

Use in vitro and high throughput screening to facilitate toxicological evaluation of engineered nanomaterials

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New consumer products



Over 2,700 types of nanomaterials (Nanowerk Nanomaterial Database, accessed Oct 4 2011)

Maynard A, Nature, 444, 267-269, 2006

How to test nanotox effects? Lessons from Conventional Chemical Safety Assessment



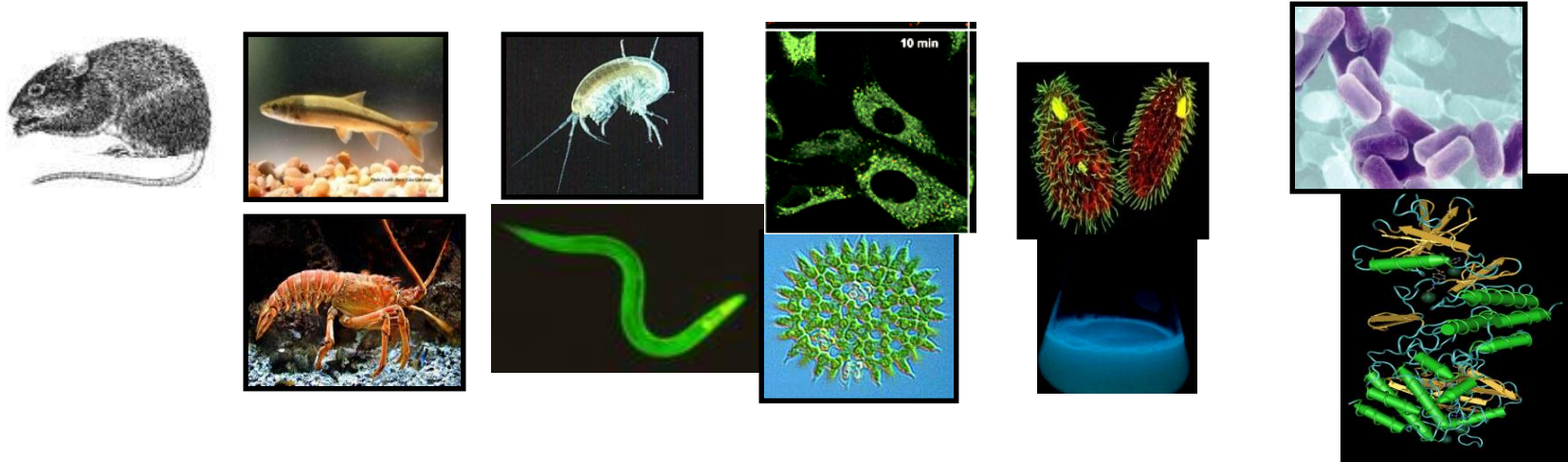
“Out of 80,000 chemicals registered in USA, only 200+ has undergone systematic toxicity testing out of which only five has been banned” (Toxic America, CNN).

	Conventional assay (Animal Model)
Time	32 Years for the chemicals so far
Cost	US\$14 billion/year ¹
Animal ethics	100 million experimental animals are used every year for toxicological studies ²
Relevance to human safety	Differences between animal and human responses to chemicals/material ^{1, 2}

1. Nature 2009, 460, 208-212

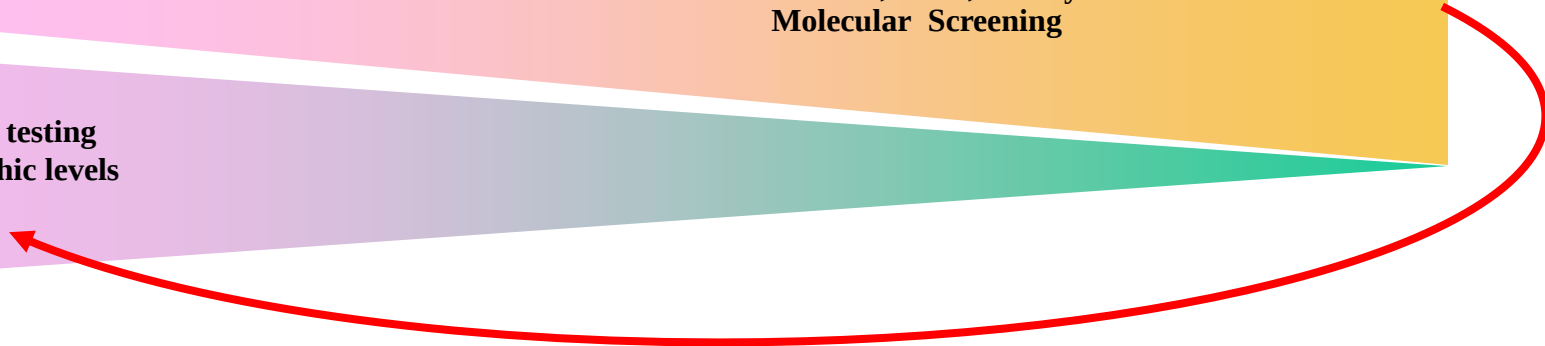
2. Environmental Health Perspectives, 1999, pp. 83-88

US National Academy of Science (NAS) Report (2007): *“Toxicity Testing in the 21st Century: A Vision and a strategy”*



High Throughput Bacterial,
Cellular, Yeast, Embryo or
Molecular Screening

Prioritize *in vivo* testing
at increasing trophic levels



We use a predictive toxicological approach for nanomaterial hazard testing

In Vivo Adverse Outcomes

Maximum of 10^2 animals
per experiment (weeks to months)

Validity of
predictions

Material
physicochemical
properties

QSARs

- mechanism of injury
- toxicological pathway

QSARs

Cellular or Bio-molecular Injury Endpoints

Up to 10^5 measurements per day

Tools to Develop Predictive Toxicology: Composition and Property-based Nanomaterial Libraries

Compositions

Transition MOx

TiO₂, ZnO, CuO, NiO,
Cr₂O₃ etc

RE Oxides

CeO₂, GdO₃, La₂O, SbO₃
etc

Metals

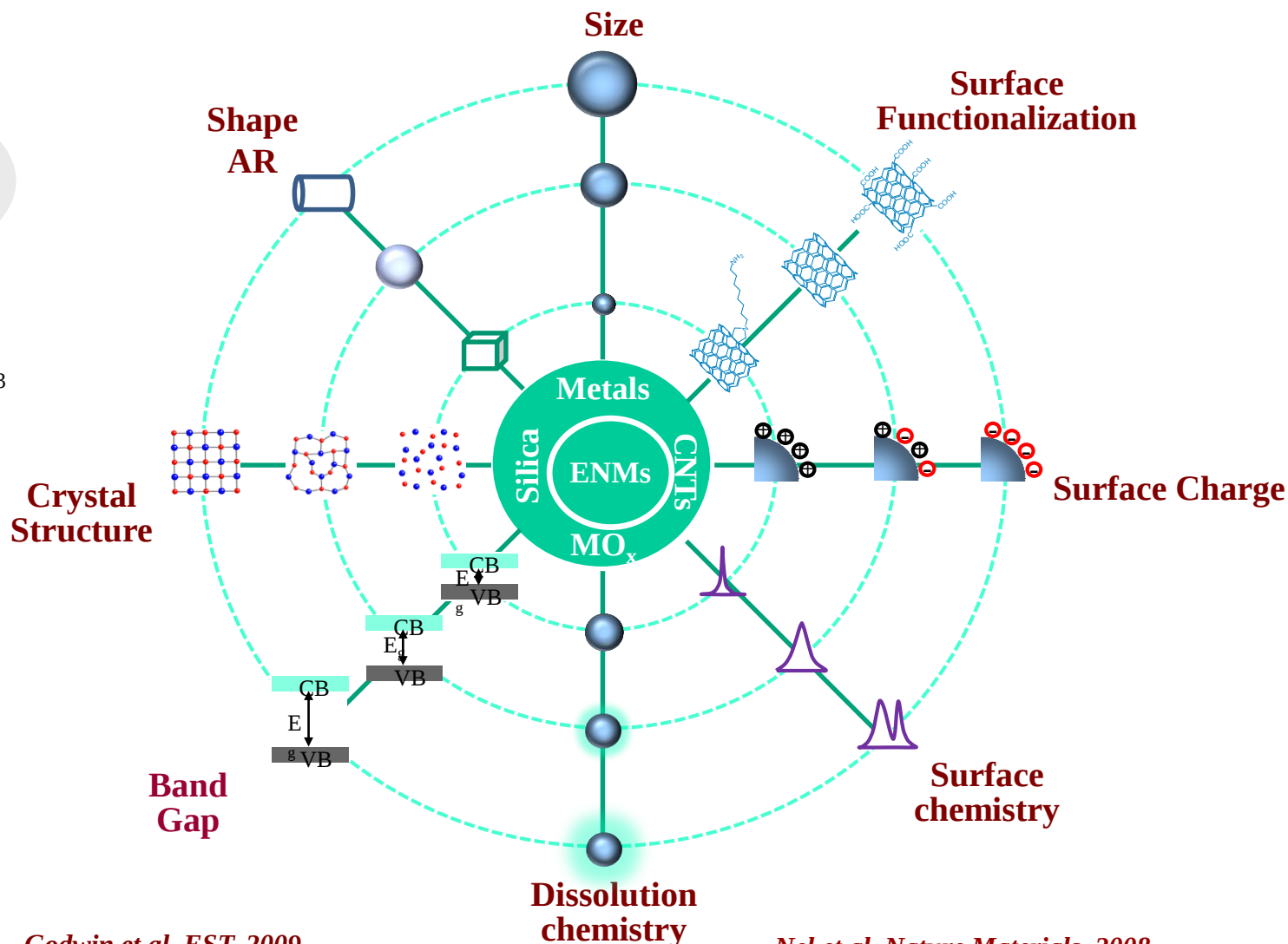
Cu, Ag, Pt, Co

Carbon Nanotubes

SWCNT
MWCNT
fCNT

Silica

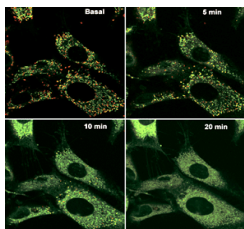
Amorphous
Fumed
Crystalline
Mesoporous



Godwin et al, EST. 2009
Thomas et al. ACS Nano. 2011
Nel et al. Small. 2012

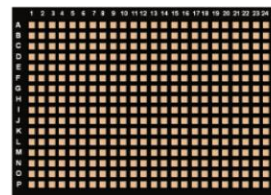
Nel et al. Nature Materials. 2008
Xia et al. ACS Nano. 2008
George et al. ACS Nano. 2011

