



# ***In Vitro Toxicity Evaluation of Nanomaterials: Importance of Materials Characterization***

**Saber Hussain, PhD  
Laura Braydich-Stolle, PhD  
Nicole Schaeublin, MS  
Air Force Research Laboratory  
Human Effectiveness Directorate  
Wright-Patterson AFB, OH**

# Report Documentation Page

*Form Approved*  
*OMB No. 0704-0188*

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE <b>28 MAR 2011</b>	2. REPORT TYPE	3. DATES COVERED <b>00-00-2011 to 00-00-2011</b>			
4. TITLE AND SUBTITLE <b>In Vitro Toxicity Evaluation of Nanomaterials: Importance of Materials Characterization</b>		5a. CONTRACT NUMBER			
		5b. GRANT NUMBER			
		5c. PROGRAM ELEMENT NUMBER			
6. AUTHOR(S)		5d. PROJECT NUMBER			
		5e. TASK NUMBER			
		5f. WORK UNIT NUMBER			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <b>Air Force Research Laboratory, Human Effectiveness Directorate, Wright-Patterson AFB, OH, 45433</b>		8. PERFORMING ORGANIZATION REPORT NUMBER			
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)		10. SPONSOR/MONITOR'S ACRONYM(S)			
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)			
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release; distribution unlimited</b>					
13. SUPPLEMENTARY NOTES <b>Presented at the 2011 DoD Environmental Monitoring &amp; Data Quality Workshop (EMDQ 2011), 28 Mar ? 1 Apr, Arlington, VA.</b>					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT <b>Same as Report (SAR)</b>	18. NUMBER OF PAGES <b>57</b>	19a. NAME OF RESPONSIBLE PERSON
a. REPORT <b>unclassified</b>	b. ABSTRACT <b>unclassified</b>	c. THIS PAGE <b>unclassified</b>			



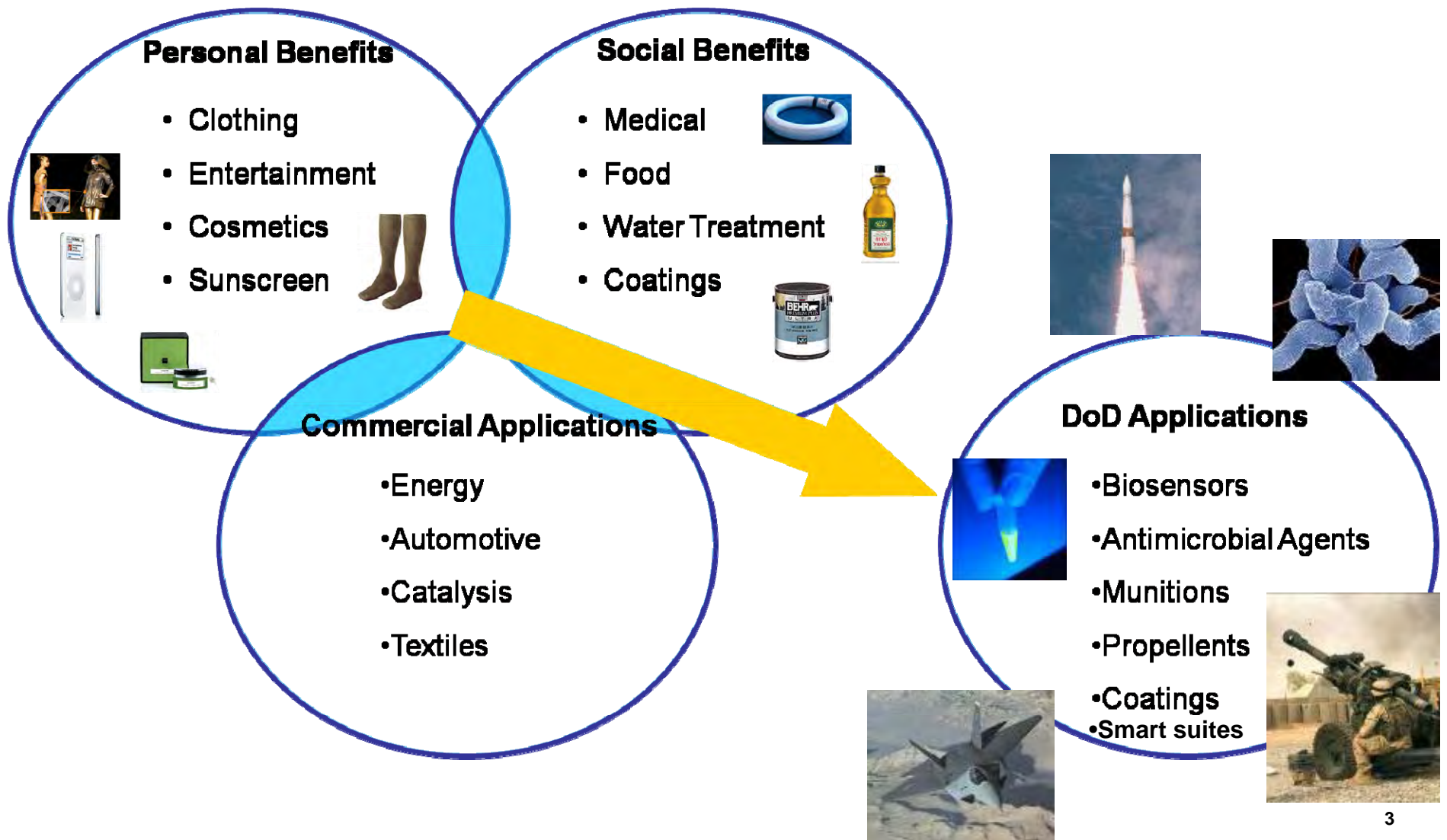
# Outline of Presentation



- **Overview**
- **Biological Interaction of NM**
- **Characterization-Technical Challenges**
- **Toxicity Response of NM**
  - Size
  - Surface Coating
  - Charge
  - Shape or Structure
- **Summary and Conclusion**



# Applications for Nanotechnology







# Applications for Nanotechnology: Silver



## Nanosilver in Footwear



<http://www.nanosilverproducts.com/>  
<http://www.latimes.com/features/health/la-he-nanosilver4-2008aug04.0,3206871.story>

## Antibacterial Nanosilver Infused in Storage Containers



## Uses of Ag NPs

## Nanosilver and Antimicrobial Personal Care



## Nanosilver Coated Surfaces of Medical Devices to Reduce Hospital Related Infections





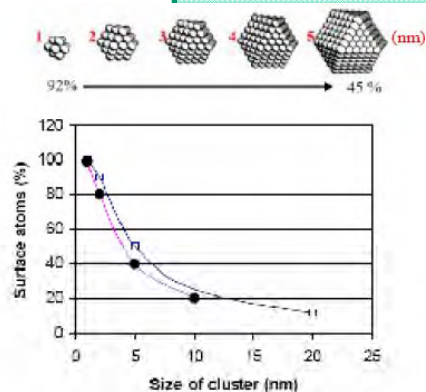


# Unique Properties of Nanoparticles



## Unique Properties

- Optical (metal & Semiconductors)
- Magnetic (metal)
- Electronic & Thermal (CN)
- Catalytic activity (high surface area)



## Challenges

- Controlled synthesis
  - Nano size (1-100 nm)
  - Large surface area relative to mass
  - Surface chemistry and dissolution
  - Surface reactivity/bioaffinities
  - Surface energy
  - Shape/Dimensional Character
- Toxicity and biocompatibility

**Do We Know Potential Risk?**



# What are Potential Routes of Exposure?



# Potential Routes of Exposure



## Application

- Nanoenergetic Materials
- Propellants & Munition
- Ultralight Soldier Clothing
- Chemical & Biological Defense
- Diagnostic and screening
- Drug delivery devices
- Optical Coating
- Consumer Products
- Antimicrobials



**Bionanotechnology-Tissue engineering, Cognition**



**Nanoparticles - Work Environment ??**



**Transport: Food chain & Bioavailability**



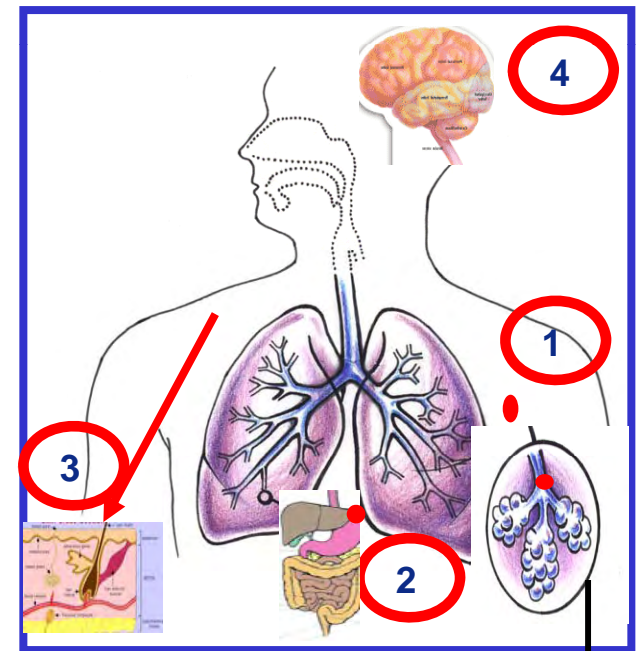
Fullerene in bass  
Oberdörster, 2004

- NP release
- Distribution (water, soil, air)
- Bio-accumulation/persistence

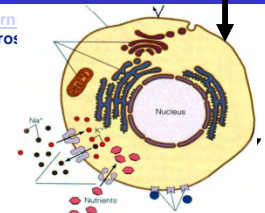
## Implication

**Human health effects of nanomaterials?**  
"Sufficient data is lacking"

**Risk Assessment ?**



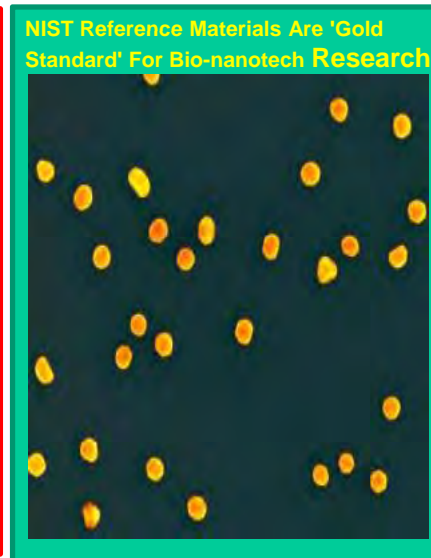
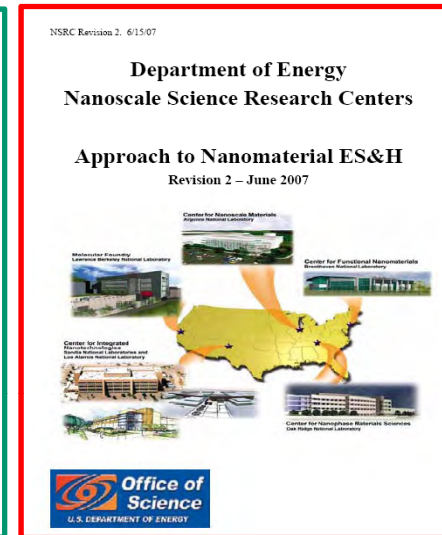
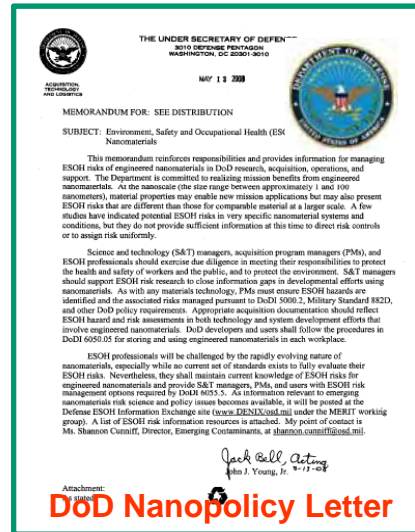
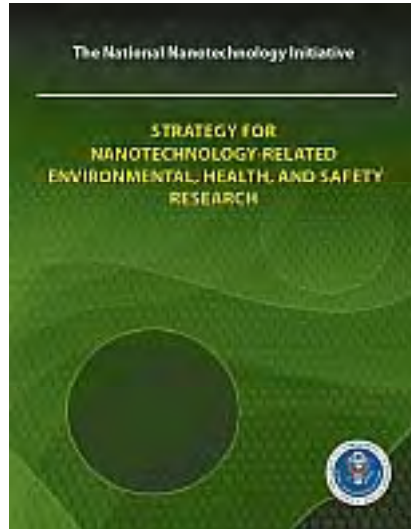
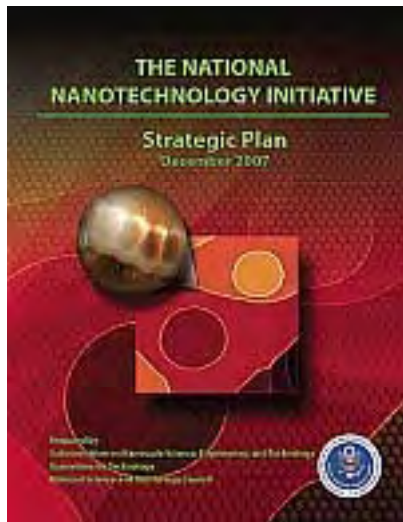
<http://www.enchantedlearnsubjects/anatomy/skin/cros>







# Government Departments and Agencies Participating in Nanotoxicity





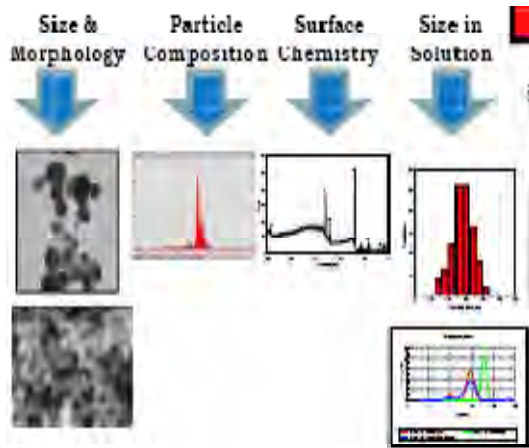
# Basic Understanding of Biological Interaction of NM



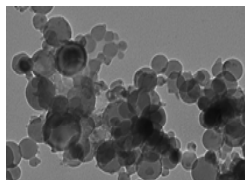
# Biological Interaction of Nanomaterials: Process Overview



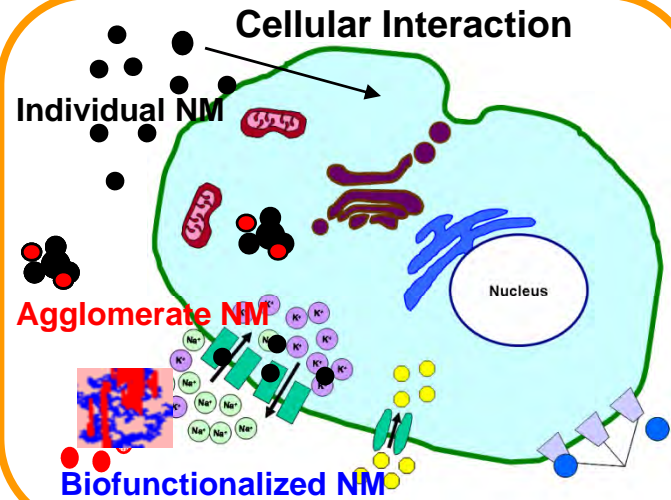
## Characterization



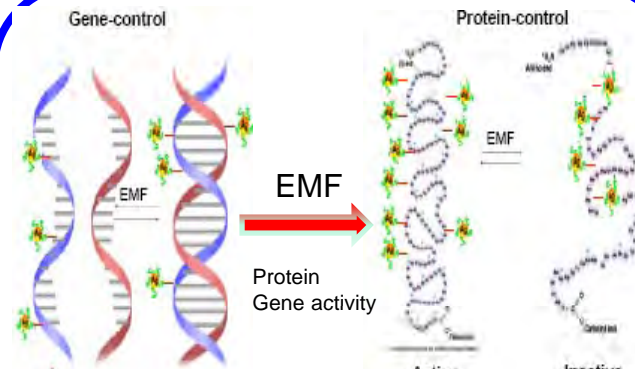
Sample	Average Nanoparticle Size after Dispersion in Lung Surfactant	
	TSM (nm)	DLSZ-Ave (d.nm) ± PDI
Al <sub>2</sub> O <sub>3</sub> 40nm	48.08 ± 21.01	878 ± 0.495
Al 50nm	32.71 ± 28.28	805 ± 0.497
Al-OA 50nm	51.09 ± 22.48	2430 ± 1.00



## Bio-Nano-Interaction



## Beneficial Effects



- Q1: Do NMs respond to EM frequency within cellular environment?
- Q2: Can we control and manipulate cellular env

## Toxicological Effects

### In vitro Toxicity

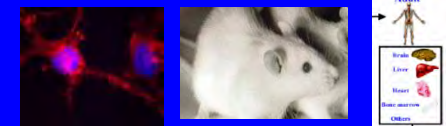
- Q1: Uptake?
- Q2: Proteins or nucleic acids
- Q3: Internal organelles?
- Q4: Overall effect to cell function ?
- Q5: EM frequency Effect ?

