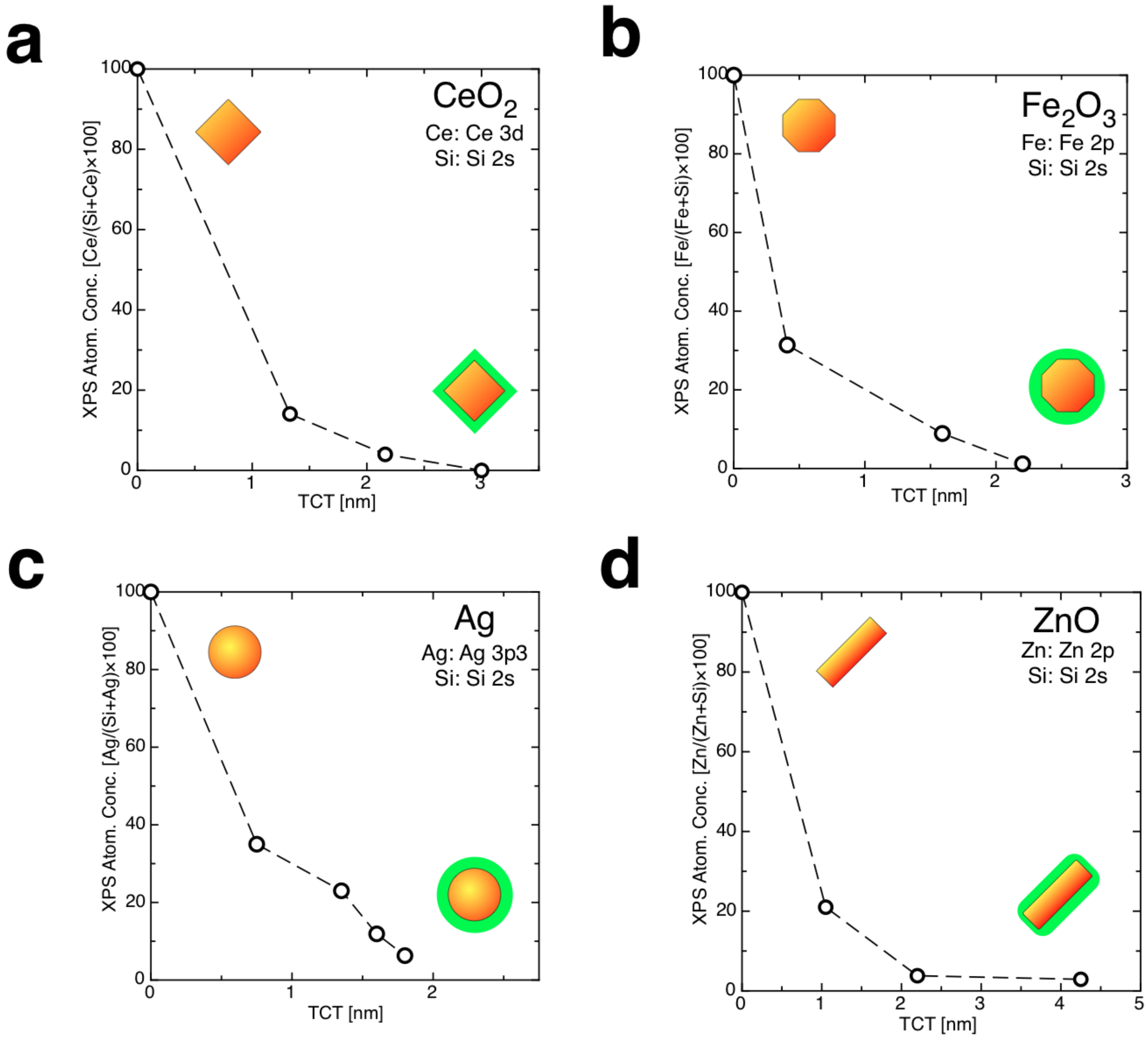
MTT results reflect LDH results in A549

(1) Kroll et al., *Particle and Fibre Toxicology* **2011**, 8, 9

(2) Xia et al., ACS Nano **2011**, 5, 1223 – 1235

Results (4/5): *Synthesis and Characterization*

Assessing SiO_2 coating efficiency quantitatively using XPS



All materials:
rapid decay in Me/(Me+Si) surface atomic fraction

-> optimized mixing conditions in coating reactor (homogenous conditions)

Results (5/6): ENM-media Interactions

Assessing SiO_2 coating influence on ENM agglomeration in liquid media.