Tools: Cellular High Throughput Screening

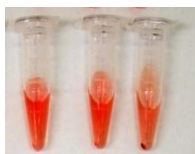


Mitochondrial damage
ROS generation
Stress response
Cellular apoptosis

Epifluorescence
microscopy



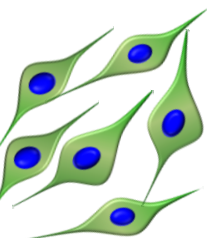
UV-Vis
spectroscopy



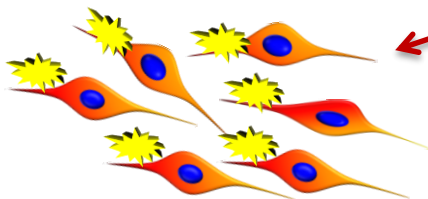
RBC lysis

Luminescence

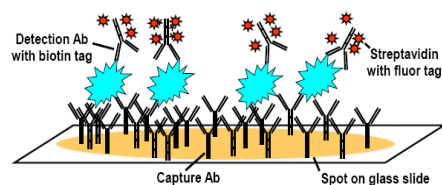
Multiplex cytokine
& Chemokine assay



Cell growth



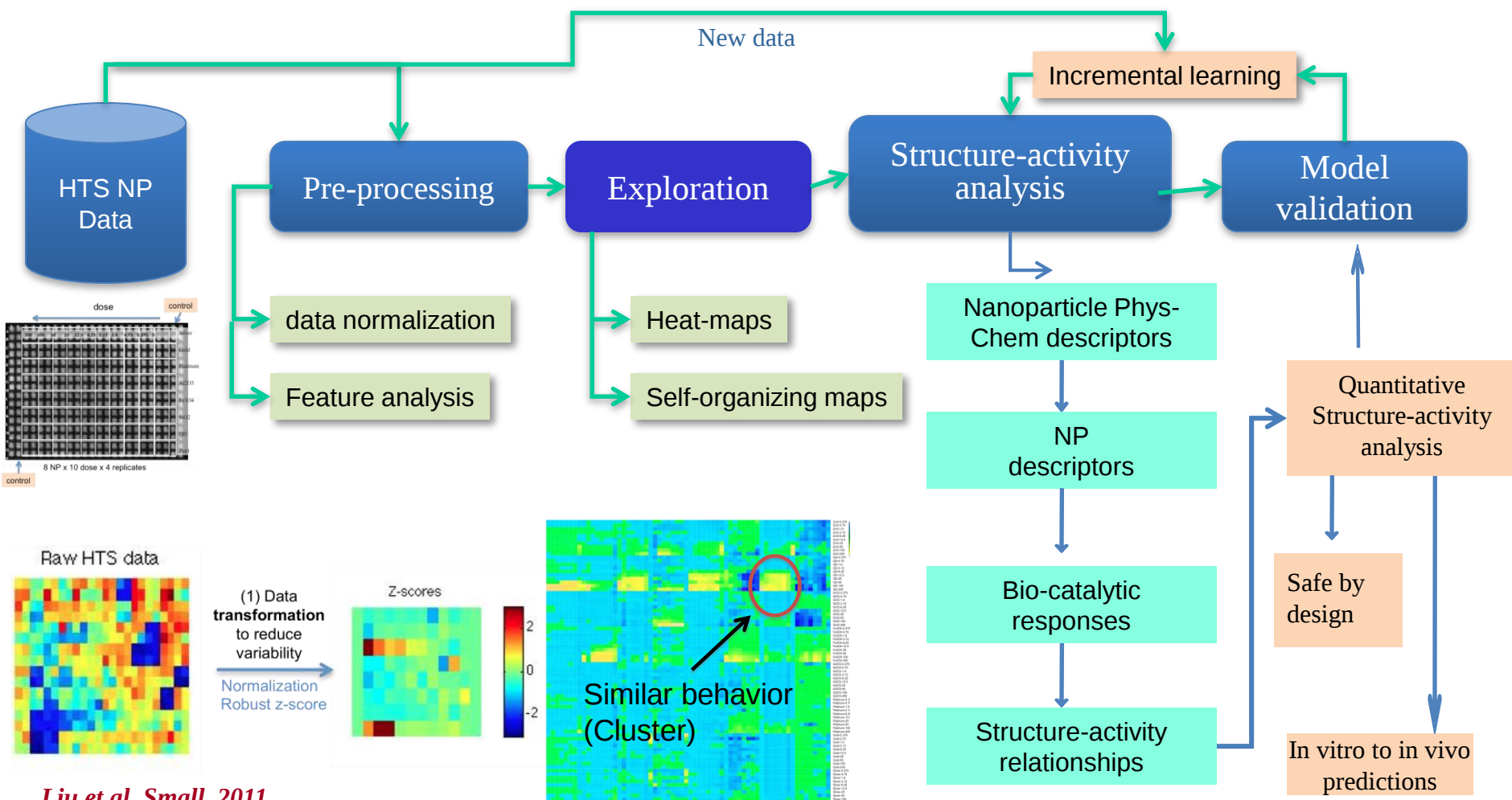
Reporter genes for
sublethal effects



Assessment of Inflammation

George et al. ACS Nano. 2010
George et al. ACS Nano. 2011
Nel et al. ACR. 2012