### In vivo Toxicity

- Q1: Exposure?
- Q2: Dose
- Q3: Acute?
- Q4: Chronic ?
- Q5: EM frequency respond ?

Nanoparticle Characterization

## Predictive Modeling



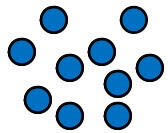




# Post Exposure Characterization of Nanoparticles



## Physical Factors:



Disperse?



Agglomerate?



Shape?

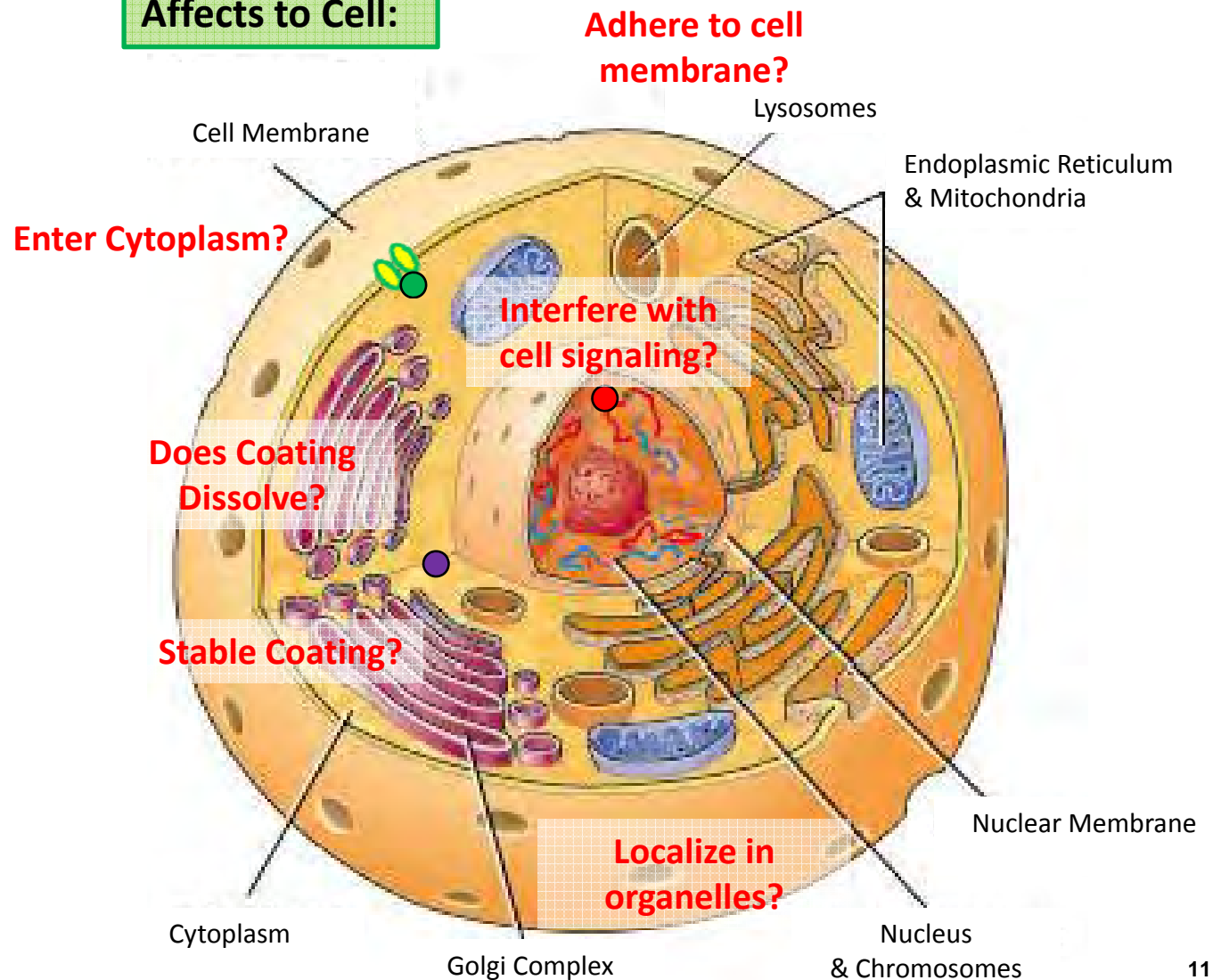


Coating?



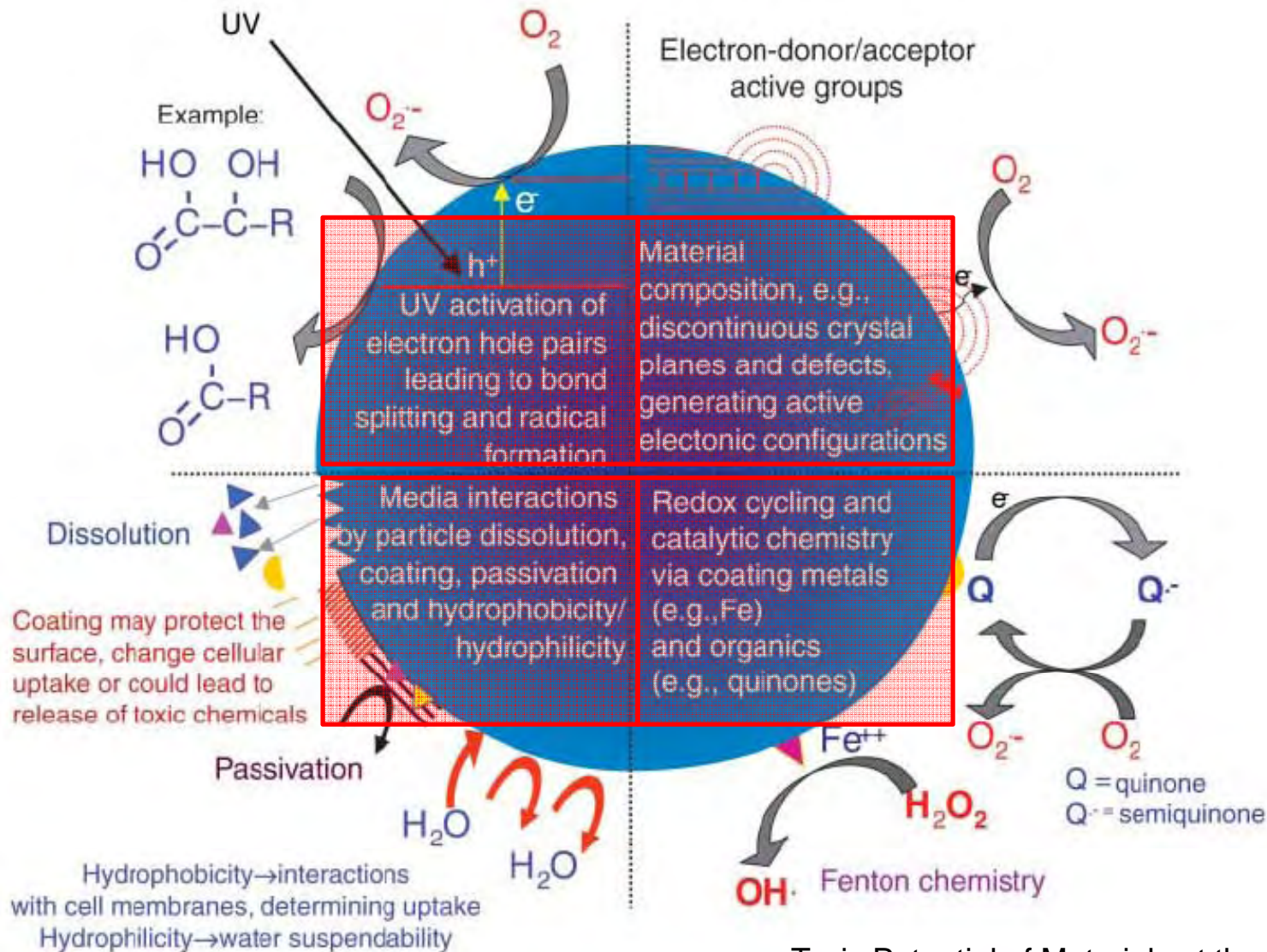
Composition?

## Affects to Cell:





# Possible mechanisms by which NM interact with biological tissue



Toxic Potential of Materials at the Nano level.  
Andre Nel et al (2006) Feb 3, 311; 622; Science



# The Hierarchical Oxidative Stress Model



	High Level of GSH/GSSG Ratio		Low Level of GSH/GSSG Ratio	
	Low Level of Oxidative stress		High Level of Oxidative stress	
Response Pathways	<b>Normal</b>	<b>I Anti Oxidant Defense</b>	<b>II Pro- inflammatory response</b>	<b>III Cytotoxicity</b>
Signaling	-	Nrf-2	MAP Kinase NF-kB cascade	<b>MMP Electron TS</b>
Gene Exp	-	Anti-oxidant response element	AP-1 NF-kB	-
Outcome	-	Antioxidant enzymes or Phase II enzymes	Cytokines & Chemokines	<b>Necrosis &amp; Apoptosis</b>

Toxic Potential of Materials at the Nano level.  
 Andre Nel et al (2006) Feb 3, 311; 622; Science

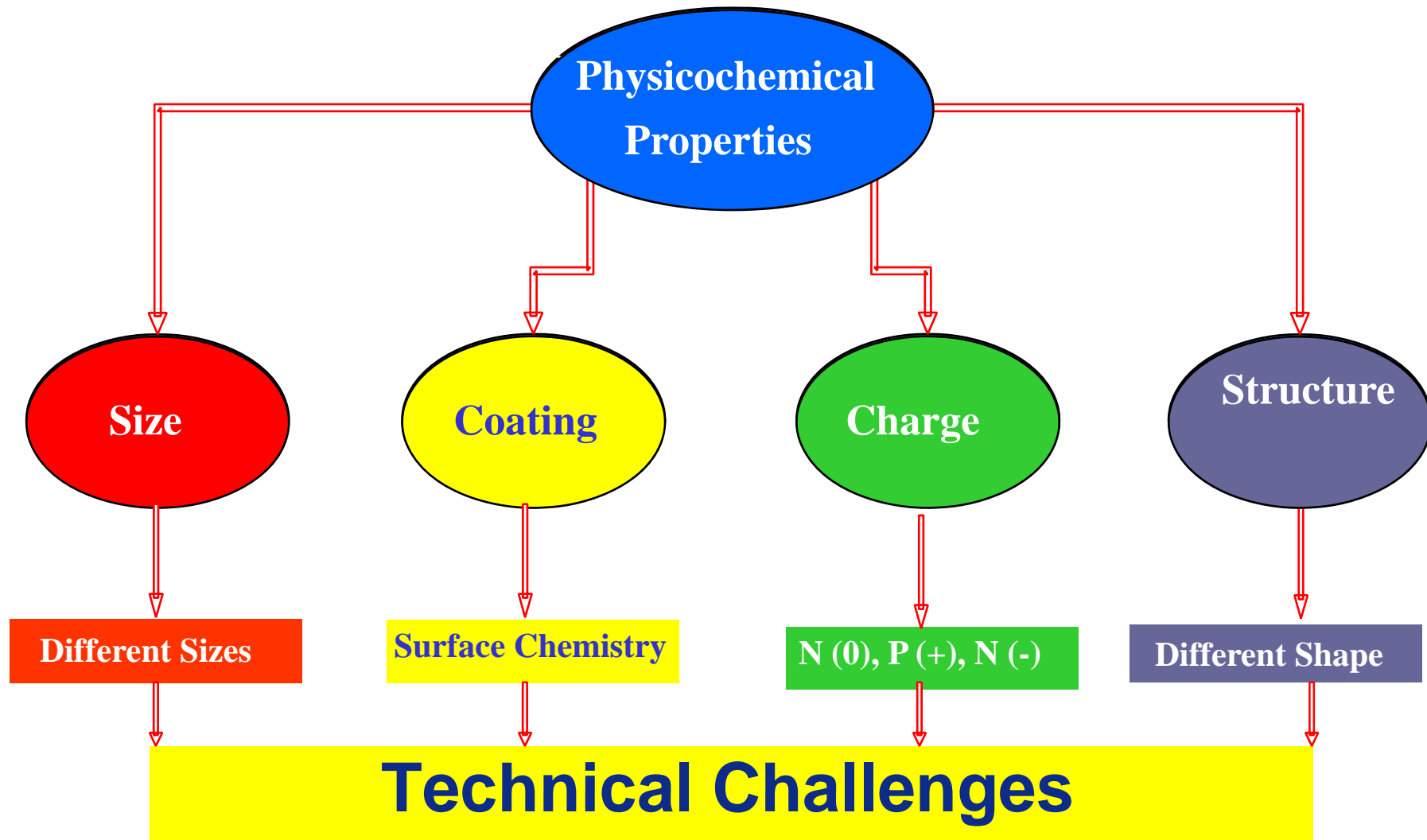




# Characterization & Challenges



# Toxicity Based on Physicochemical Properties

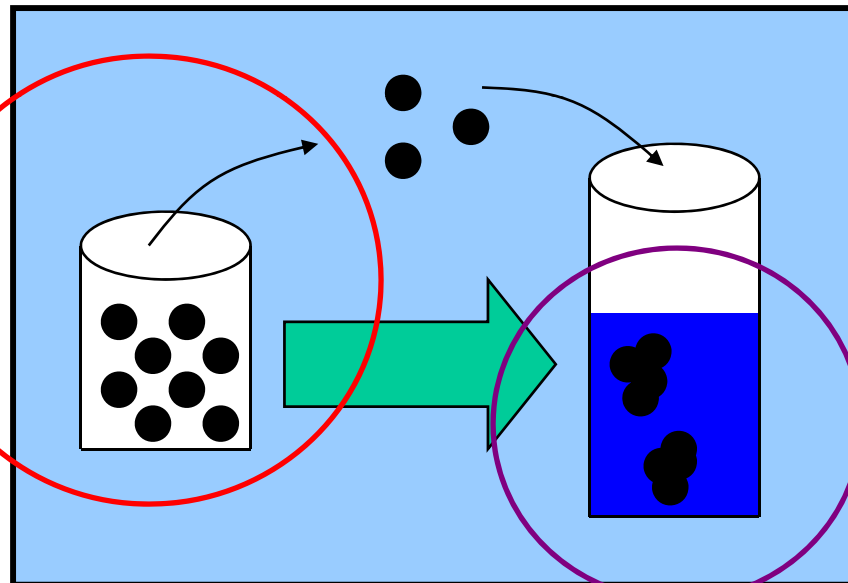




# Nanoparticle Size vs. Toxicity



Is the primary size related to the toxicity?

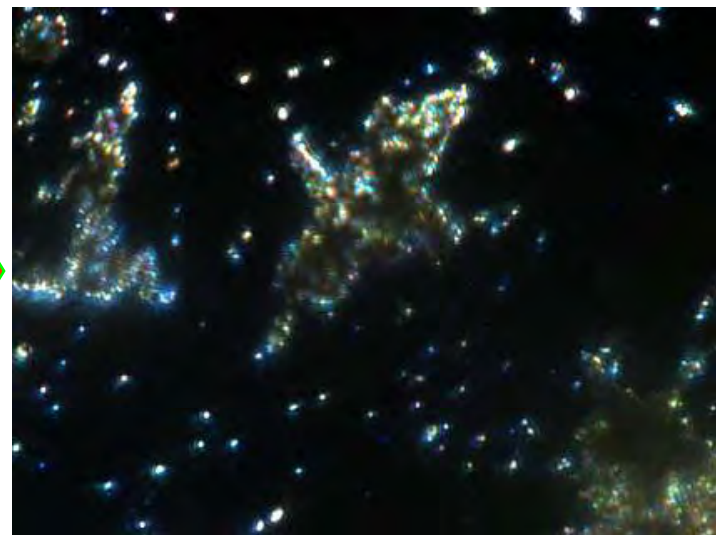
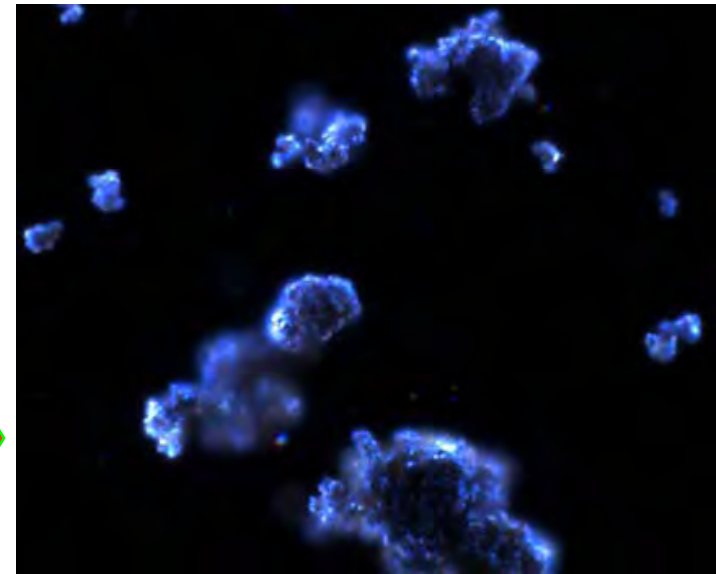
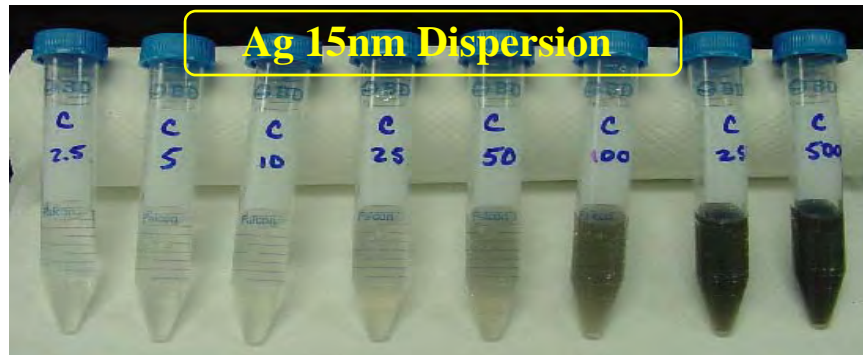


Or is the agglomerated size related to the toxicity?





# Turbidity & Dispersion Issues

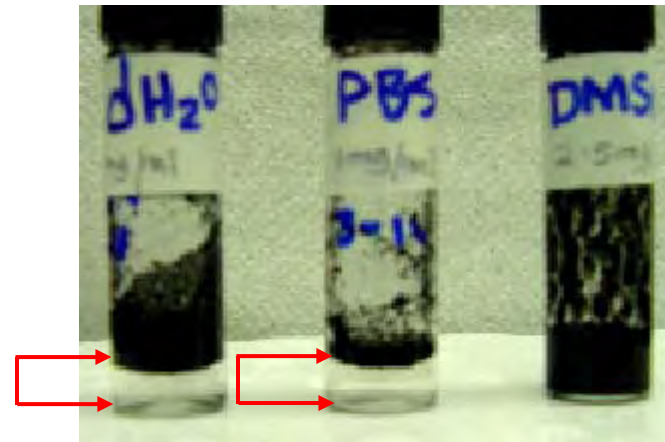




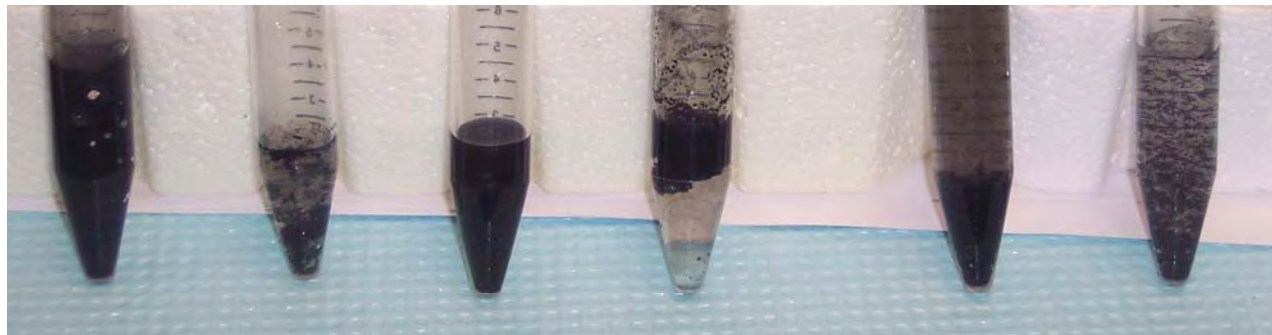
# Non-homogeneous Dispersion & Agglomeration



dH<sub>2</sub>O      PBS      DMSO



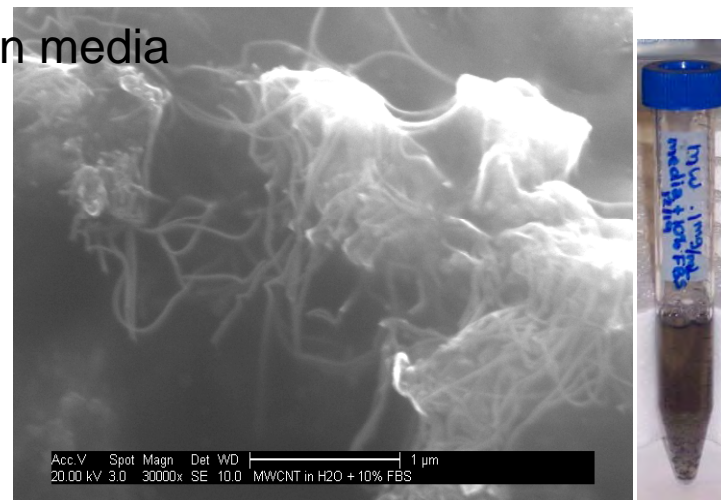
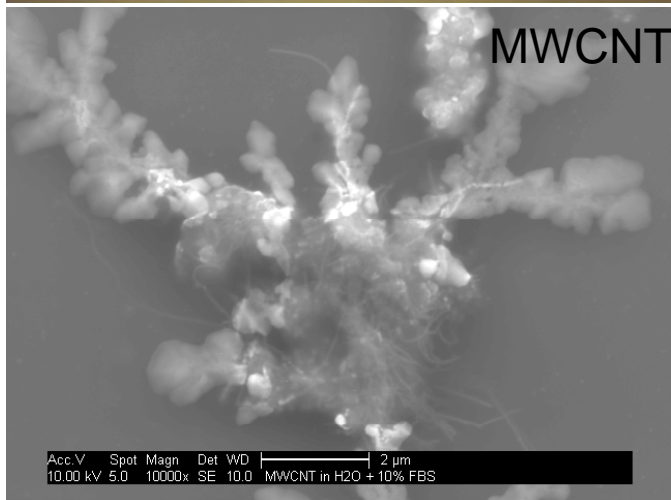
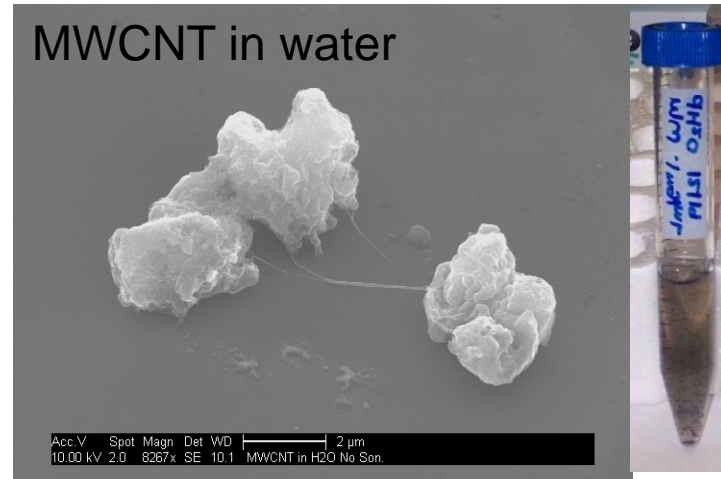
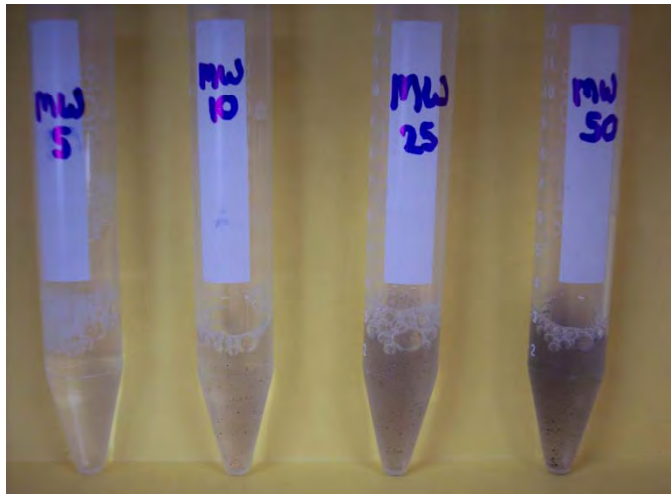
Single Walled Carbon Nanotubes



Carbon Nanofibers



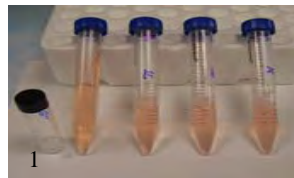
# MWCNT Agglomerate Structure in Solution



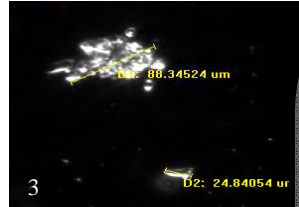
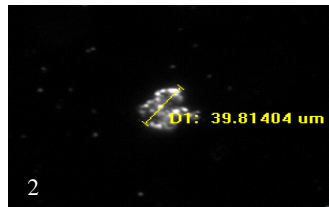




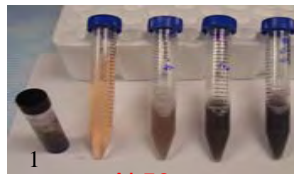
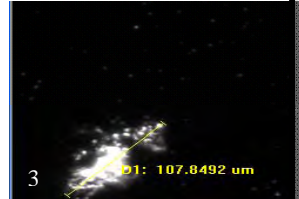
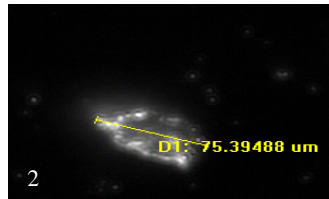
# Agglomeration Issues



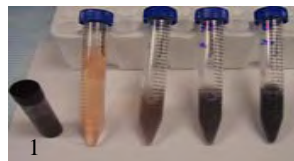
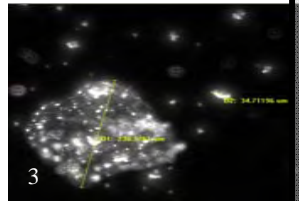
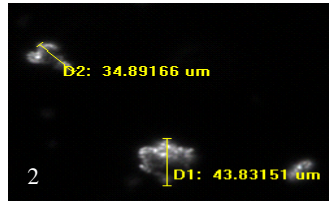
Al<sub>2</sub>O<sub>3</sub> 30nm



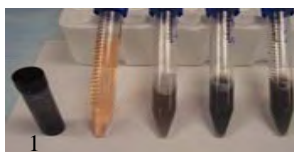
Al<sub>2</sub>O<sub>3</sub> 40nm



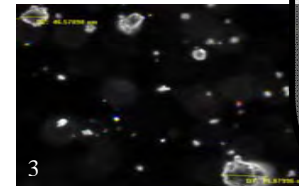
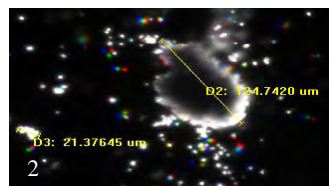
Al 50nm



Al 80nm



Al 120nm



## Dynamic Light Scattering

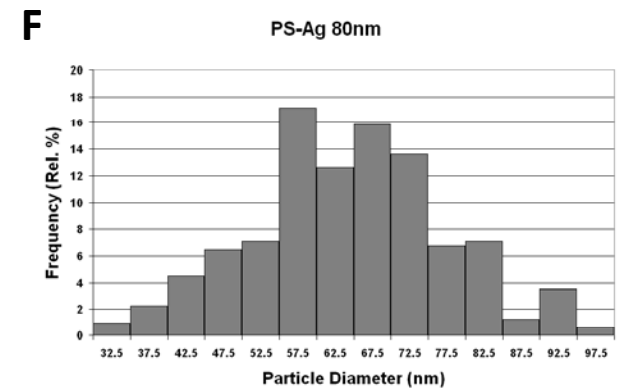
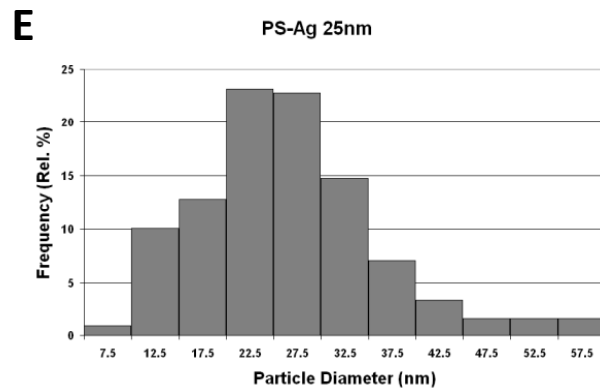
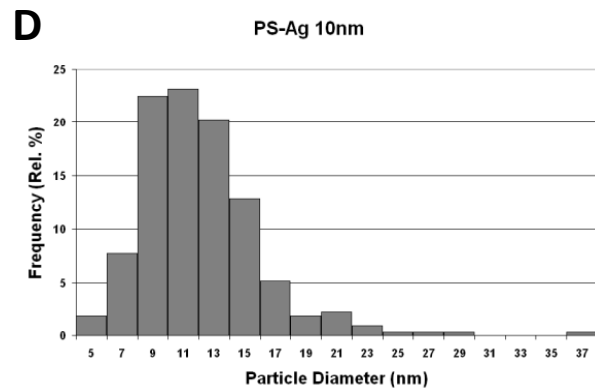
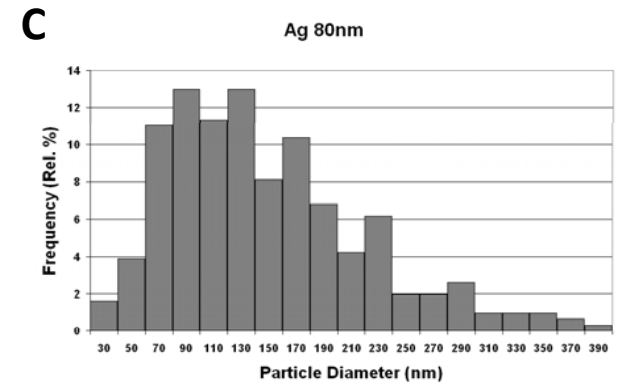
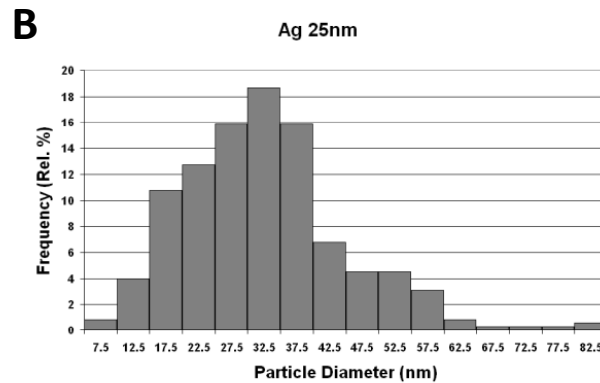
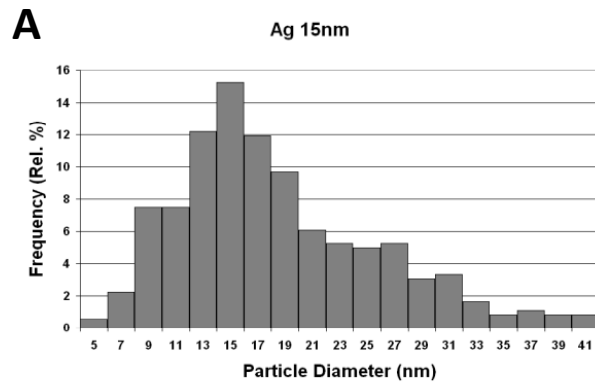
Particle	DLS	
	Diameter (nm)	PDI
<b>Al<sub>2</sub>O<sub>3</sub> 30nm</b>		
Water	210	0.125
Media	1430	0.373
Media w/ 20% Serum	223	0.23
<b>Al<sub>2</sub>O<sub>3</sub> 40nm</b>		
Water	237	0.145
Media	1050	0.232
Media w/ 20% Serum	251	0.252
<b>Al 50nm</b>		
Water	253	0.224
Media	1170	0.247
Media w/ 20% Serum	395	0.393
<b>Al 80nm</b>		
Water	378	0.422
Media	1390	0.268
Media w/ 20% Serum	355	0.398
<b>Al 120nm</b>		
Water	342	0.341
Media	1610	0.25
Media w/ 20% Serum	535	0.821

DLS trend of particles highly agglomerating in media without serum, and then decreasing agglomeration with presence of serum.