Tools: High Content Data exploration and SAR modeling

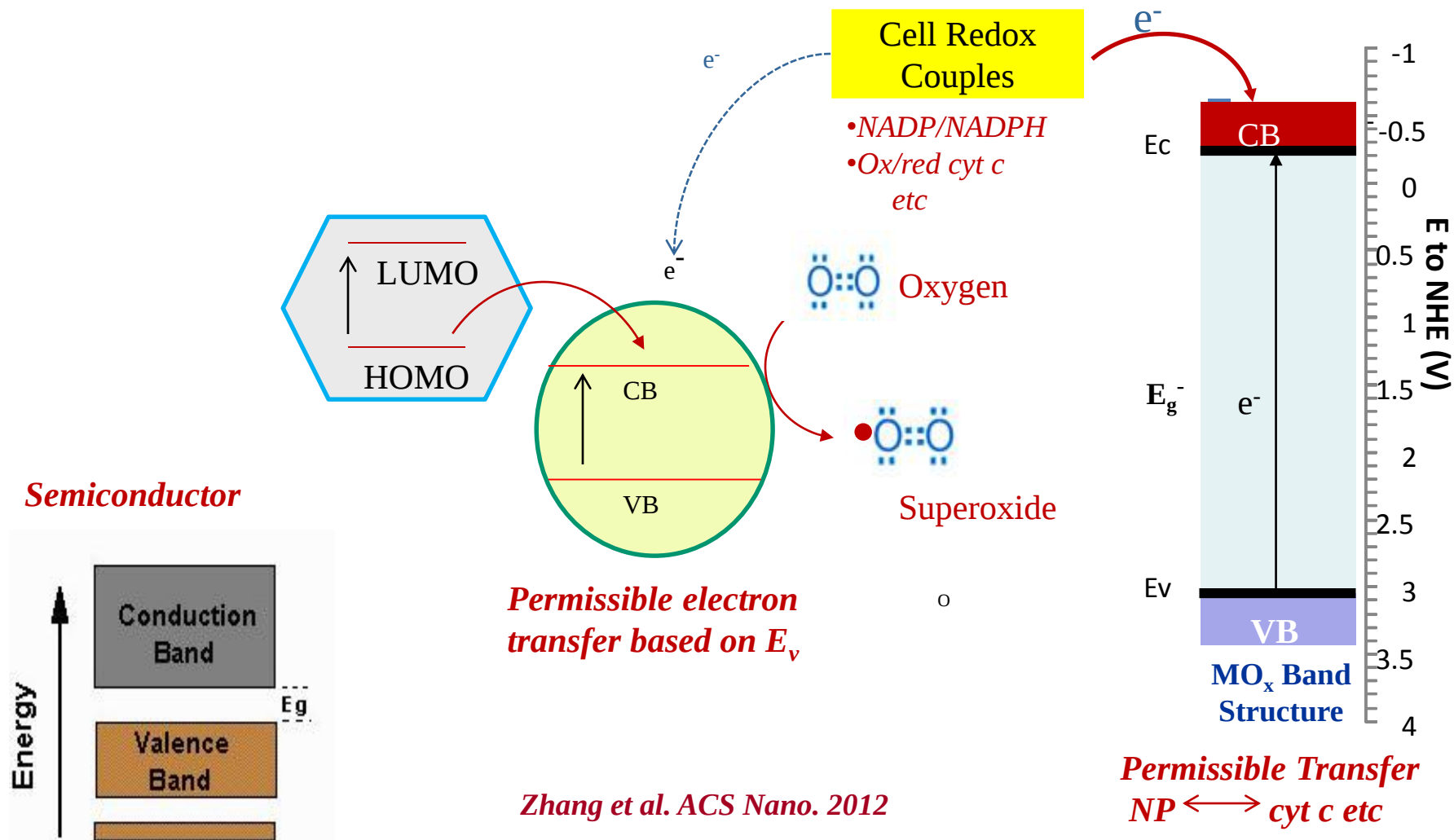


Liu et al. Small. 2011

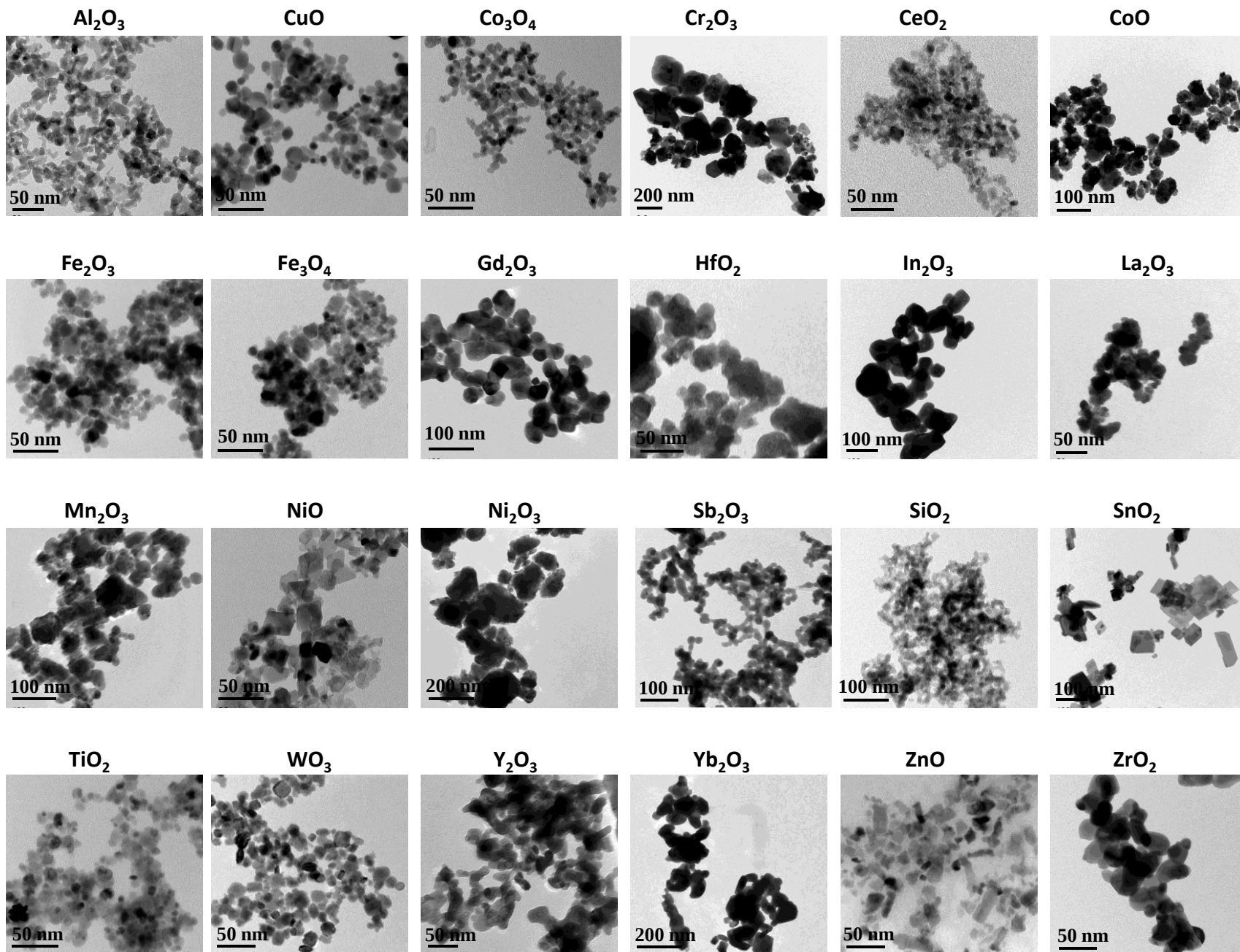
Rallo et al. EST. 2011

Damoiseaux et al. Nanoscale. 2011

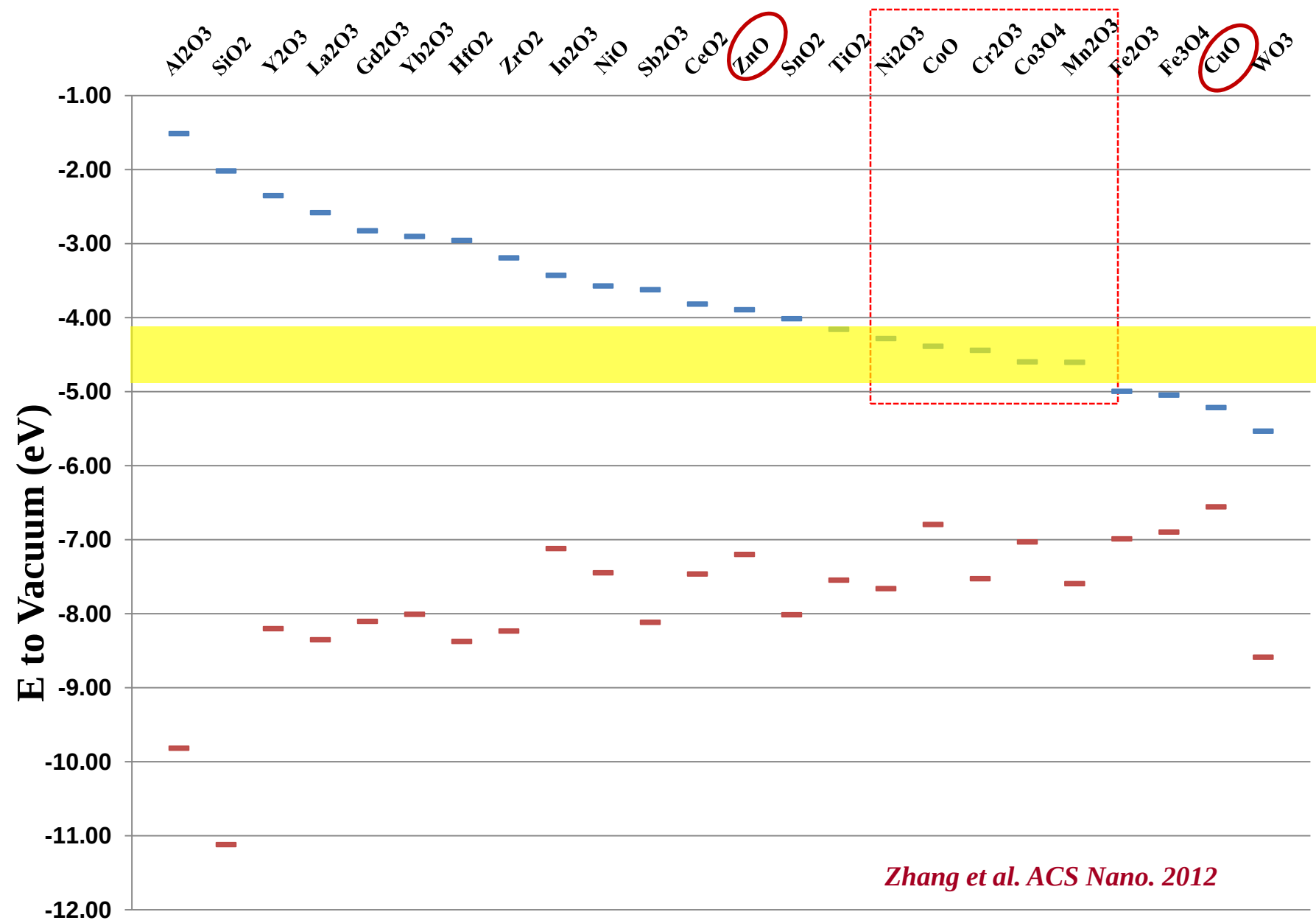
Use of Metal Oxide Band gap to pursue A High Throughput Predictive Toxicological Paradigm for Oxidative Stress



TEM images for 24 metal oxide nanoparticles



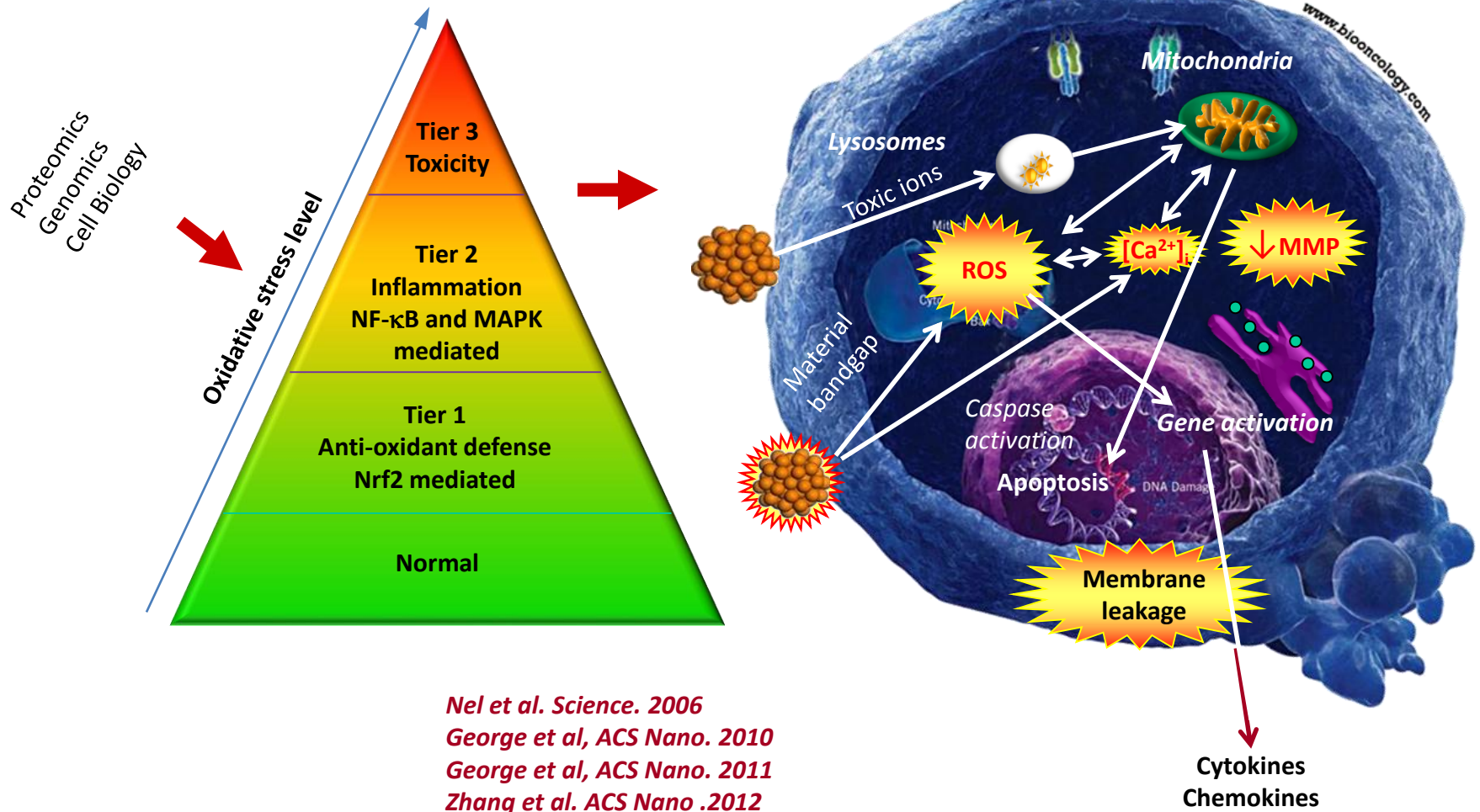
Prediction of MOx Toxicity based on Bandgap Energy



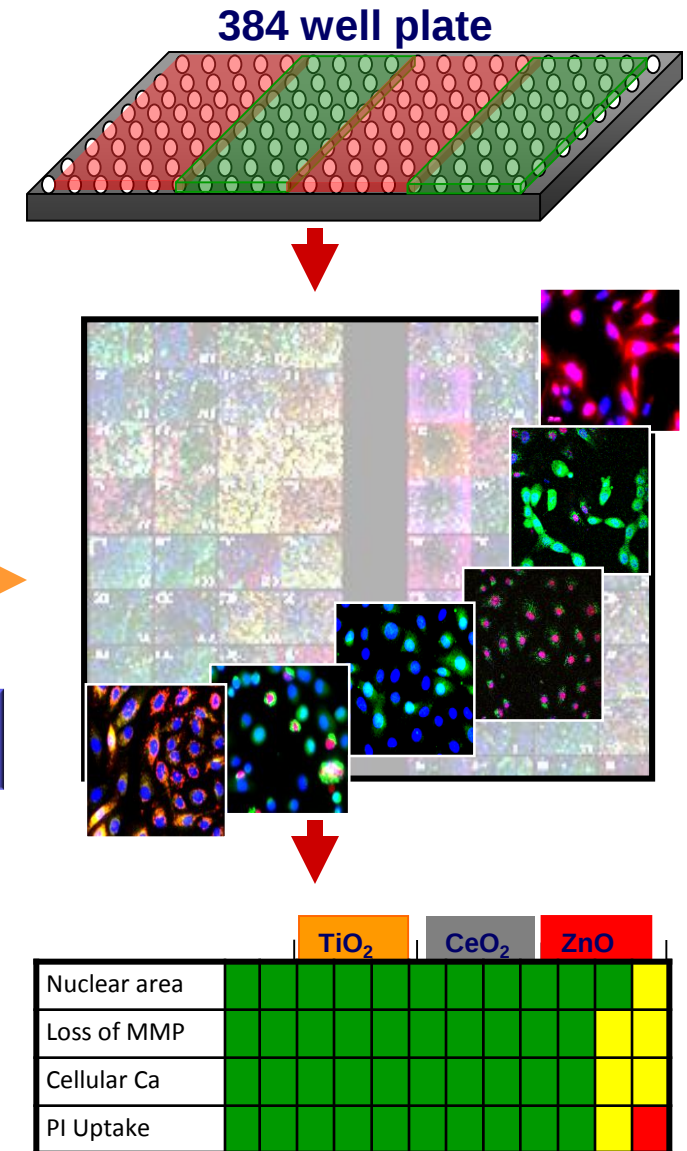
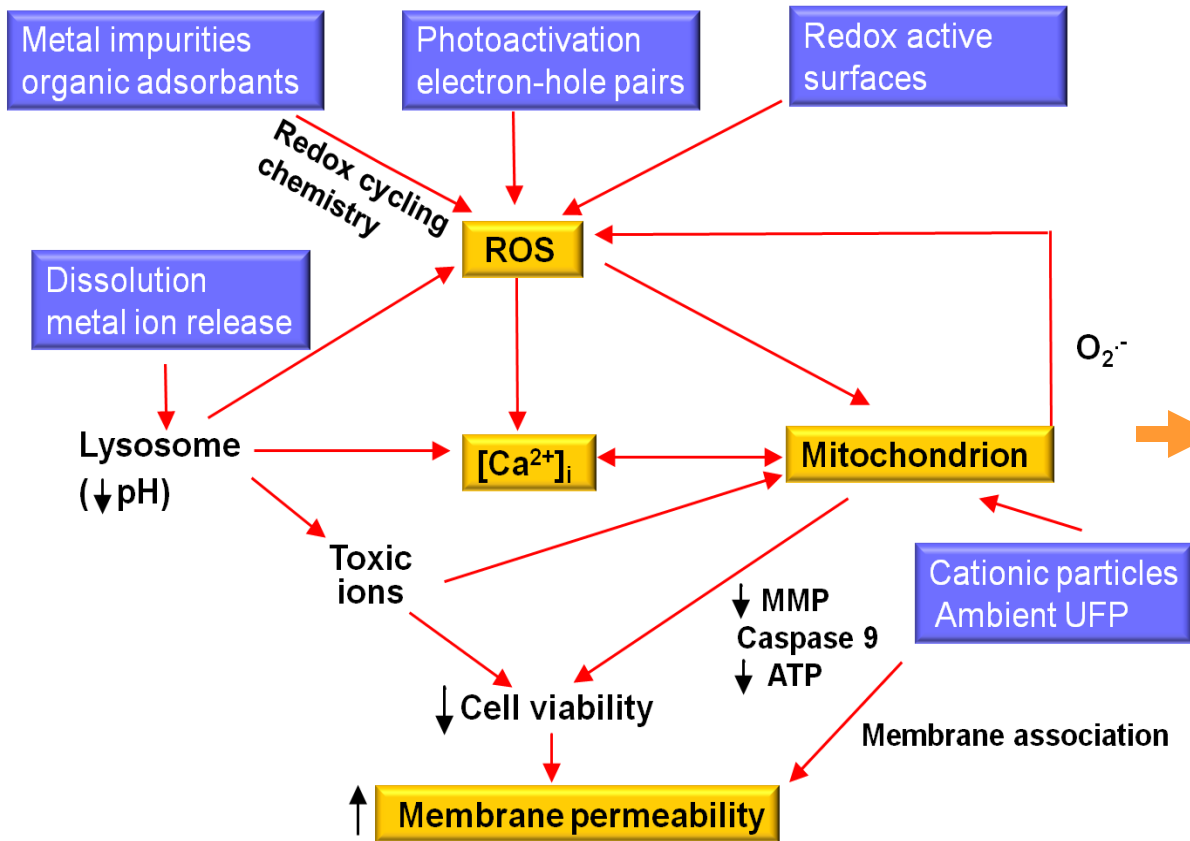
Zhang et al. ACS Nano. 2012