# TEM Measured Size Distributions





# Constant Mass of Sample: 10 $\mu$ g



Sample	Estimate of Total Number of Nanoparticles	Estimate of Surface Area Per Particle (nm <sup>2</sup> )	Estimate of Volume Per Particle (nm <sup>3</sup> )	Surface Area to Volume Ratio
<i>Ag 15nm</i>	7.8E+11	469	1226	0.382
<i>Ag 25nm</i>	1.6E+11	1267	5840	0.217
<i>Ag 80nm</i>	3.0E+09	16818	321920	0.052
<i>PS-Ag 10nm</i>	1.8E+12	283	519	0.546
<i>PS-Ag 25nm</i>	2.5E+11	997	3824	0.261
<i>PS-Ag 80nm</i>	9.2E+09	10143	104010	0.098

## Constant Number of Particles: 1.00x10<sup>12</sup>

Sample	Estimate of Mass (mg/mL)	Estimate of Total Surface Area (nm <sup>2</sup> )	Estimate of Total Volume (nm <sup>3</sup> )
<i>Ag 15nm</i>	0.05	1.2E+15	5.0E+15
<i>Ag 25nm</i>	0.28	3.8E+15	2.7E+16
<i>Ag 80nm</i>	32.23	8.5E+16	3.1E+18
<i>PS-Ag 10nm</i>	0.01	5.1E+14	1.3E+15
<i>PS-Ag 25nm</i>	0.15	2.5E+15	1.4E+16
<i>PS-Ag 80nm</i>	1.65	1.4E+16	1.6E+17



# Summary: Technical Challenges



- **Variation in size distribution**
- **Non-homogeneous dispersion**
  - Maintaining homogenous dispersion
  - Stability of solution
- **Agglomeration after gentle mixing**
  - Proper mixing protocols need to be developed
  - Effect of carbon coating
- **Increasing turbidity**
  - Turbidity may have impact on cells

***Collaboration between materials scientists and toxicologists is key to establish safety risk of nanotechnology***



# Editorial Highlight in Toxicological Sciences

## IMPACT



### TOXICOLOGICAL HIGHLIGHT

## How Meaningful are the Results of Nanotoxicity Studies in the Absence of Adequate Material Characterization?

Editor

David B. Warheit<sup>1</sup>

*DuPont Haskell Global Centers for Health and Environmental Sciences, Newark, Delaware*

Received November 6, 2007; accepted November 6, 2007

In their publication in this issue, Murdock *et al.* (2007) have focused on the importance of developing adequate physico-chemical characterization of nanomaterials prior to undertaking experiments for *in vitro* toxicity assessments. These authors have correctly suggested that for *in vitro* toxicity studies, particle size, size distribution, particle morphology, particle composition, surface area, surface chemistry, and particle reactivity in solution are important factors which need to be accurately characterized as prerequisites for implementing nanoparticle toxicity studies. This point cannot be overstated,

Murdock and coworkers concluded that many metals and metal oxide nanomaterials tend to agglomerate in solution. Moreover, other variables, such as the addition of serum in the culture media, can affect toxicity measurements, likely due to influences affecting agglomeration and/or surface chemistry of nanoparticles. These factors represent important considerations that have not been previously recognized.

Therefore, in the Murdock *et al.* study, these investigators have focused on characterizing a wide range of nanomaterials including metals, metal oxides, and carbon-based structures using dynamic light scattering (DLS) concomitant with transmission electron microscopy, for particles dispersed under wet conditions in cell culture media, with and without serum. Some basic cell viability and morphology studies were correlated with DLS particle size characteristic experiments to assess toxicity from observed agglomeration alterations under the various experimental conditions.

Perhaps the most significant impact of the Murdock *et al.* publication is to raise the issue of the importance of adequately characterizing the nanomaterial preparation prior to the initiation of toxicological experimentation.





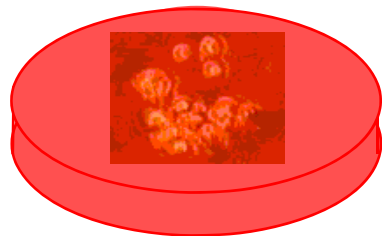
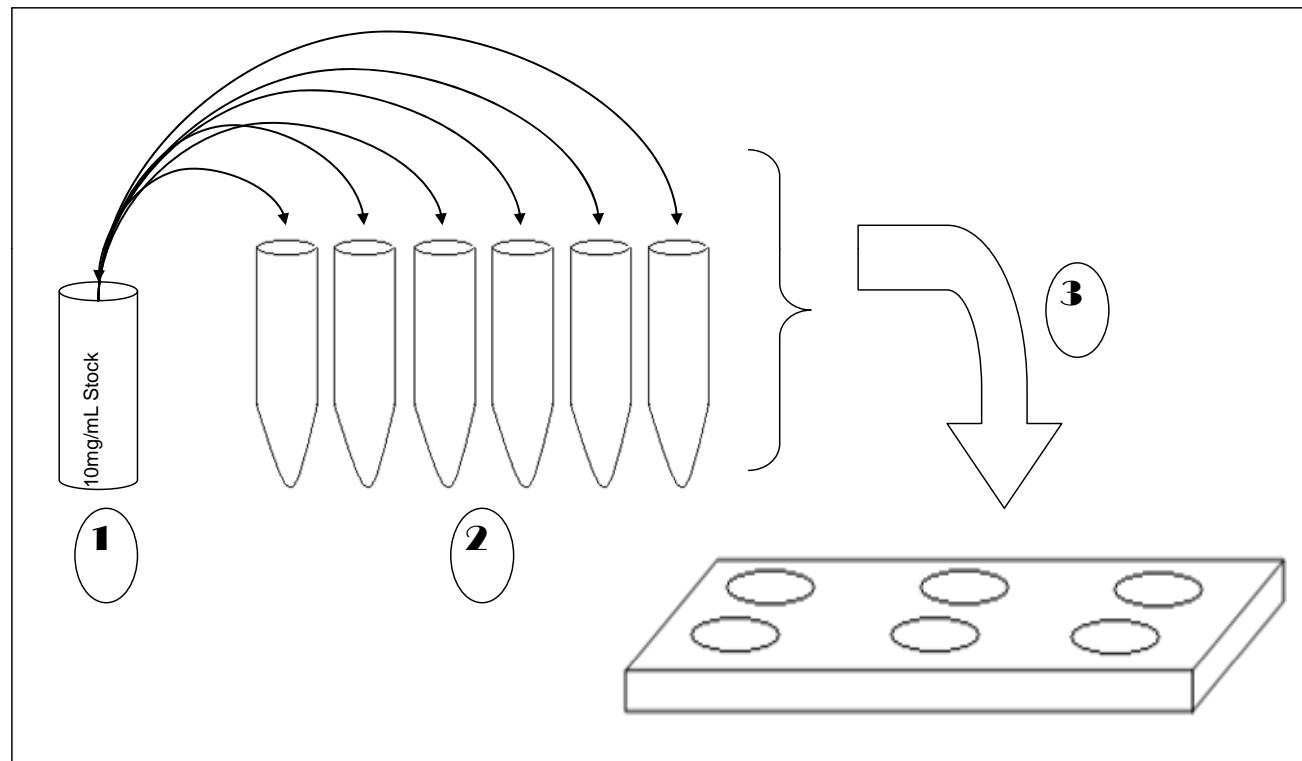
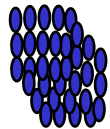
# Nanotoxicity Studies Experimental Design



# Schematic Representation of Dosing Cells



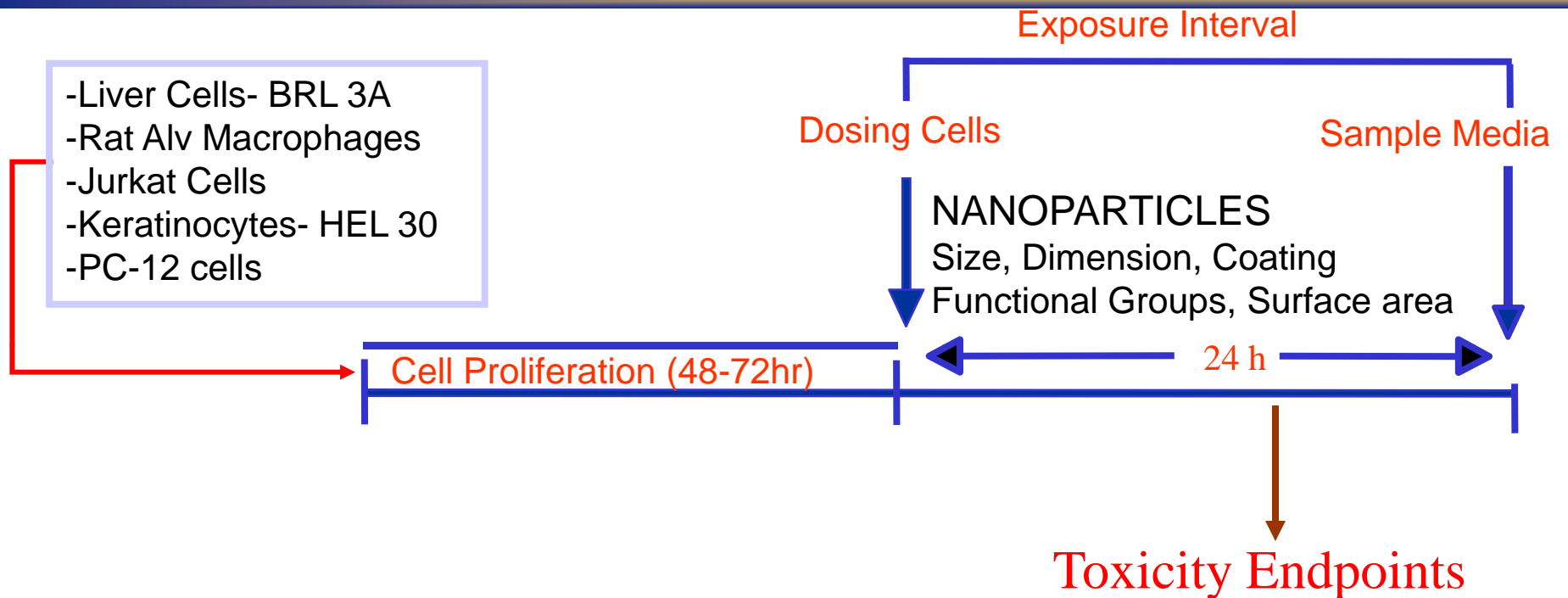
Nanoparticles



In Vitro Cell Culture



# Experimental Design



- Liver Cells- BRL 3A
- Rat Alv Macrophages
- Jurkat Cells
- Keratinocytes- HEL 30
- PC-12 cells

- Membrane Leakage- **LDH**
- Mitochondrial Function- **MTT**
- Reactive Oxygen Species- **ROS**
- Mitochondrial Mem Pot- **MMP**
- Cytokines- **TNF- $\alpha$ , IL-6, MIP-2**
- Gene Expression
- Protein Expression



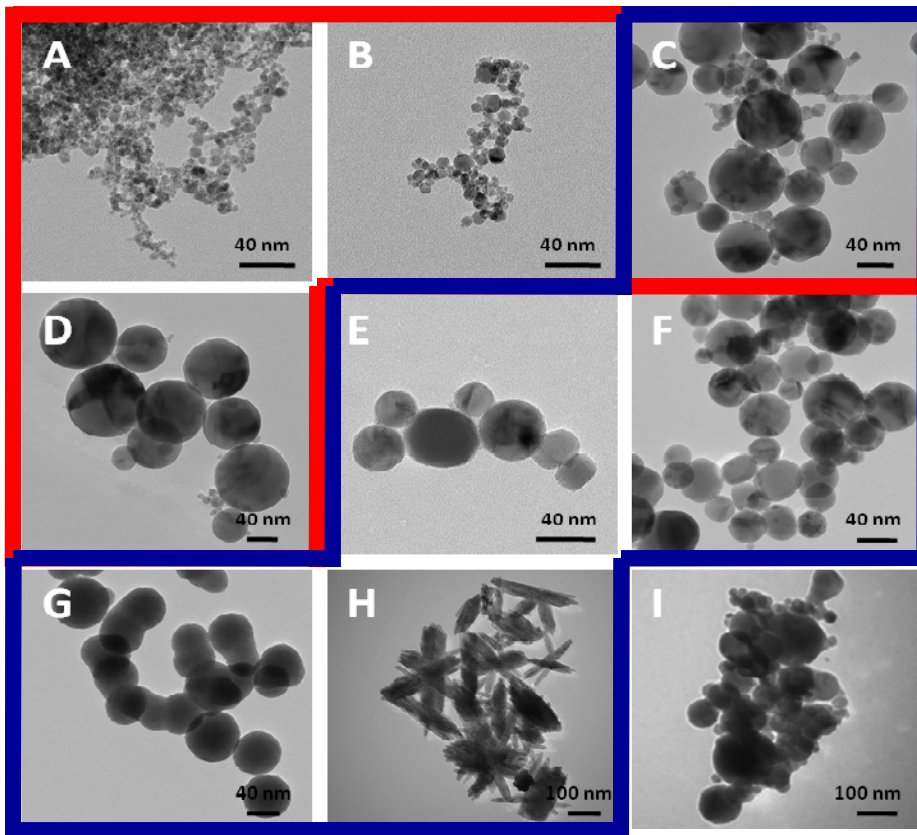
# Nanoparticles Size vs. Toxicity



# Size vs. Crystal Structure in TiO<sub>2</sub> Nanotoxicity

## Study Design

The bioeffects of TiO<sub>2</sub> were studied in mouse keratinocytes using the following nanoparticles:



Size Dependent Study with 100% Anatase TiO<sub>2</sub>

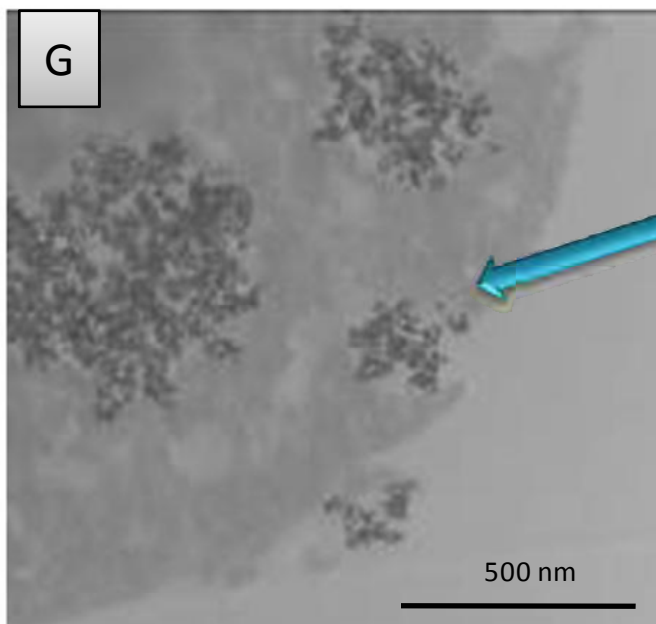
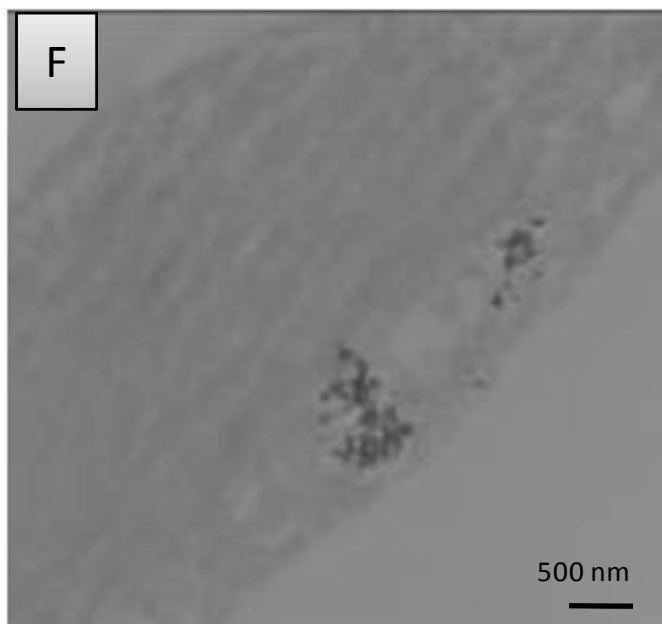
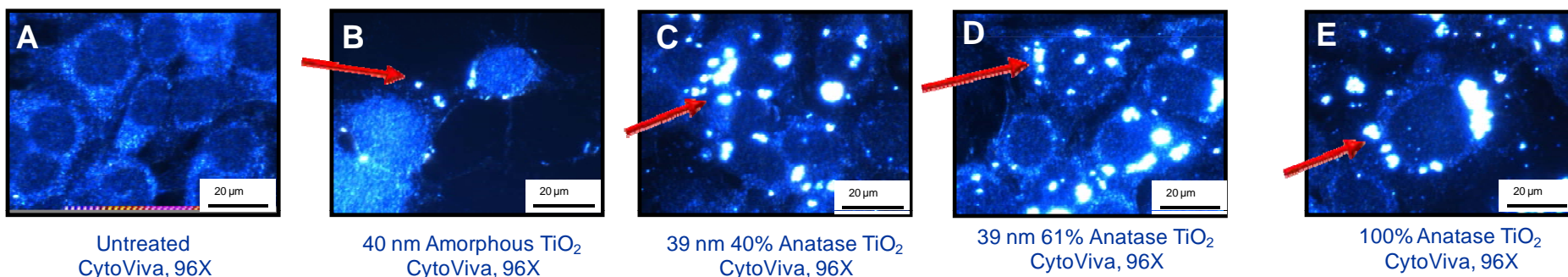
A: 6.3 nm  
B: 10 nm  
C: 50 nm  
D: 100 nm

Crystal Structure Study with TiO<sub>2</sub>

C: 100% Anatase 50 nm  
E: 40% Anatase 39 nm  
F: 61% Anatase 39 nm  
G: Amorphouse 40 nm  
H: 100% Rutile 51 nm

# Size vs. Crystal Structure in TiO<sub>2</sub> Nanotoxicity

## Uptake of TiO<sub>2</sub> Using CytoViva and TEM Imaging



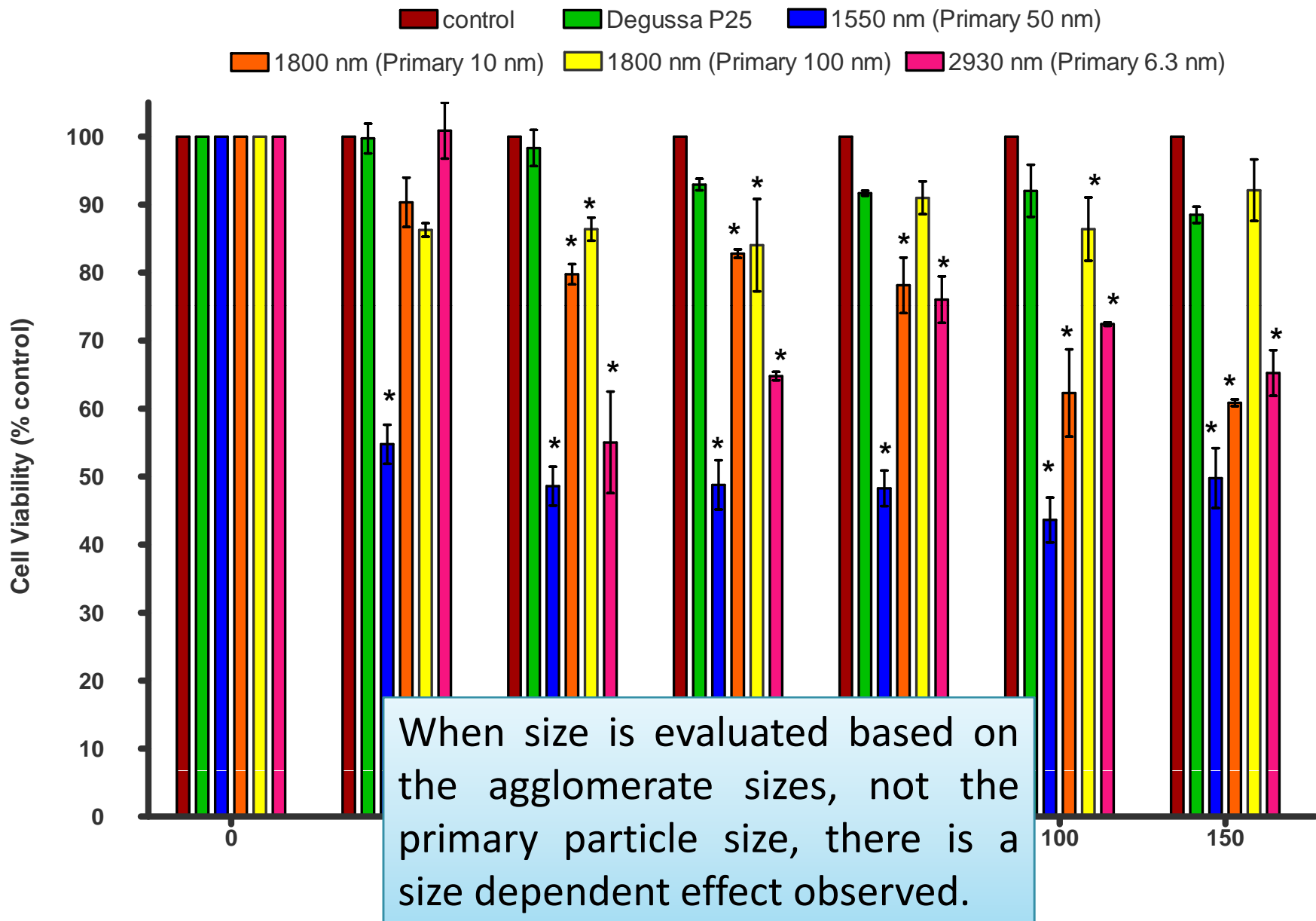
TEM of the 61% anatase

Cell appears to be “eating” the NP, indicates endocytosis as most likely mechanism of uptake.

# Size vs. Crystal Structure in TiO<sub>2</sub> Nanotoxicity

	Particle	DLS		LDV	
		Average Diameter (nm)	PDI	Zeta Potential $\zeta$ (mV)	Electrophoretic Mobility U ( $\mu\text{mcm}/(\text{Vs})$ )
Crystallinity	<b>*TiO<sub>2</sub> 40 nm Amorphous</b>				
	DI H <sub>2</sub> O	1300	0.282	-21.2	-1.66
	DMEM/F-12 Media	2040	0.349	***	***
	<b>*TiO<sub>2</sub> 39 nm, 39% R, 61% A</b>				
	DI H <sub>2</sub> O	796	0.654	-23.3	-1.83
	DMEM/F-12 Media	2510	0.408	***	***
	<b>*TiO<sub>2</sub> 39 nm, 60% R, 40% A</b>				
	DI H <sub>2</sub> O	519	0.661	-20.1	-1.58
	DMEM/F-12 Media	2030	0.743	***	***
	<b>*TiO<sub>2</sub> 50 nm 100% A</b>				
DI H <sub>2</sub> O	749	0.435	-13.7	-1.07	
DMEM/F-12 Media	1550	0.861	***	***	
<b>TiO<sub>2</sub> 51 nm, 100% R</b>					
DI H <sub>2</sub> O	582	0.604	-21.8	-1.71	
DMEM/F-12 Media	1110	0.647	***	***	
Size	<b>TiO<sub>2</sub> 6.3 nm</b>				
	DI H <sub>2</sub> O	476	0.552	-29.0	-2.27
	DMEM/F-12 Media	2930	1	***	***
	<b>TiO<sub>2</sub> 10 nm</b>				
	DI H <sub>2</sub> O	216	0.439	-2.79	1.63
	DMEM/F-12 Media	1800	0.402	***	***
	<b>TiO<sub>2</sub> 50 nm</b>				
	DI H <sub>2</sub> O	749	0.435	-13.7	-1.07
	DMEM/F-12 Media	1550	0.861	***	***
	<b>TiO<sub>2</sub> 100 nm</b>				
DI H <sub>2</sub> O	1000	0.301	-21.3	-1.67	
DMEM/F-12 Media	1800	0.402	***	***	
Control	<b>TiO<sub>2</sub> Degussa</b>				
	DI H <sub>2</sub> O	542	0.499	19.4	1.52
	DMEM/F-12 Media	3500	0.303	***	***
	<b>TiO<sub>2</sub> Ruthenium</b>				
	DI H <sub>2</sub> O	663	0.689	-17.9	-1.41
DMEM/F-12 Media	5870	1	***	***	

# Size vs. Crystal Structure in TiO<sub>2</sub> Nanotoxicity





# Size vs. Crystal Structure in TiO<sub>2</sub> Nanotoxicity

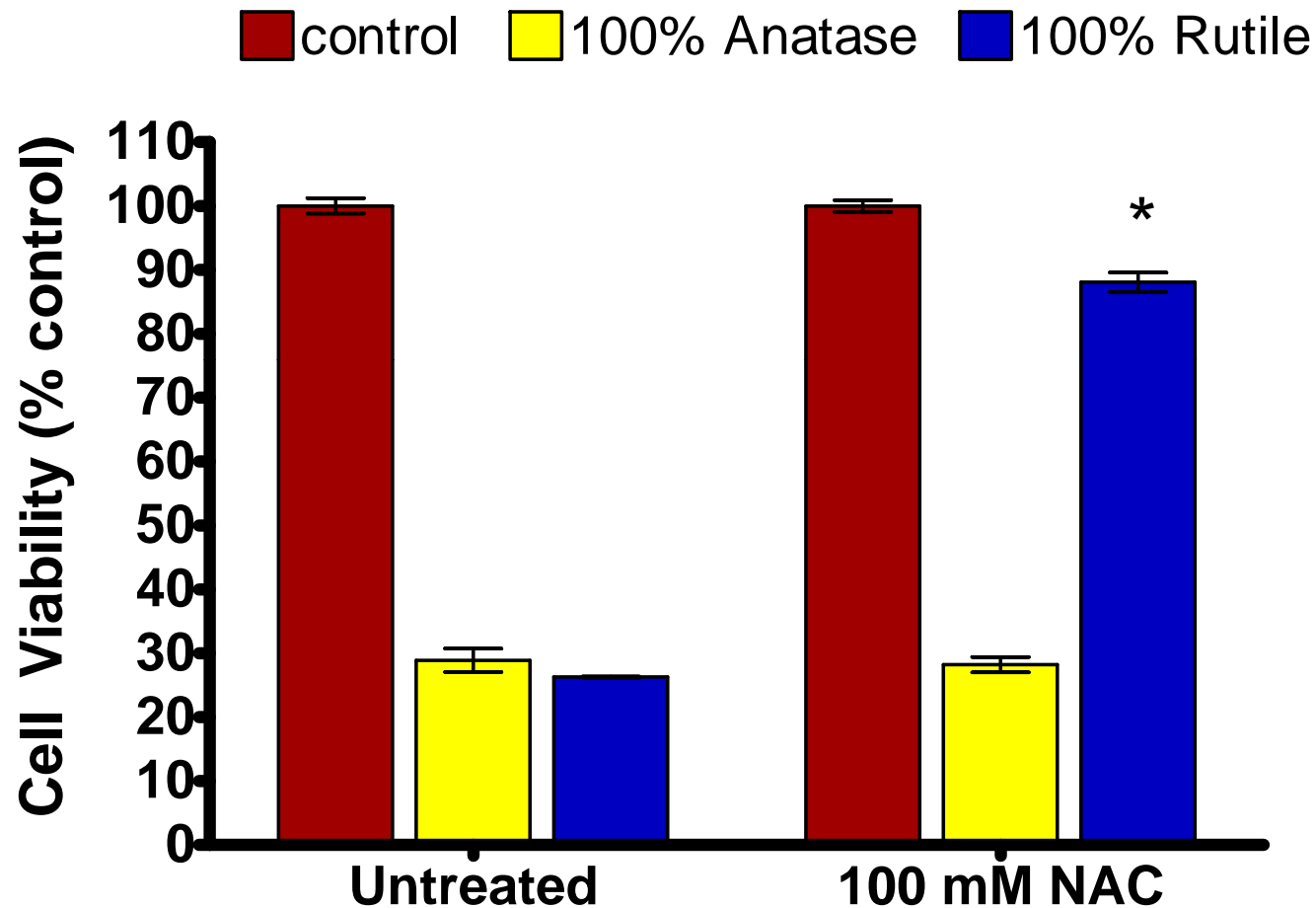
## Summary of Cellular Effects Data

	Particle	Nanoparticle Characterization				Cellular Response to Nanoparticles			Mode of Cell Death
		TEM Average Diameter (nm)	DLS Hydrodynamic Diameter (nm)	LDV Zeta Potential (mV)	Acellular ROS Fold Increase over Control at 100µg/mL	MTS % to Control at 100µg/mL	LDH % to Control at 100µg/mL	ROS Fold Increase over Control at 100µg/mL	
Crystallinity	<i>TiO<sub>2</sub> 40nm Amorphous</i>	40 ± 16	2040	-21.2	1.04	40.98 ± 7.97	3.71 ± 0.34	2.83	Apoptosis
	<i>TiO<sub>2</sub> 39nm, 39%R, 61%A</i>	39 ± 10	2510	-23.3	1.42	32.12 ± 2.36	2.12 ± 0.12	1.74	Apoptosis
								3.29	Apoptosis
								1.32	Necrosis
								5.13	Apoptosis
Size	<i>TiO<sub>2</sub> 100nm</i>							0.84	Necrosis
								0.95	Necrosis
								1.32	Necrosis
		100 ± 23	1800	-21.3	1.00	75.48 ± 0.13	124.50 ± 30.16	0.52	Necrosis
Control	<i>TiO<sub>2</sub> Degussa P25</i>	30	3500	19.4	1.73	91.92 ± 5.43	0.39 ± 0.22	1.75	Not toxic
	<i>TiO<sub>2</sub> Ruthenium</i>	40 ± 14	5870	-17.9	***	***	***	***	***
Cellular Impact Level		Low			Moderate			High	

**If the apoptosis correlates to the formation of ROS can antioxidants control this effect?**

**Crystal structure appears to be mediating the mechanism of cell death**

## Size vs. Crystal Structure in TiO<sub>2</sub> Nanotoxicity



Conclusion: The rutile TiO<sub>2</sub> ROS initiated apoptosis can be controlled for by treatment with antioxidants, thus making the anatase structure more toxic than the rutile.

## Summary and Conclusions

The TiO<sub>2</sub> nanoparticles are being taken up by the keratinocytes, most likely, through endocytosis.

The TiO<sub>2</sub> nanoparticles agglomerate when dispersed in exposure media. When describing size dependent toxicity, agglomerate size and primary particle size must be taken into account

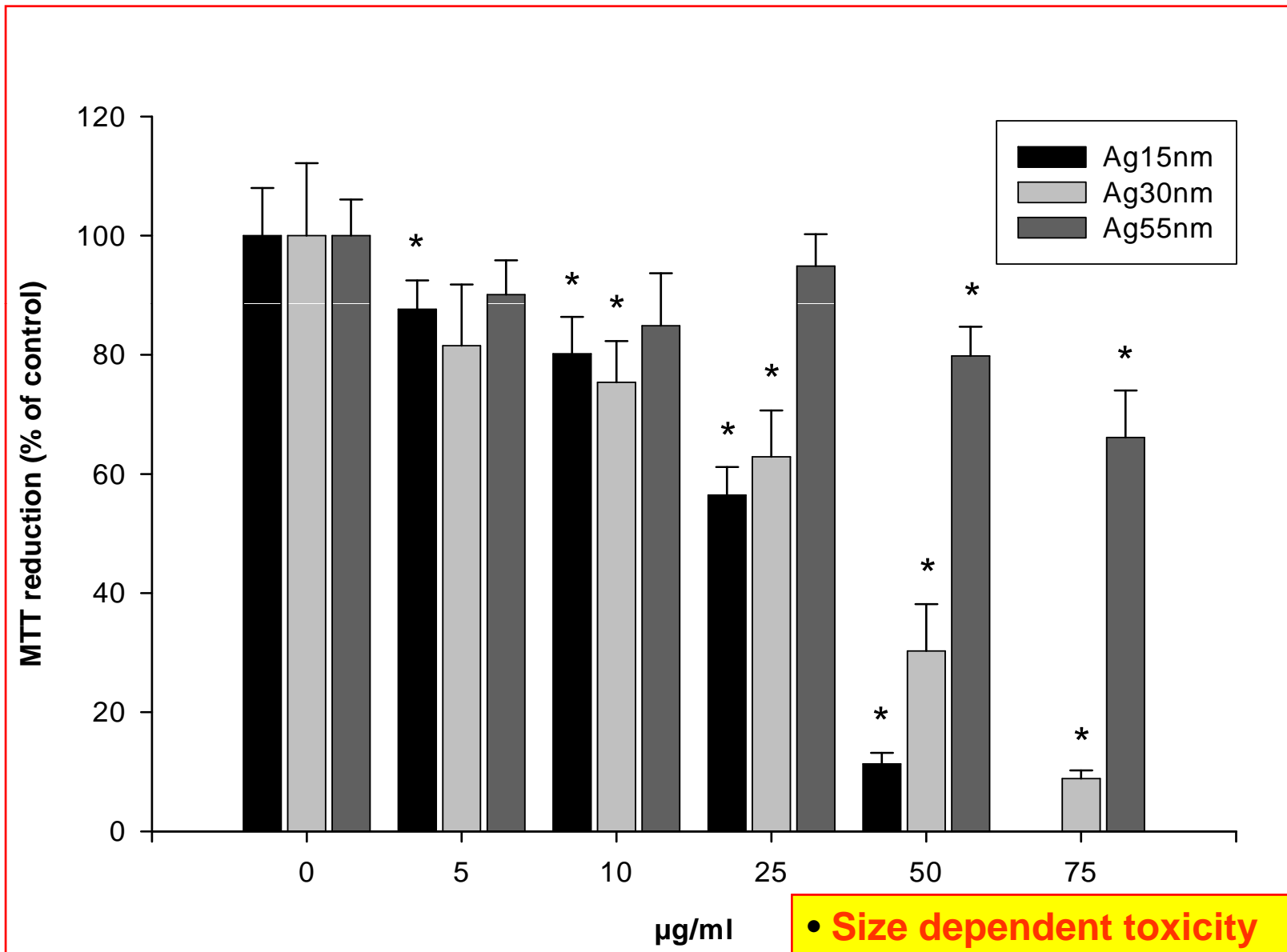
Crystal structure appears to be determining the type of cell death  
High LDH leakage was associated with the anatase but not the rutile nanoparticles (indicates necrosis).