A Multi-parameter Assay that was built on an Oxidative Stress Paradigm

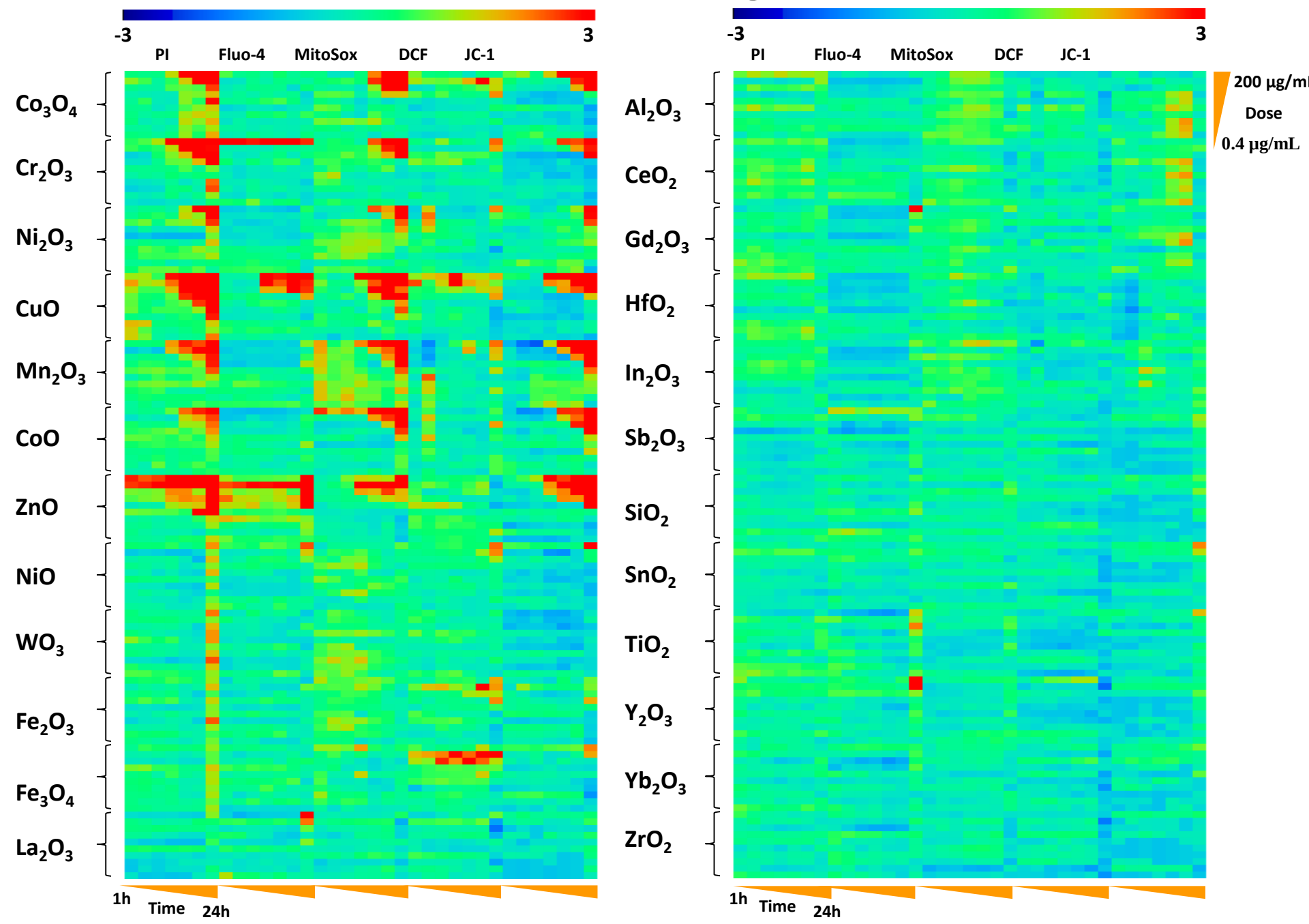
Hierarchical Oxidative Stress Paradigm



In vitro Multi-parametric High Throughput Screening Assays



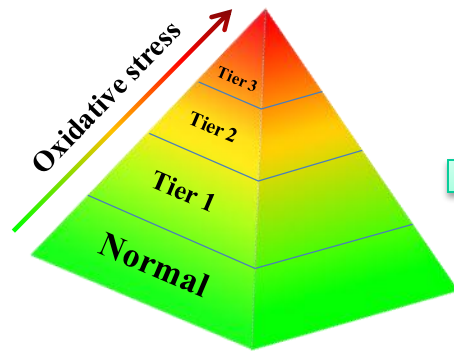
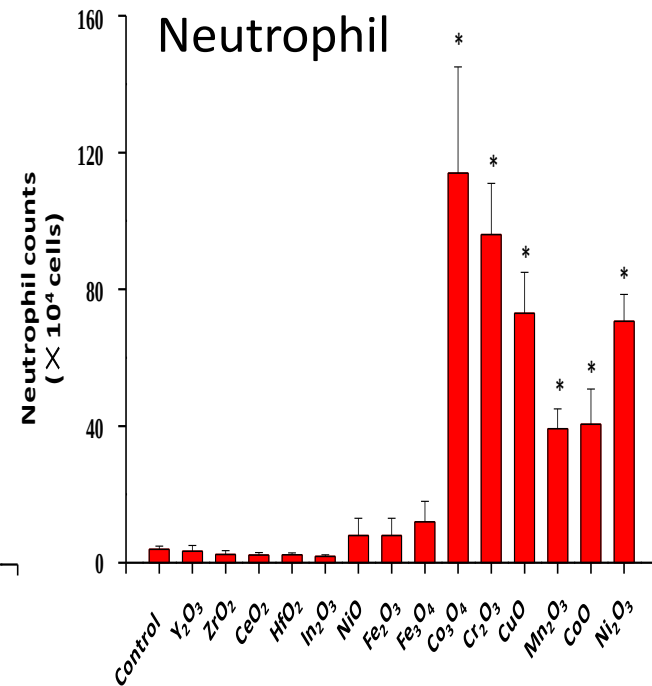
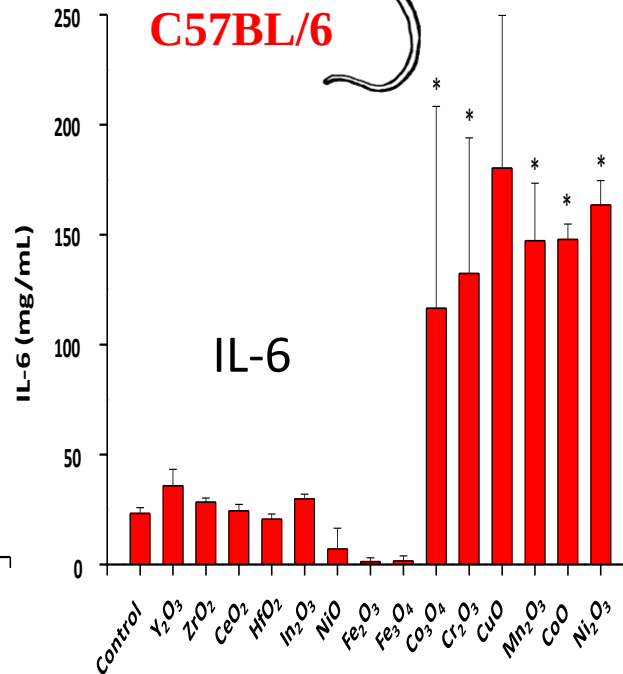
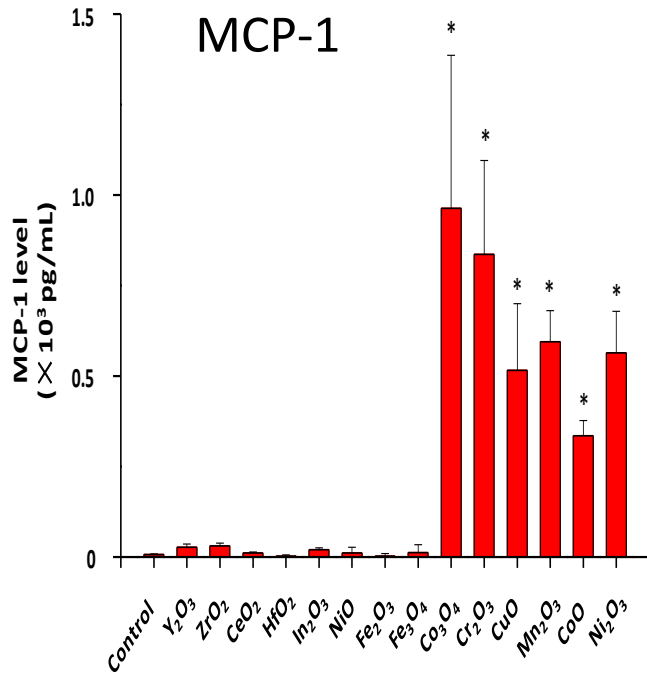
Heatmap for metal oxides in lung epithelial cell BEAS-2B



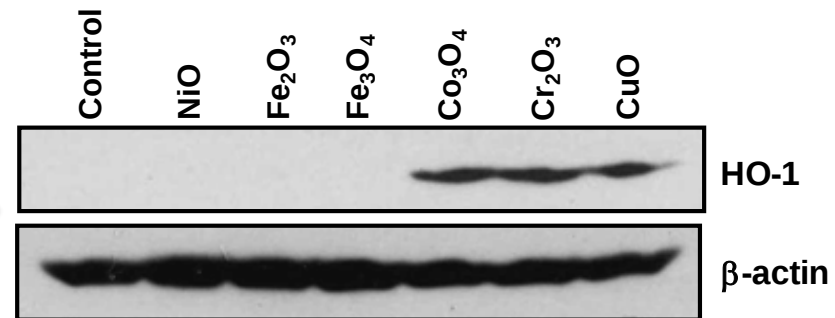
In vivo acute pulmonary inflammation in C57 BL/6 mice.