High levels of ROS production was associated with the rutile but not the anatase nanoparticles (indicates apoptosis).

Antioxidants can control the cell death induced by the rutile nanoparticles



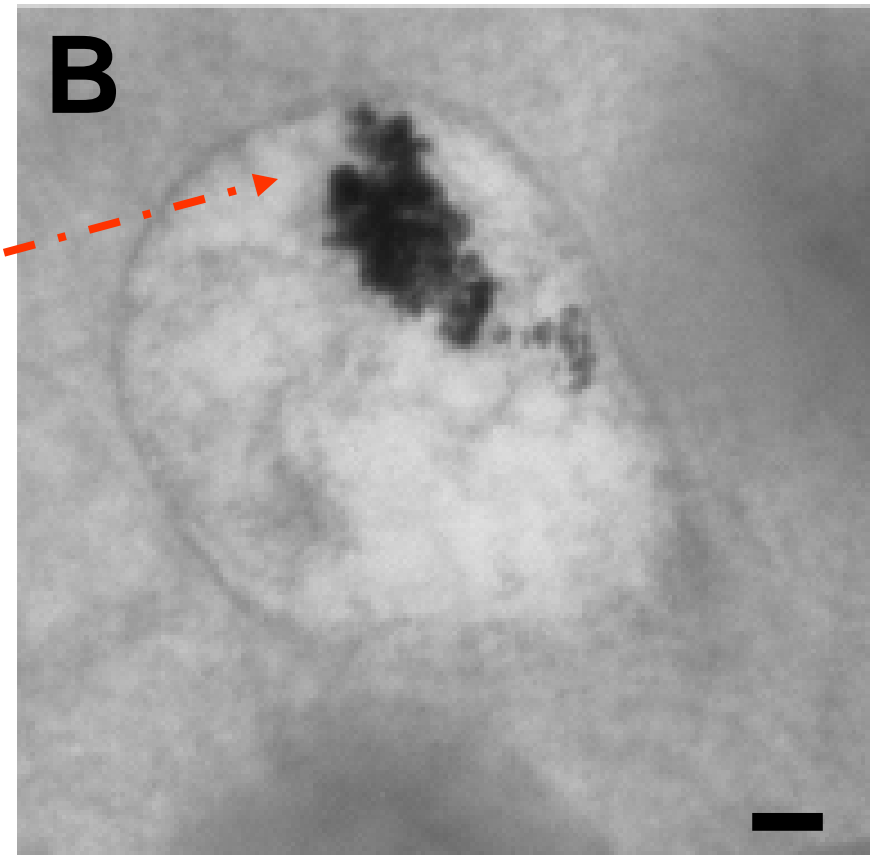
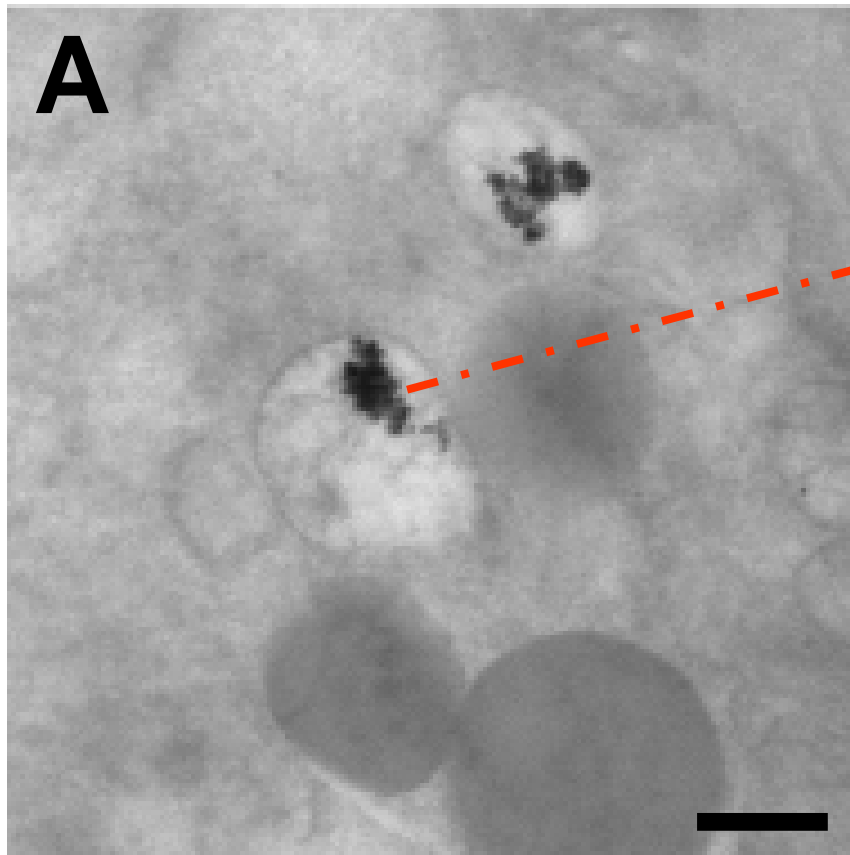
# Toxicity of Silver NP Depends on Particle Size





# Biological Interaction of Nanomaterials

## Silver Nanoparticles (Ag-NP)



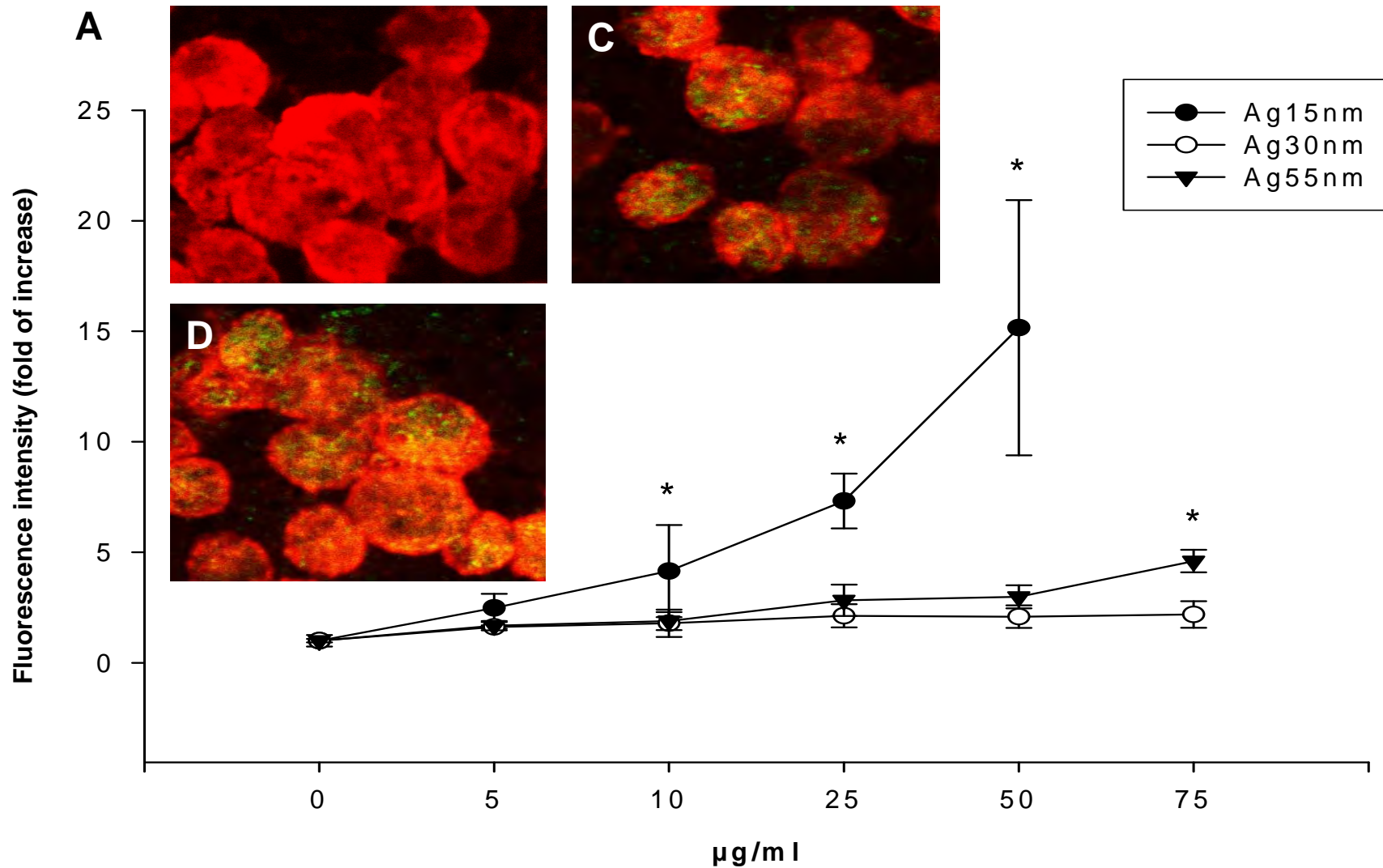
**Internalization and localization of Ag nanoparticles to intracellular vacuoles demonstrated by TEM:**

**Can Silver Nanoparticles be Useful as Potential Biological Labels?**





# Silver-NP induced ROS Generation Based on Size



• Size dependent induction of oxidative stress



# Nanoparticle Coating vs. Toxicity



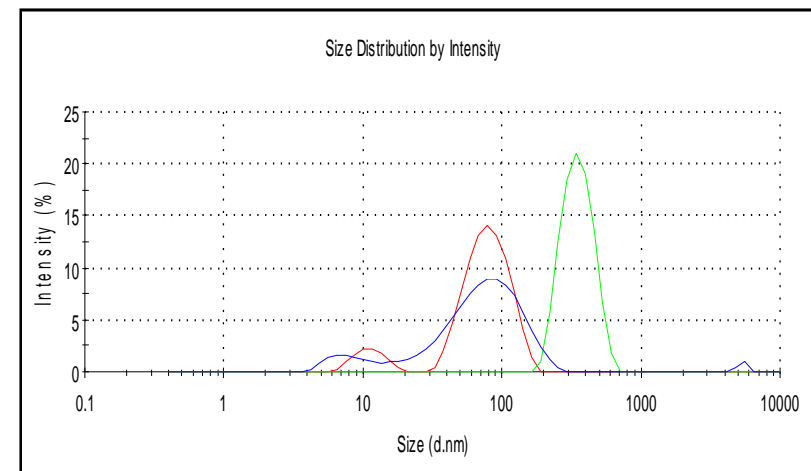
# PS coated Silver Nanoparticles: Size Determination by DLS



## DLS

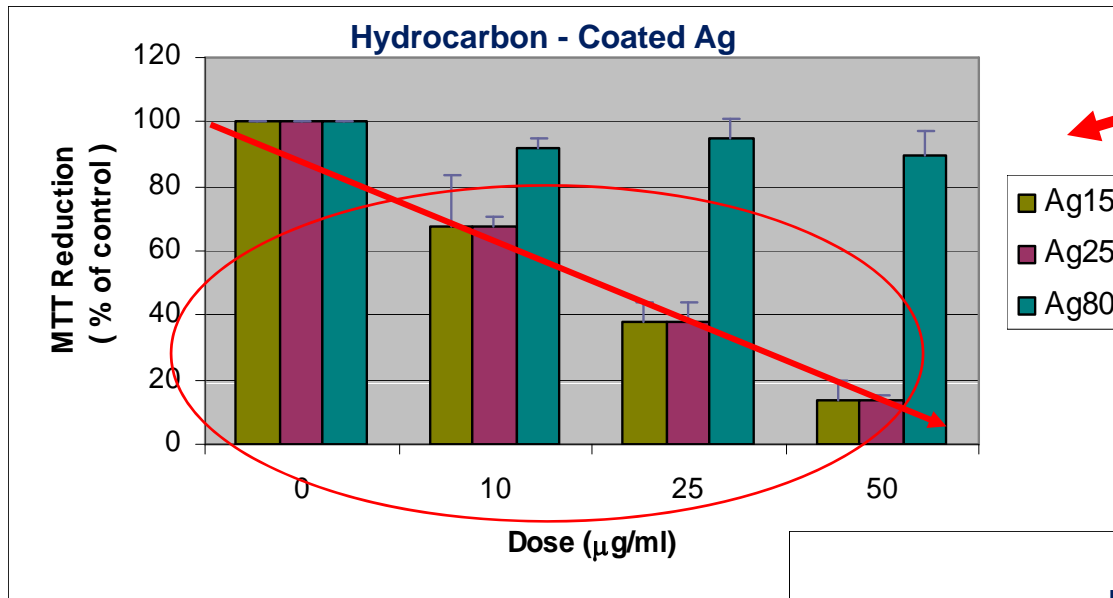
Particle	Average diameter (nm)
----------	-----------------------

PS-Ag 10 nm	
DI H <sub>2</sub> O	72.8
RPMI-1640 media	413
RPMI-1640 media wt/	49.4
20% serum	
PS-Ag 25–30 nm	
DI H <sub>2</sub> O	128
RPMI-1640 media	261
RPMI-1640 media wt/	118
20% serum	
PS-Ag 80 nm	
DI H <sub>2</sub> O	250
RPMI-1640 media	743
RPMI-1640 media wt/	1230
20% serum	