C57BL/6

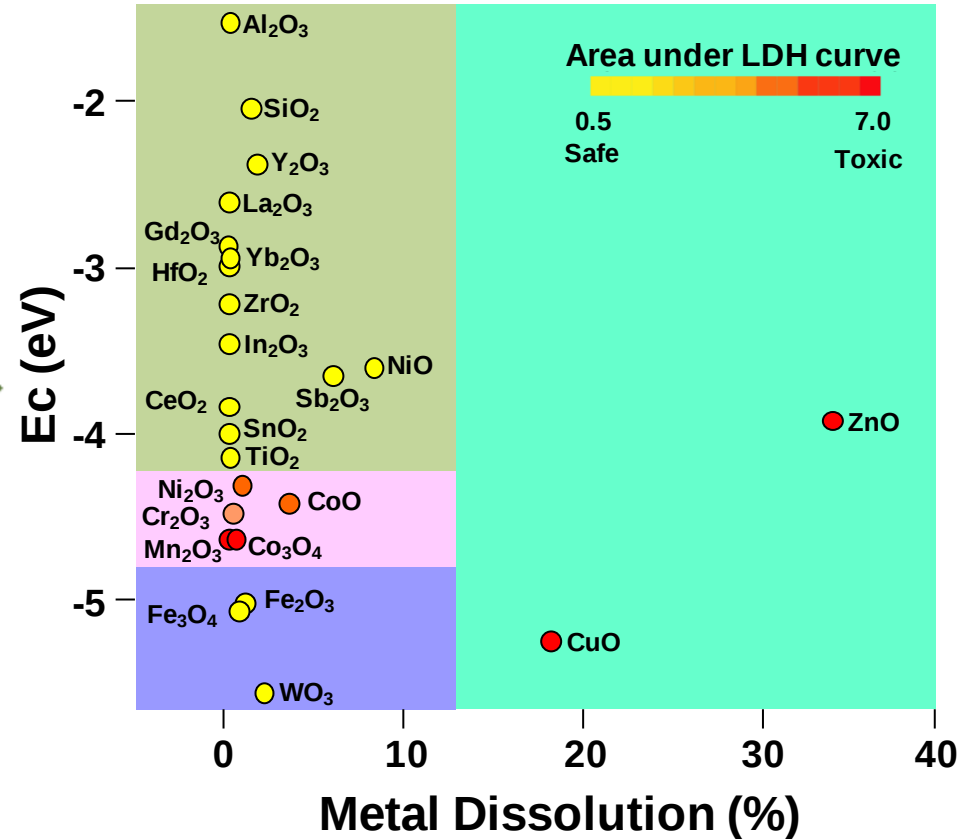
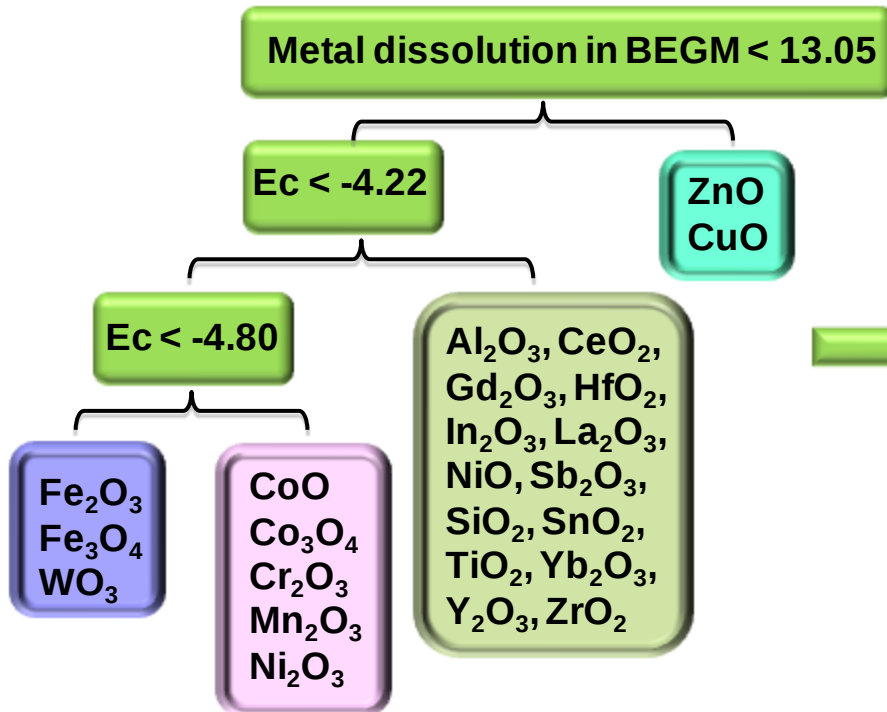


Tier 1



Regression tree analysis for the toxicological impact of metal dissolution *versus* conduction band energy.

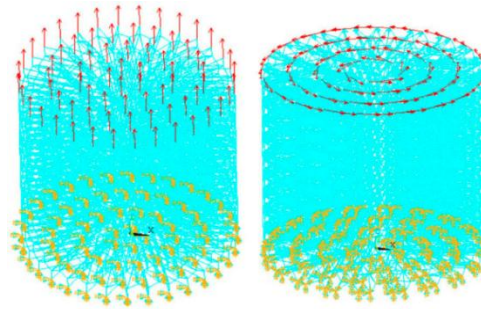
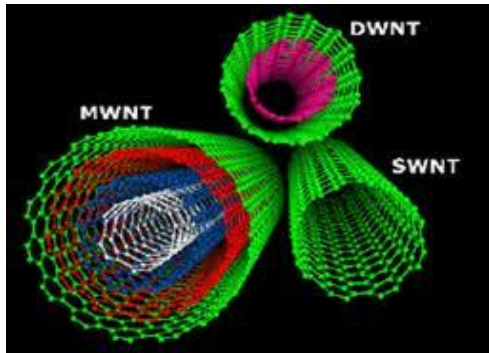
Regression Tree



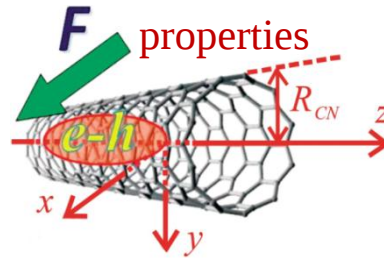
CNT Physicochemical Properties that could determine Disease outcome

Google images

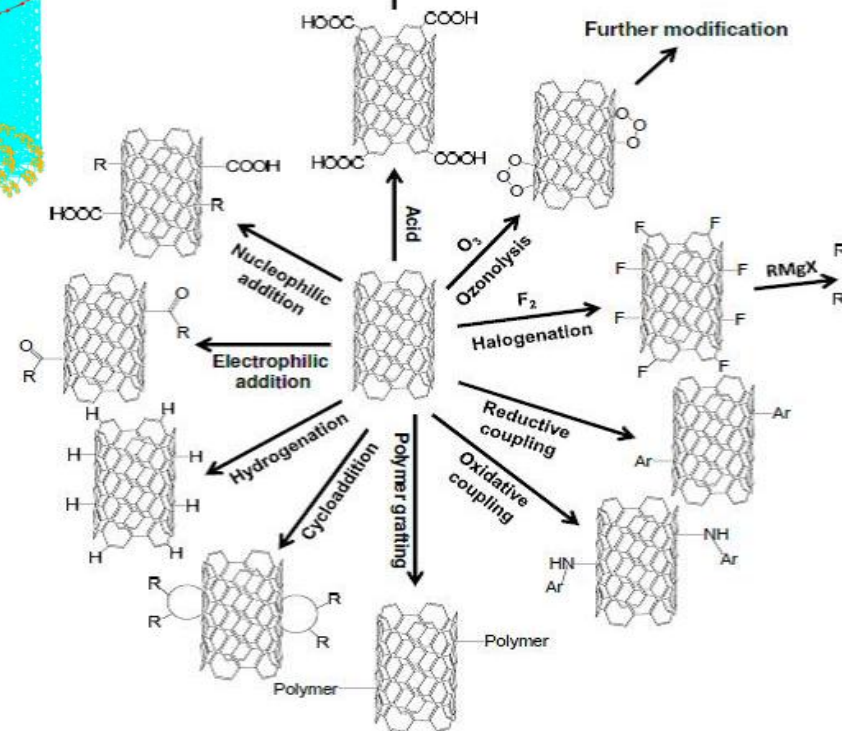
SWCNT/MWCNT



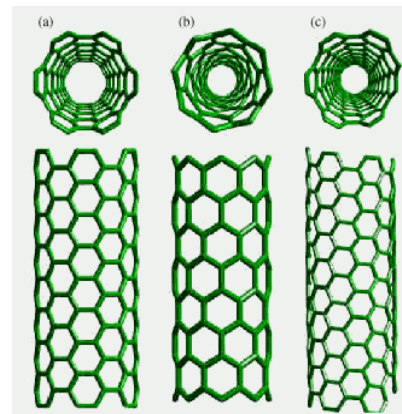
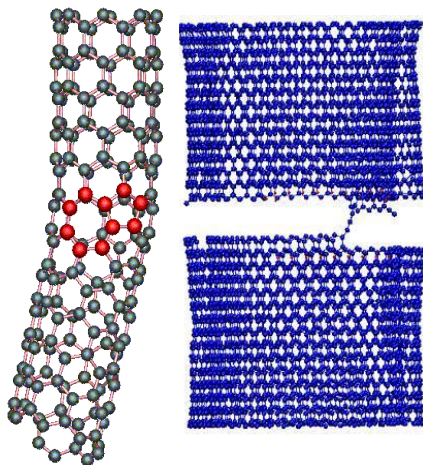
Different electronic properties