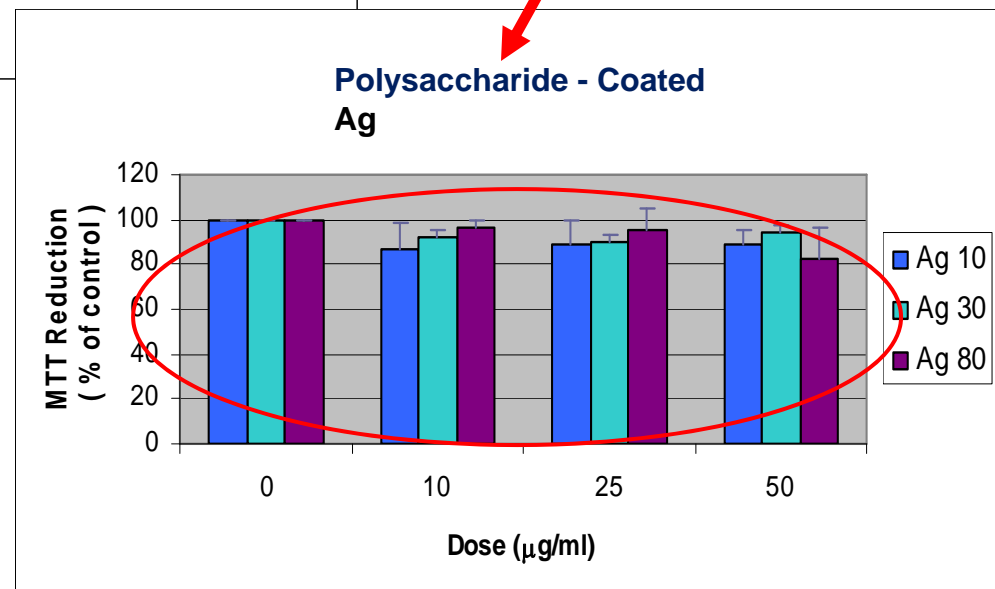


# Surface Coating Protect from Silver-NP Toxicity



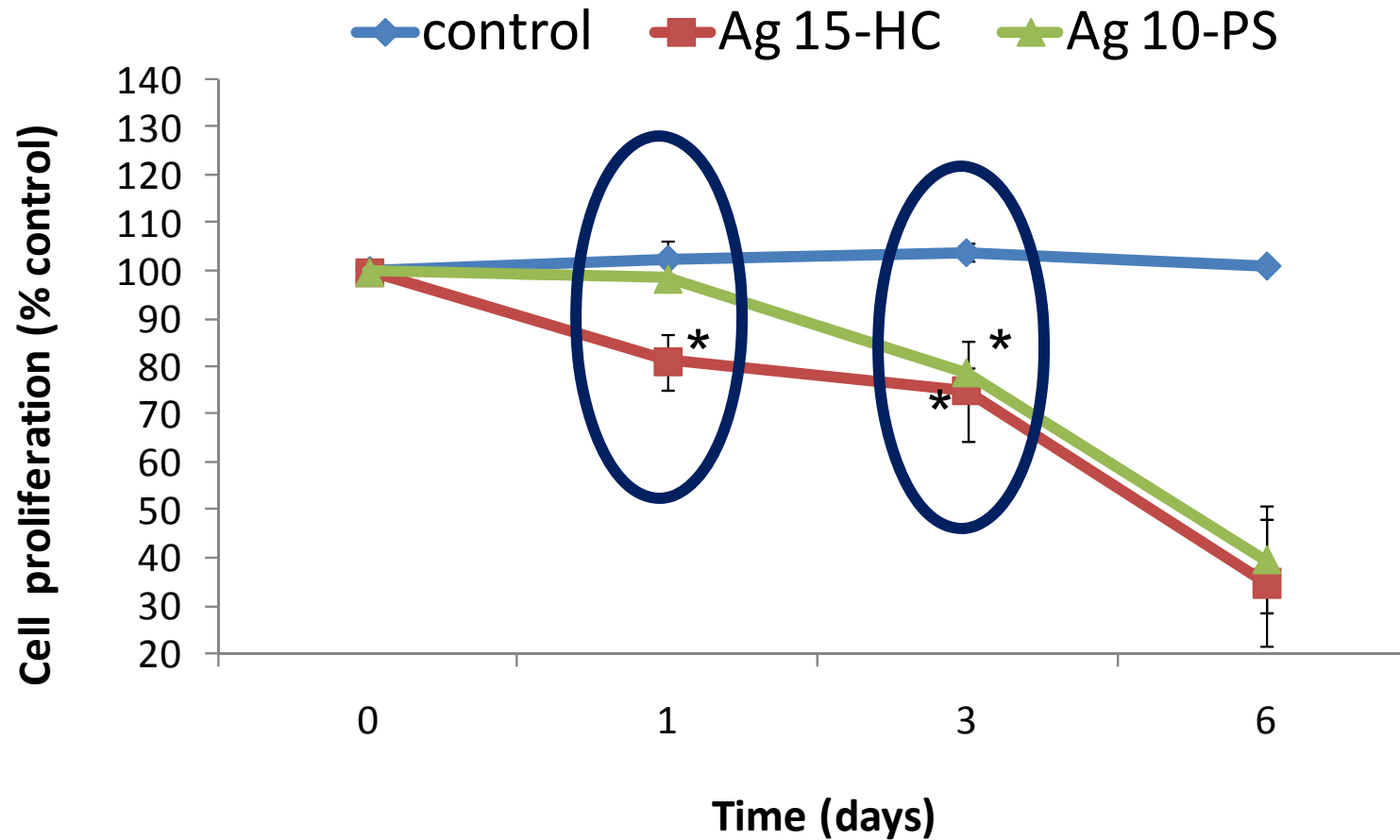
**Different coatings decreased toxicity of Ag nanoparticles**

**Cell viability was significantly higher when dosed with PS-Ag, even at 50 µg/mL, when compared to HC-Ag samples.**





# Is PS Coating Stable?

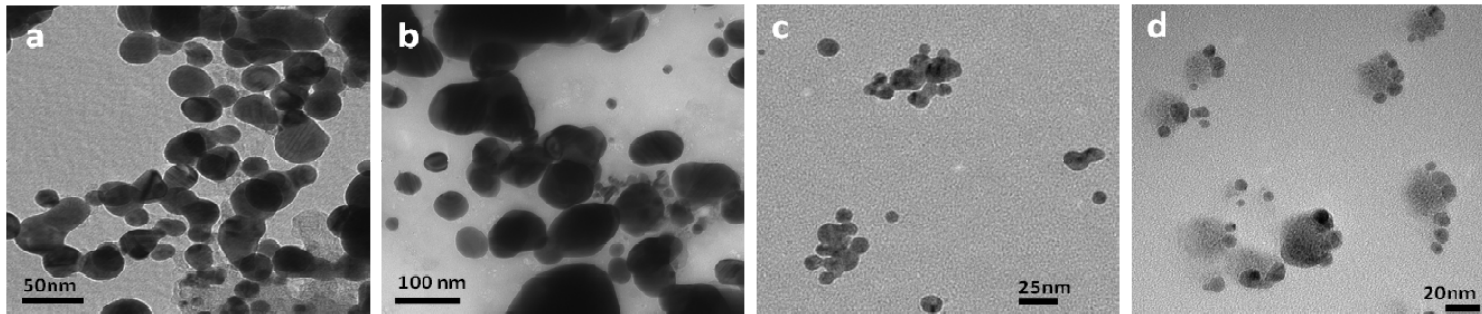


**Mostly likely cause---degradation of NP coating**





# Characterization of Coated Silver-NP

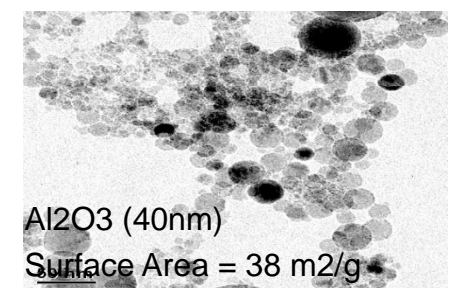
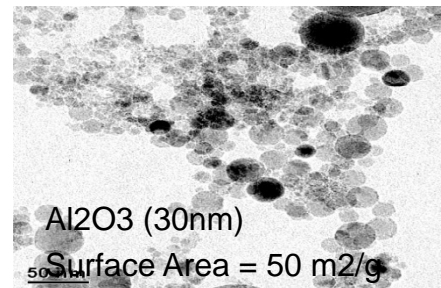
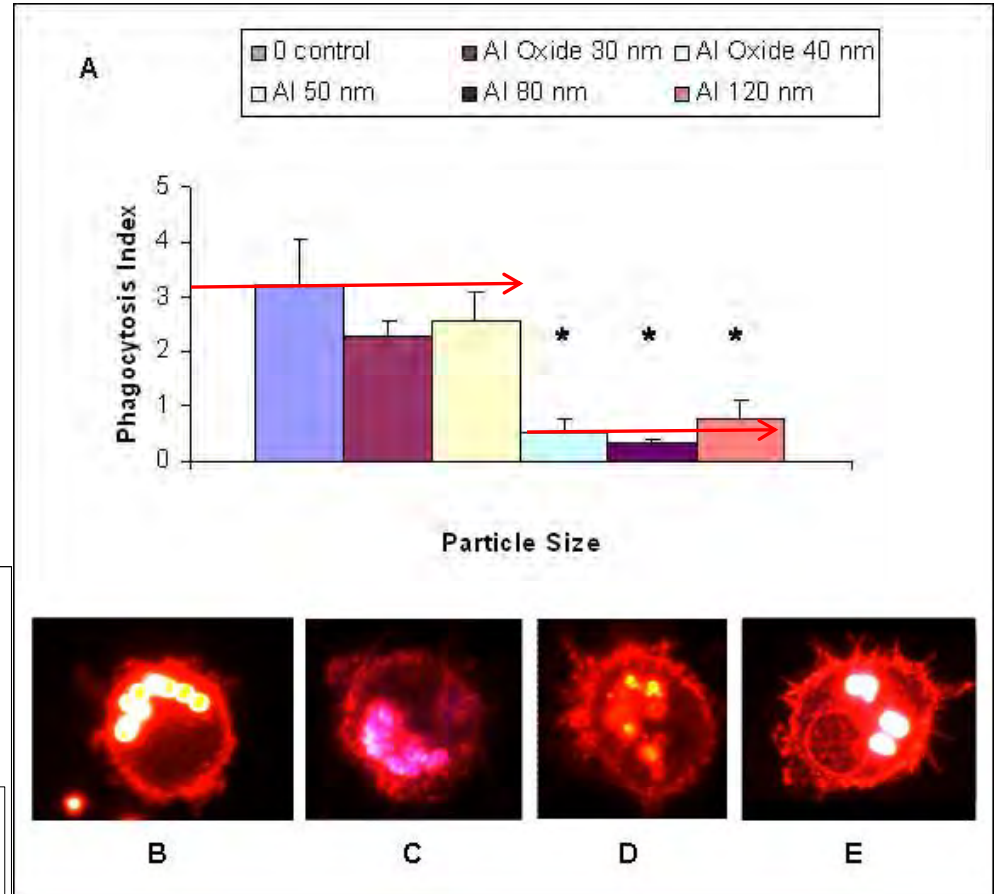
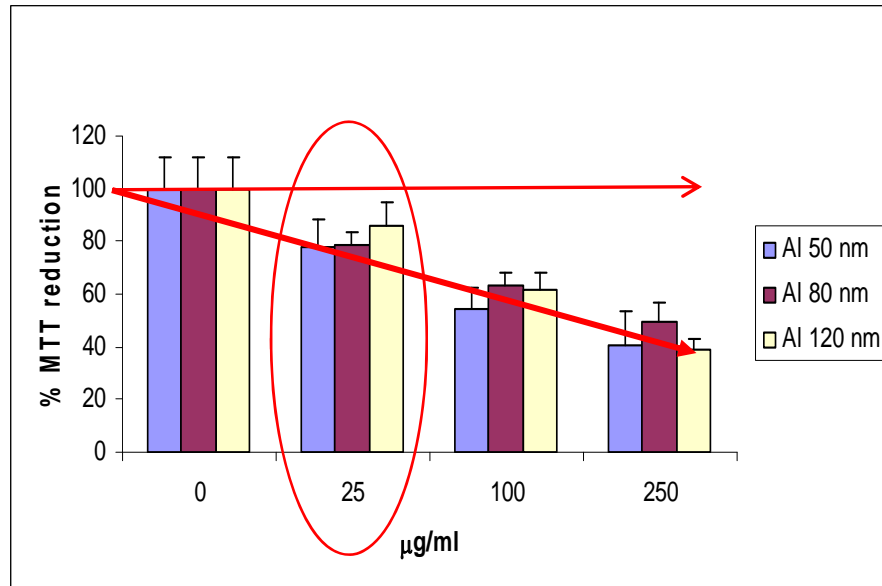
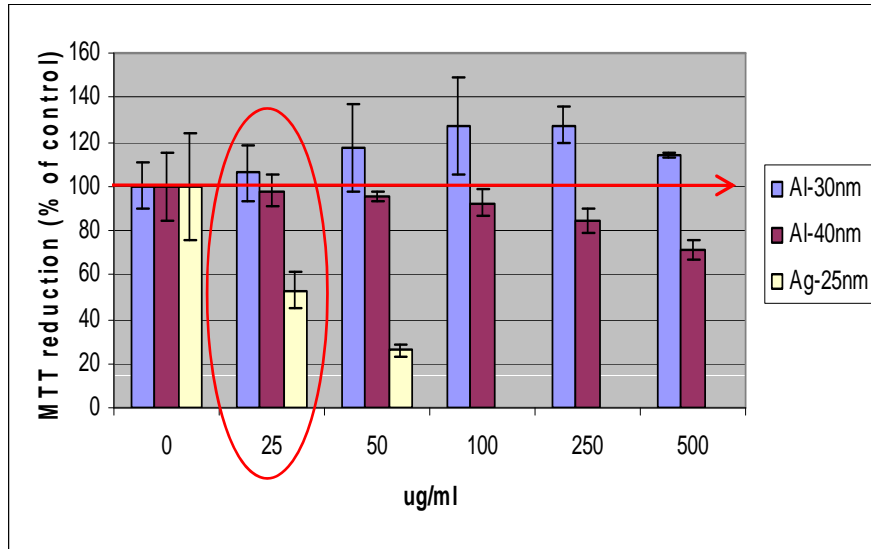


e Sample	Mean Primary Particle Diameter $\pm$ SD (nm) (TEM)			Z-Average Particle Diameter (nm) (DLS)	
	Pre-Exposure	Intracellular	Post-Exposure	Pre-Exposure	Post-Exposure
Ag 25-HC	32.5 $\pm$ 12.4	20.1 $\pm$ 10.3	34.4 $\pm$ 22.8	208*	155
Ag 10-PS	12.0 $\pm$ 3.9	34.6 $\pm$ 7.8	6.9 $\pm$ 2.2	72.8*	298

f Sample		C			O	Na	N	Ag	Al	Cl	S	
		C=O	C-O	C-H, C								
Pre-Exposure	Ag 25-HC	-1	4.9	7.6	28	33.5	---	---	22.4	3.7	---	---
	Ag 10-PS	-1	1.2	3.8	62.6	20	9	0.5	0.2	---	---	2.7
		-2	1.2	4.3	61.8	20.2	8.8	0.7	<0.1	---	---	2.9
Post-Exposure	Ag 25-HC	-1	3.7	11.3	30	20.9	---	---	30.4	---	3.8	---
	Ag 10-PS	-1	4.8	12.7	8.4	44.4	---	---	28.2	---	1.4	---
		-2	4.6	16.2	7.2	42.7	---	---	27.5	---	1.9	---