Surface functionalities



Surface Defects

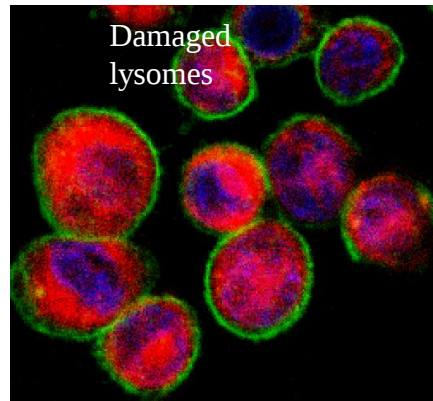
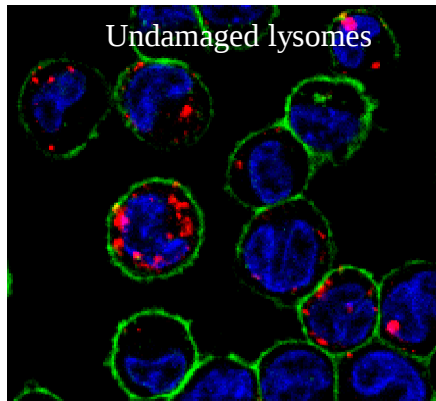
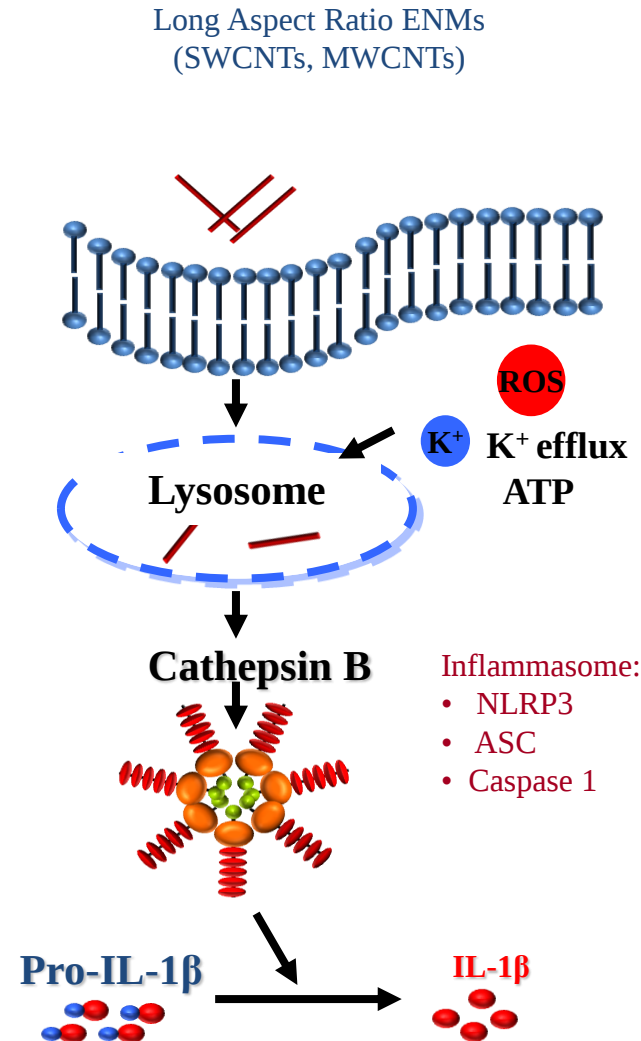
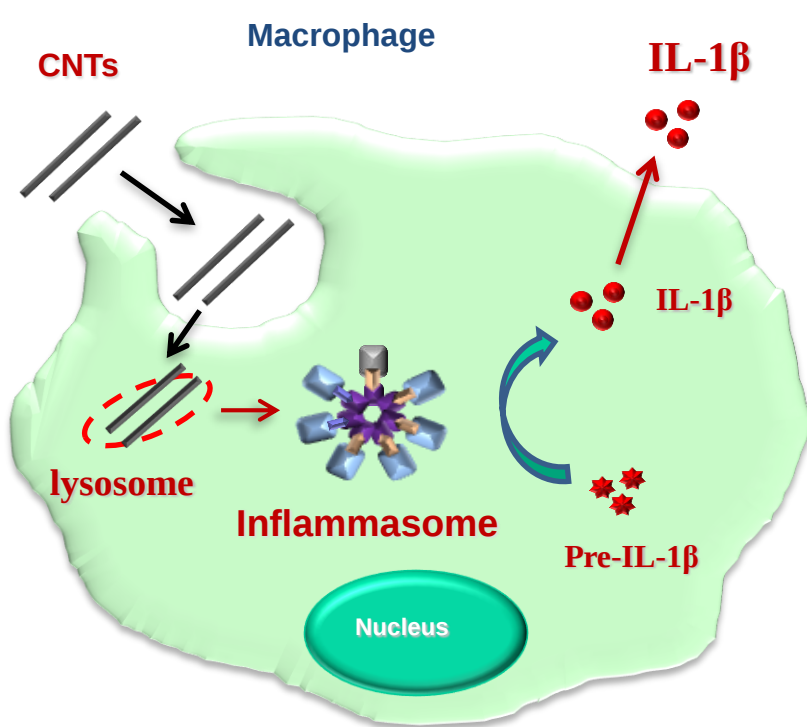


armchair, zigzag, chiral

Surface coatings



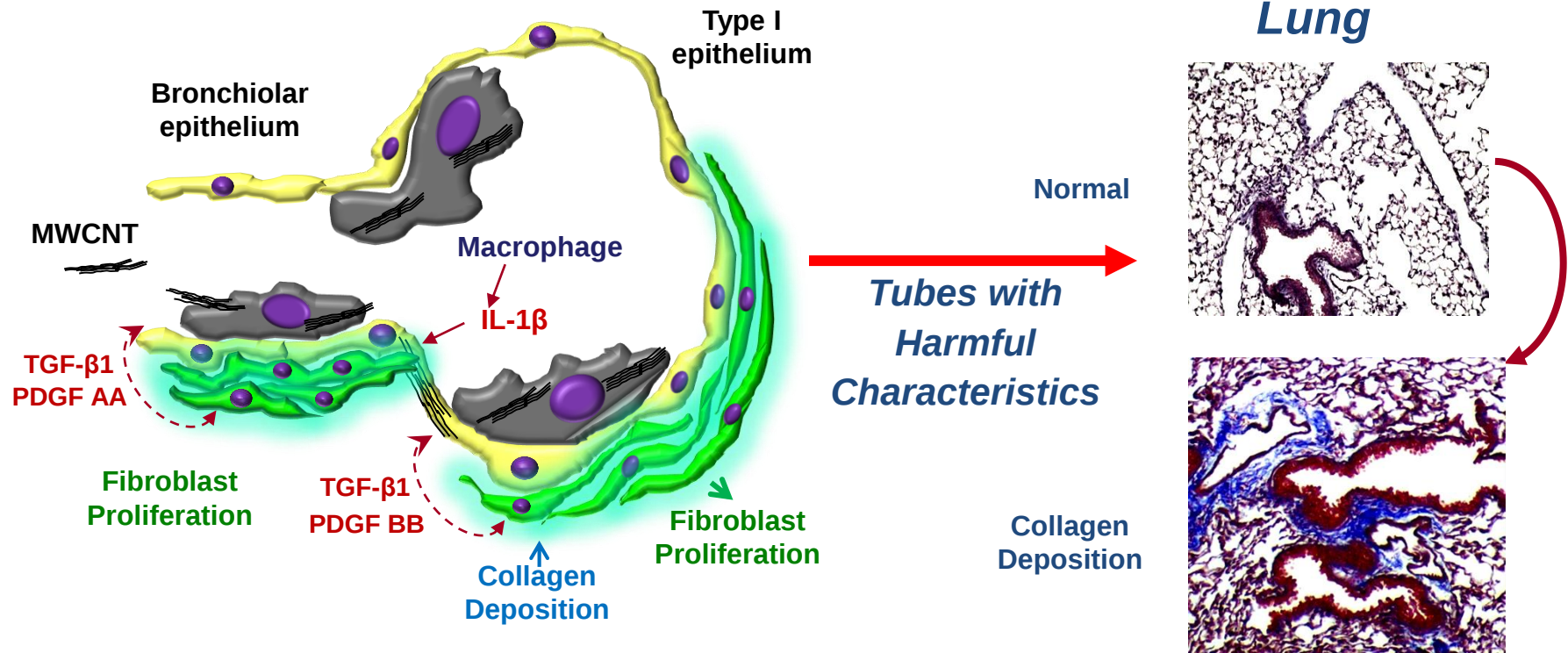
Use of the Macrophage to develop a Predictive Toxicological Paradigm for Lung Damage



Wang et al. ACS Nano. 2010

Wang et al ACS Nano. 2011

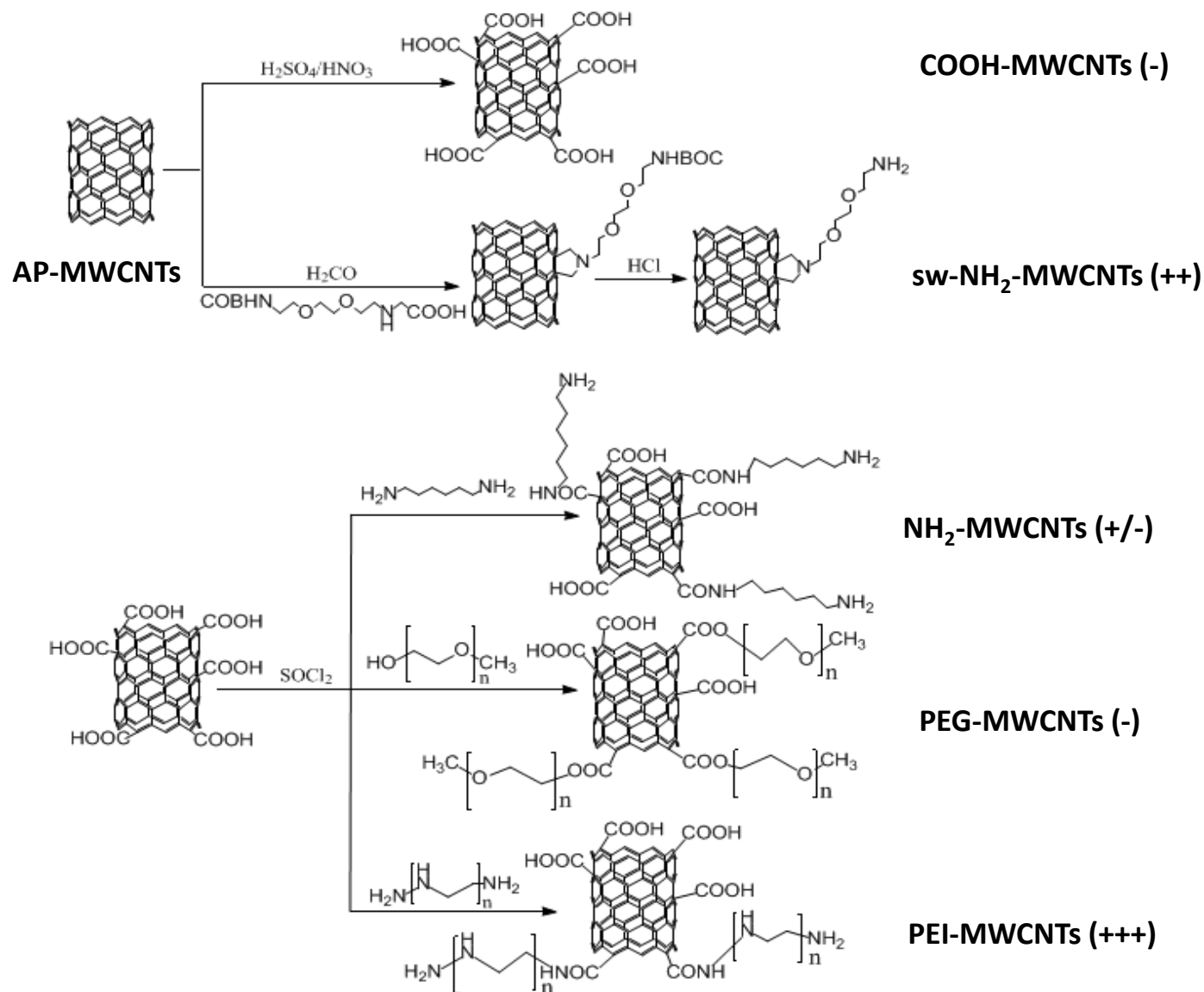
Quantifiable Cooperative Cellular Interactions as Biomarkers for CNT Disease Pathogenesis in the Lung



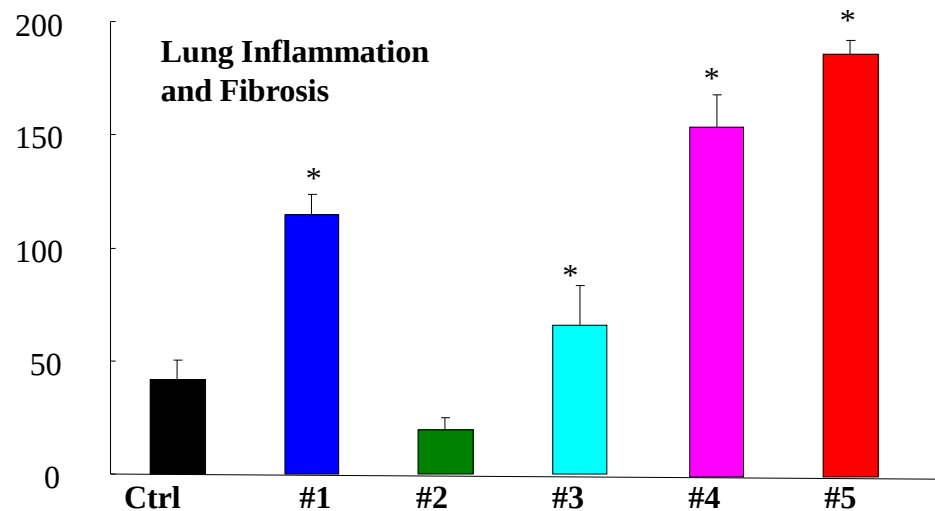
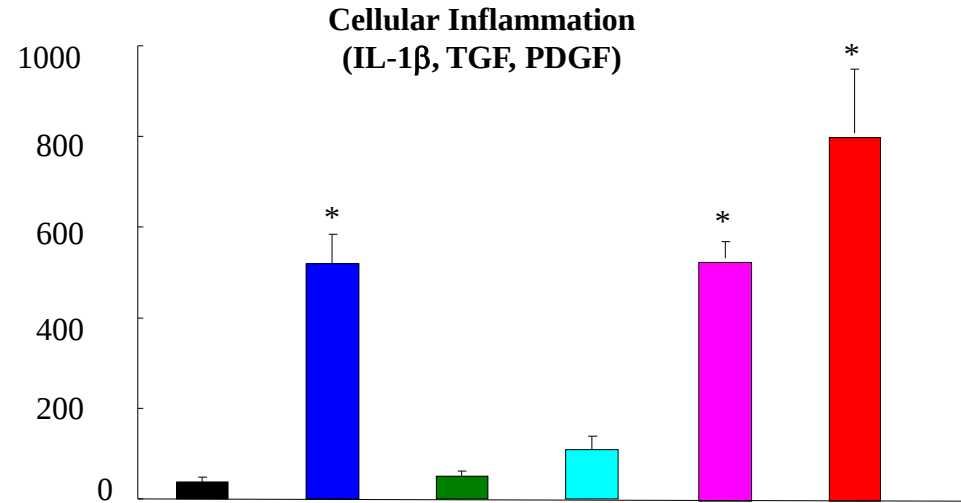
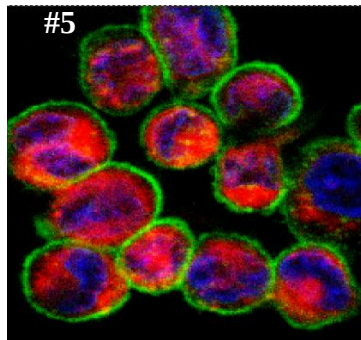
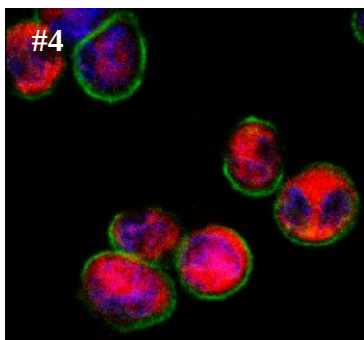
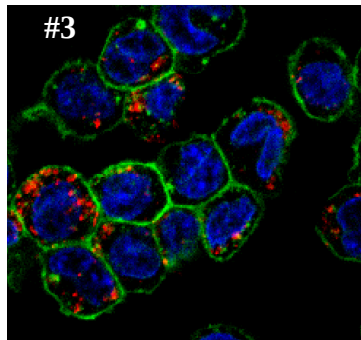
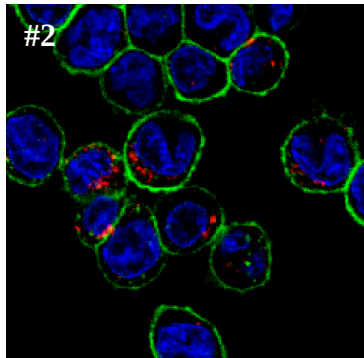
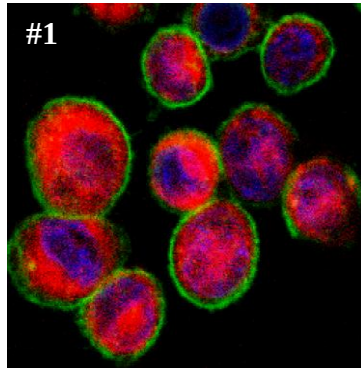
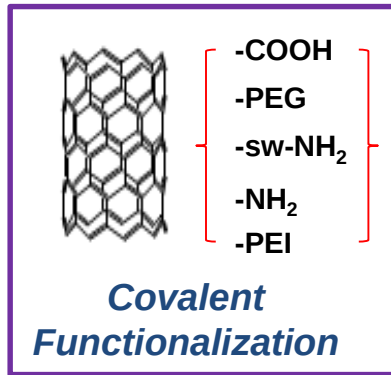
In vitro



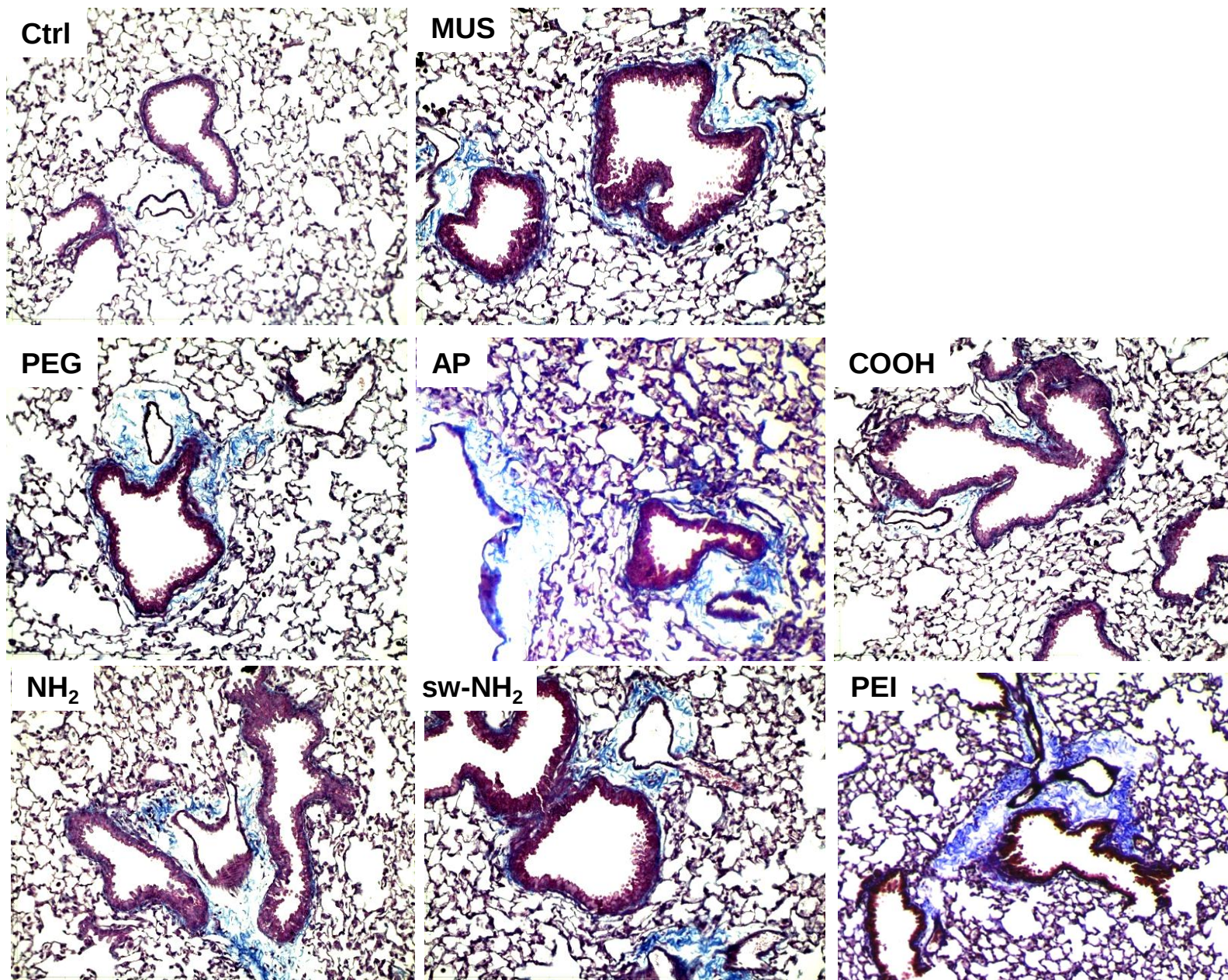
In-House Synthesis of Covalently Functionalized MWCNTs



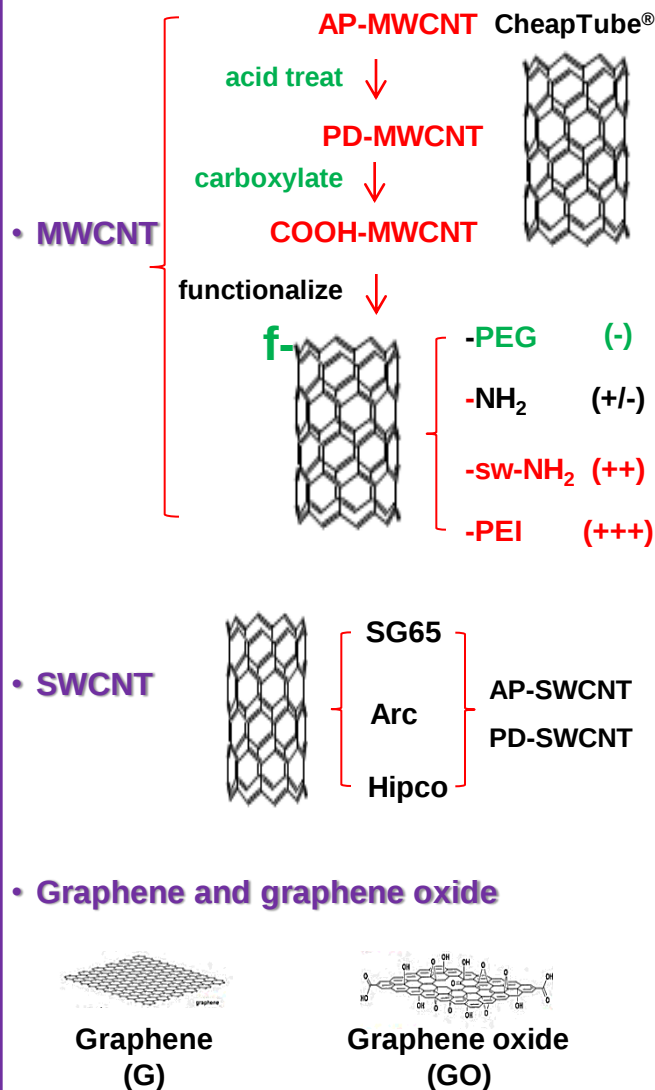
High Content Screening using Cellular Biomarkers to show Pro-fibrinogenic Potential of functionalized CNTs