# Toxicity of Al-NP Depends on Surface Coating



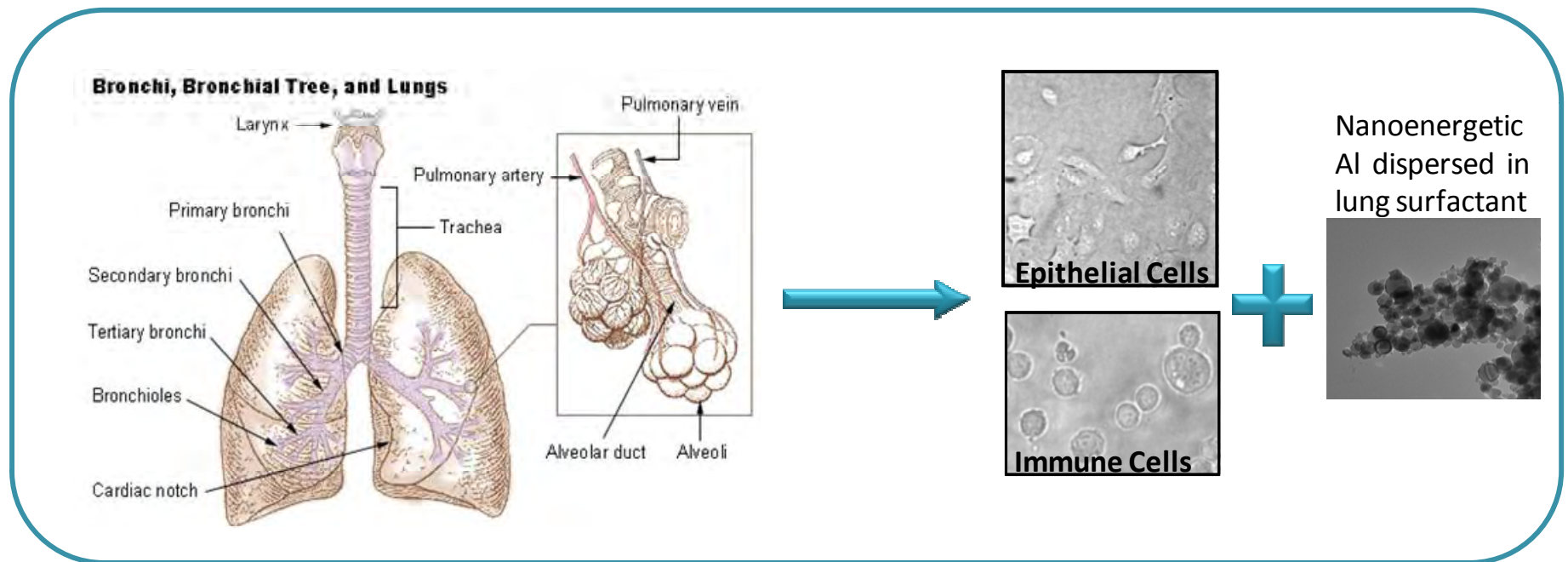


# Evaluation of Nanoenergetic Aluminum



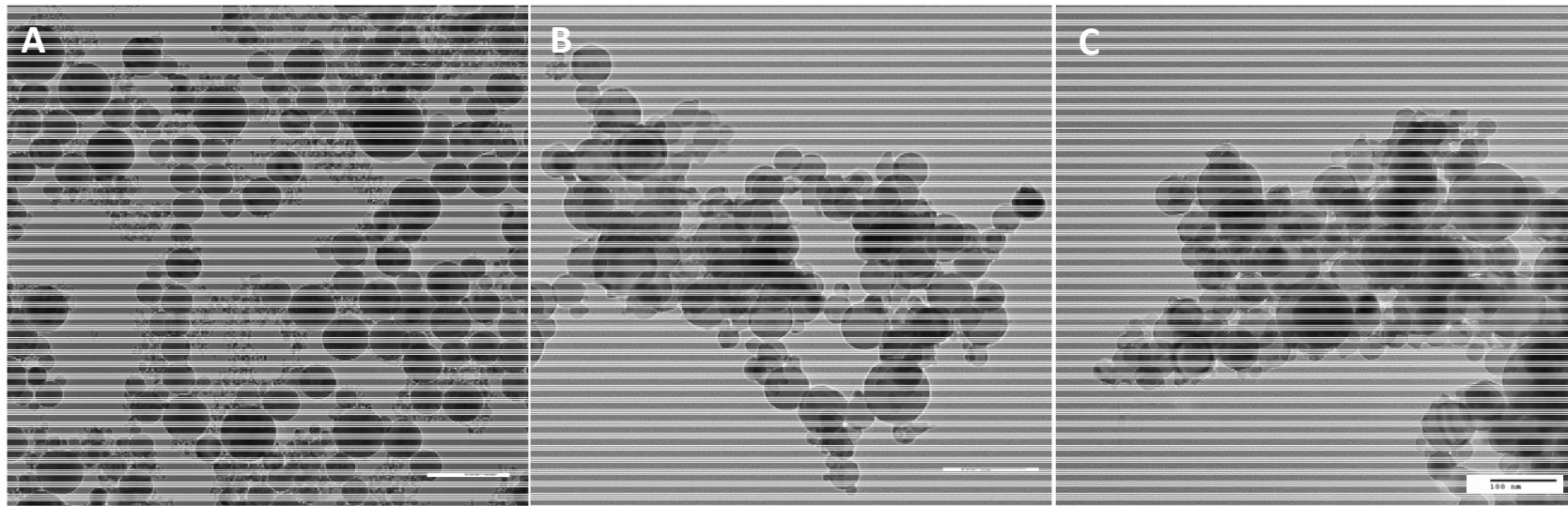
Inhalation the most likely exposure scenario.

Development of co-culture model to assess cell viability, phagocytic activity, activation of immune response, and secretion of inflammatory responses.





# Characterization of Nanoenergetic Aluminum



D	Sample	Primary Nanoparticle Size TEM (nm)	DLS Z-Ave (d.nm) $\pm$ Pdl			
			Dispersion in Water		Dispersion in Artificial Lung Surfactant	
			Exposure Media	Growth Media	Exposure Media	Growth Media
	Al <sub>2</sub> O <sub>3</sub> -40nm	48.08 + 21.01	859 $\pm$ 0.25	309 $\pm$ 0.359	878 $\pm$ 0.495	486 $\pm$ 0.603
	Al-50nm	32.71 + 28.28	698 $\pm$ 0.598	839 $\pm$ 0.661	805 $\pm$ 0.497	948 $\pm$ 0.618
	Al-OA-50nm	51.09 + 22.48	5700 $\pm$ 1.0	138 $\pm$ 0.179	2430 $\pm$ 1.0	195 $\pm$ 0.307





# Establishment of Co-culture



Cell were treated with 25 µg/ml of nanoparticles

Bronchi, Bronchial Tree, and Lungs



The immune cells protected the epithelial cells in the co-cultures. There was a drastic reduction in cell death when compared to when the epithelial cells were cultured alone. Provides a more realistic model to assess exposure.

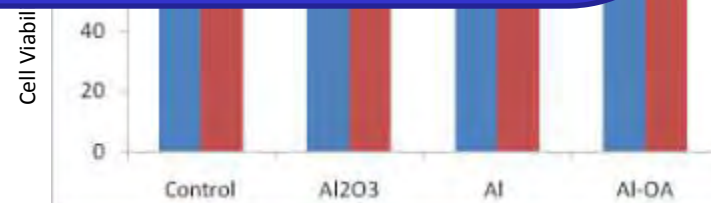
Ma  
such a

3:1 ratio of A549:U937 cells<sup>1</sup>

The nanoparticles were dispersed in an artificial lung surfactant<sup>2</sup>

<sup>1</sup> Wang et al. (2002) Toxicology 173: 211-219.

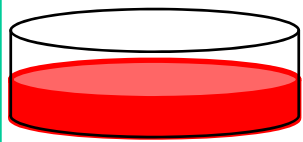
<sup>2</sup> Ansoborlo et al. (1999) Health Physics 77: 638-645



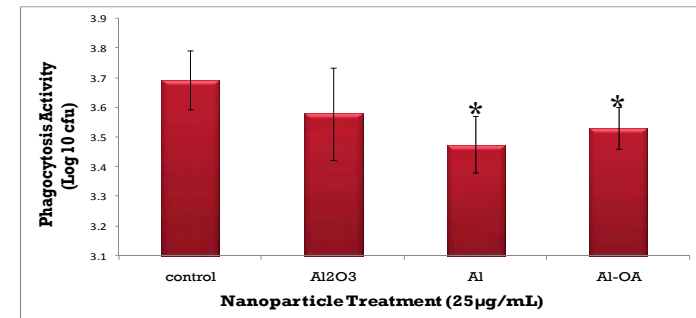
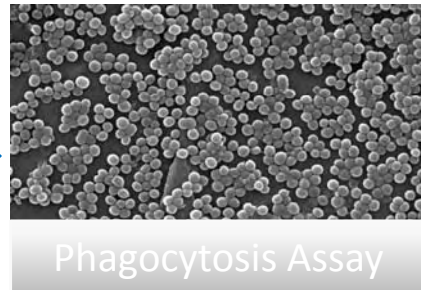




# Evaluation of Macrophage Function



24 h Al NP  
Exposure  
25  $\mu\text{g}/\text{ml}$



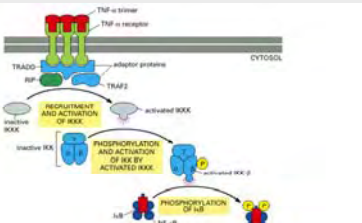
Al reduced the macrophages ability to phagocytose bacteria.

Once phagocytosis occurs the NF $\kappa$ B pathway is activated to produce inflammatory cytokines.

Will the reduction in phagocytic function impact the NF $\kappa$ B pathway and cytokine secretion???

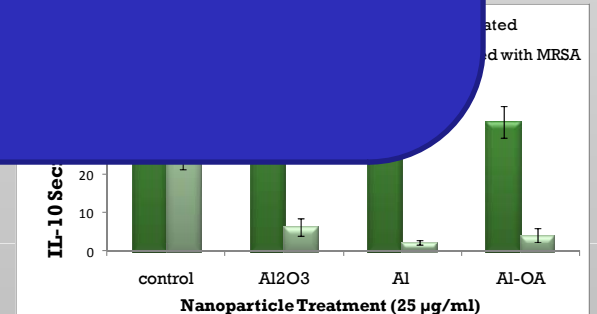
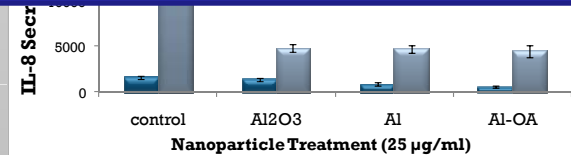
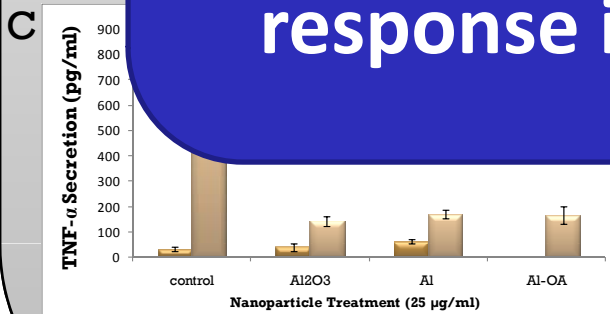


# Secretion of Inflammatory Cytokines



Secretion of IL-6, IL-1 $\beta$ , TNF $\alpha$ , IL-8, and IL-10 was evaluated.

**For all 5 cytokines assessed, the Al nanoparticles caused a massive down-regulation in the secretion of cytokines. The Al altered the immune response in the co-cultures.**





## Summary

Established a co-culture system to simulate the alveolar microenvironment

The Al nanomaterials show more localization in the immune cells in comparison to the epithelial cells.

When a respiratory pathogen is introduced into these co-cultures there is a difference in how these cells respond when the Al nanoparticles are present.

Cytokine secretion is comparable to control levels in the Al NP treatments even when infected with MRSA.

Despite the low toxicity, the presence of the Al NPs interferes with the cells natural ability to respond to pathogens



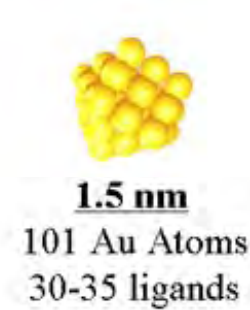
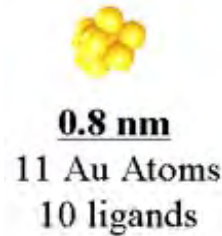
# Surface Charge vs. Toxicity



# Gold Nanoparticles: Size and Surface Functionalization



Courtesy of Bettye L.S. Maddux, Ph.D.  
University of Oregon, Materials Science Institute

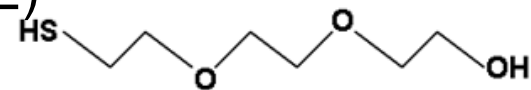


## Surface Functionalization

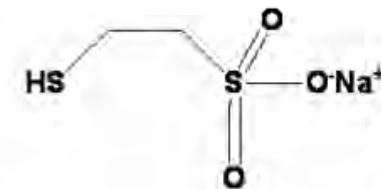
Neutral: 2-(2-mercaptoethoxy)ethanol (MEE)  
0.8 and 1.5 nm AuNPs



Neutral: 2,2,2-[mercaptoethoxy(ethoxy)]ethanol (MEEE)  
0.8, 1.5 and 10 nm AuNPs



Anionic: 2-mercaptoethanesulfonate (MES)  
0.8 and 1.5 nm AuNPs



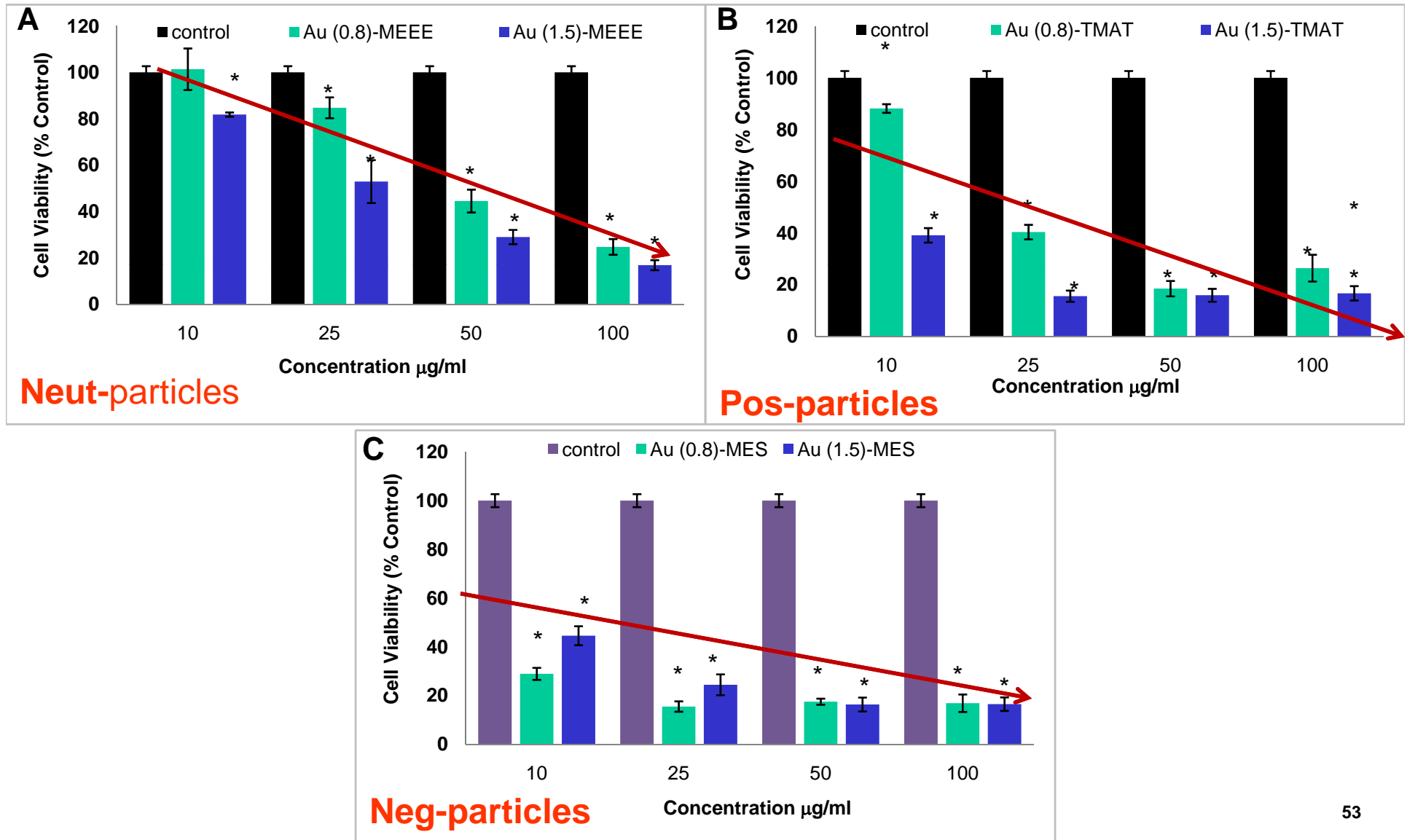
Cationic: N,N,N-trimethylammoniummethanethiol (TMAT)  
0.8, 1.5 and 10 nm AuNPs







# Toxicity of Au-NP Depends on Surface Charge





# Summary of Characterization!!!



- **Characterization of nanomaterials before and after exposure**
- **Supply of well characterized nanomaterials**
- **Toxicity dependent on size, coating, charge & Shape**
  - **Size: Ag (15, 25, 55 nm) = Size dependent toxicity**
  - **Size: Ag (15 nm) induces oxidative stress**
  - **Agglomeration: Al (50,80,120 nm) decreased phagocytosis but not size dependent**
  - **Coating: Ag-PS (10, 25-30 nm) = No toxicity (> 100ug/ml)**
  - **Coating: Al<sub>2</sub>O<sub>3</sub> (30, 40 nm) = Not toxic**
  - **Charge & Shape: Gold Particles**

***Characterization of nanomaterials (understanding surface Properties) is key to establishing safety of nanotechnology***



# What's the Message?

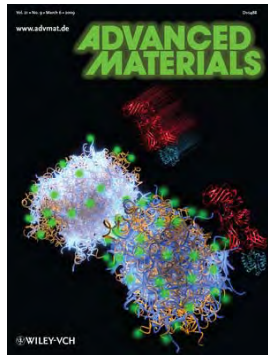


- Size matters, but not always
- Physicochemical character matters
  - Crystallinity
  - Chemical reactivity
  - Shape, charge
  - Coating
- Contaminants must be considered
- Agglomeration vs. dispersion- Critical point
- Charge matters (affects reactivity and dispersion)

**Good Nanotoxicology Requires  
Good Characterization**



# Scientific Impact



**Schrand A, Braydich-Stolle L, Schlager LL, Hussain SM. Can silver nanoparticles be useful as potential biological labels? Nanotechnology 19 (June 11 2008) 235104**

- Accessed >1 million times since publication.
- 1 of only 3 2008 biology & medicine articles selected for free download.



**Hussain SM, Braydich-Stolle LK, Schrand AM, Murdock RC, Yu KO, Mattie DM, Schlager JJ, Terrones M. Toxicity evaluation for safe use of nanomaterials: Recent achievements and technical challenges.**

Adv Mater. 2009 (2): 1-11.

- *Impact Factor 9*





Thank you

Questions??

Saber.hussain@wpafb.af.mil