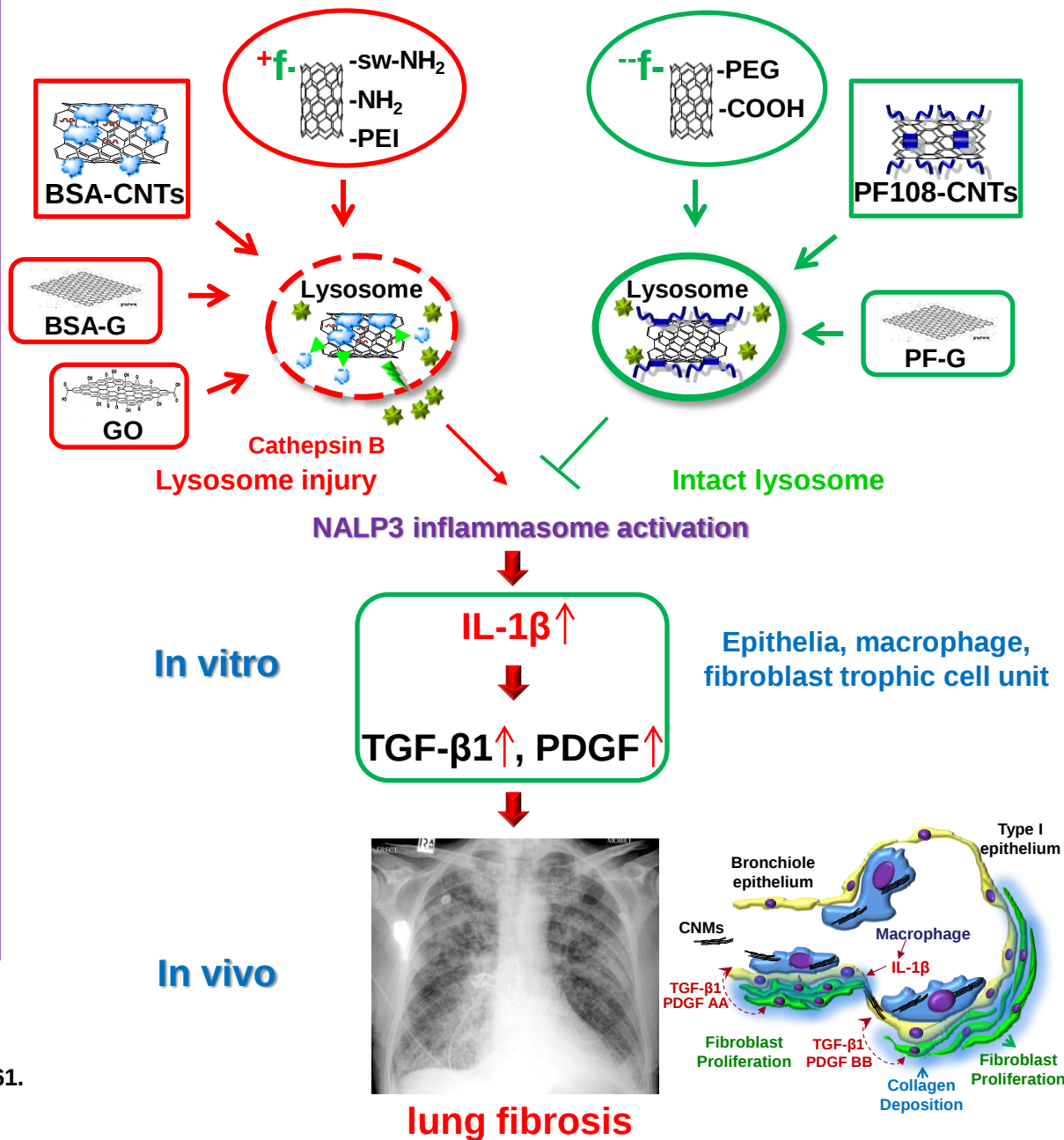
MWCNTs induced collagen deposition in animal lungs



Carbonaceous Nanomaterials Libraries



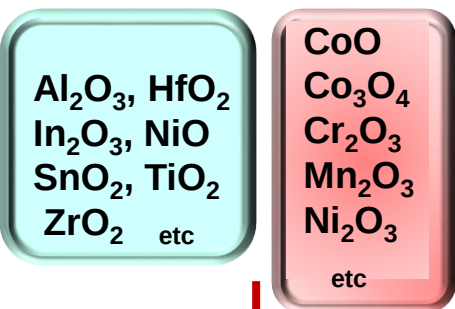
Mechanism of Carbonaceous NMs Toxicological Effects



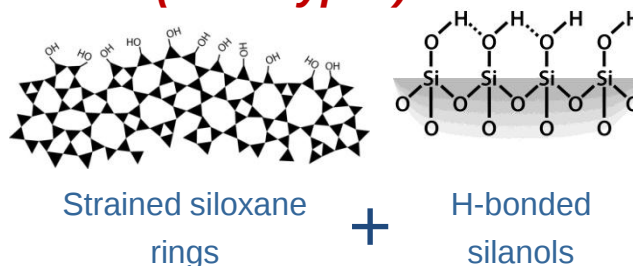
1. Wang. et al. ACS Nano, 2010, 4 (12): 7241–7252.
2. Wang. et al. ACS Nano, 2011, 5 (12): 9772–9787.
3. Wang. et al. Nano Letters, 2012, 12 (6): 3050–3061.
4. Li. et al. ACS Nano, 2013, 7 (3): 2352–2368.

Predictive Toxicology allows Large Numbers of Materials to be grouped into Hazard and Mechanistic Categories

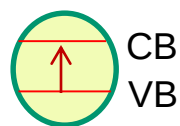
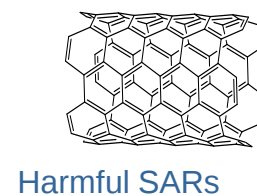
Transition MOx's (>30)



High and Low Temp Silicas (>5 Si types)



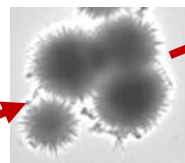
SWCNT & MWCNT Libraries (>5 batches)



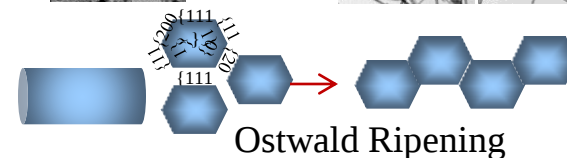
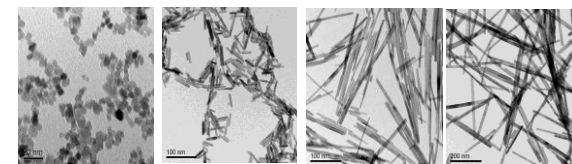
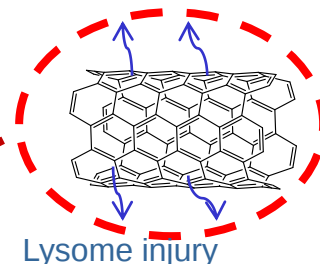
Oxidative stress



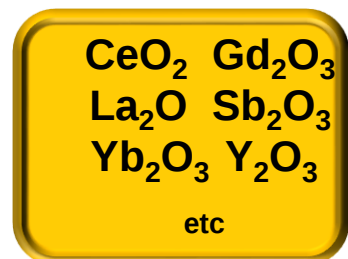
Inflammation



NLRP3



LAR Metal oxides (2)



George et al. ACS Nano. 2010
 Xia et al. ACS Nano. 2011
 Zhang et al. ACS Nano. 2012
 Nel et al. ACR. 2012

Rare Earth Oxides (>10)

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Dr. Ning Li
Dr. Meiyang Wang
Ms. Linjiang Li**

UCLA

Chemistry & Biochemistry

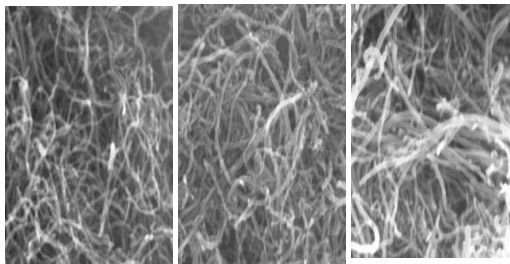
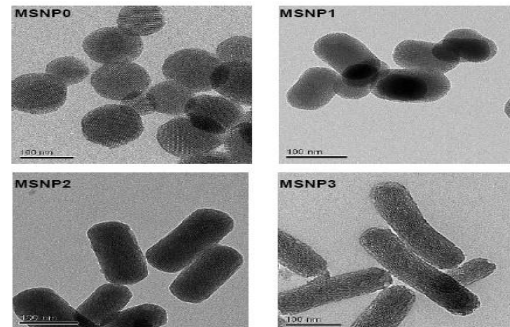
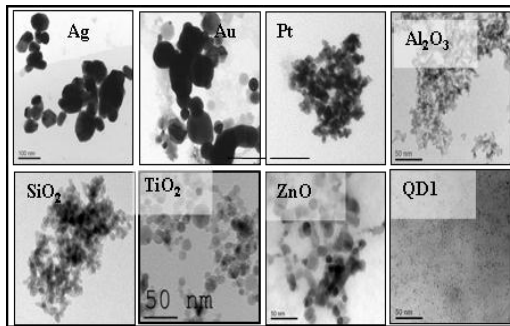
**Dr. Jeff Zink
Min Xue
Zongxi Li
Dr. Monty Liong
Dr. Sarah Angelos
Courtney Thomas
Sui Yang
Sanaz Kabehie
Dr. Yanli Zhao**

Support:

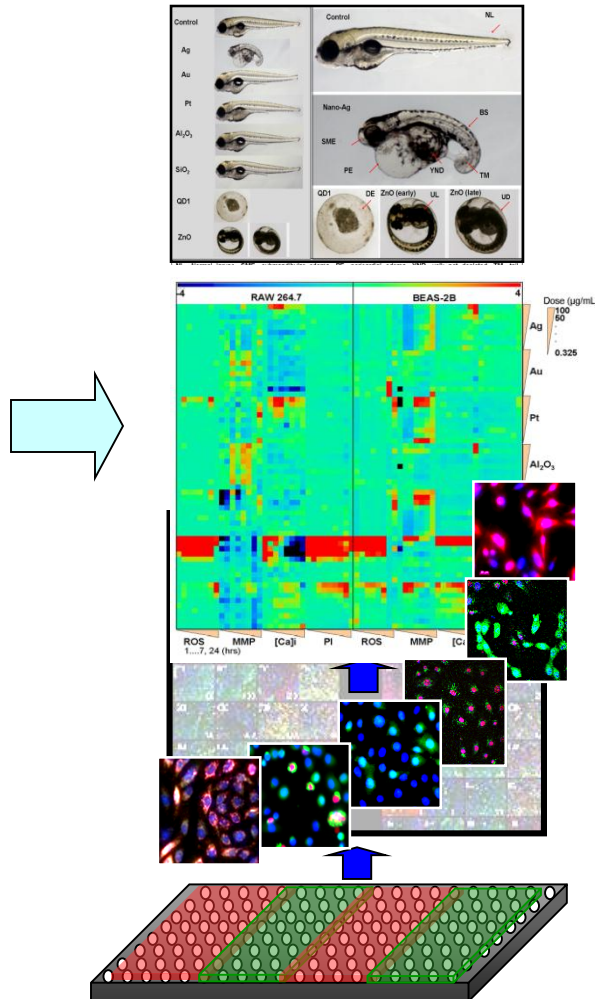


Questions?

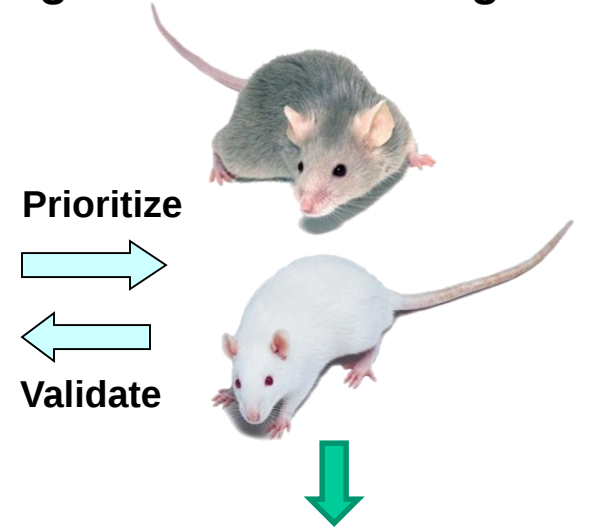
Nanomaterial libraries



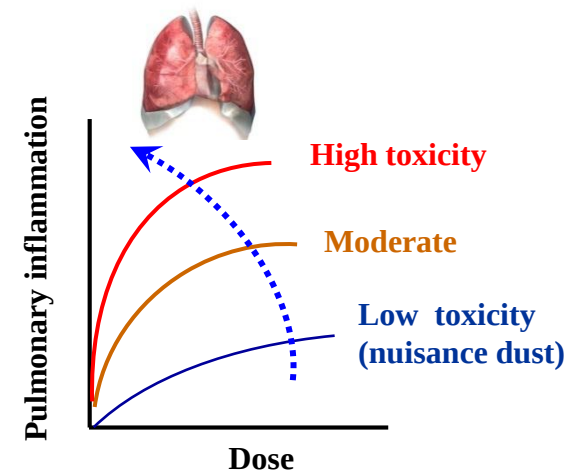
High throughput screening



Animal testing



In vivo hazard ranking



Property

Activity