Biosafety of Nanoparticles:

Evaluation of Nanomaterials By a Suite of Cellular Assays

Mellisa Theodore[1], Nathan Boggs[1], Bakhtiyor Rasulev PhD[2], Jerzy Leszczynski PhD[2], and Joany Jackman PhD[1]

1- JHU APL
2- Jackson State University

The Johns Hopkins University
APPLIED PHYSICS LABORATORY

Science & Technology Business Area

Biosafety of Nanoparticles

Our Approach

- **Is there a need for concern?**
  - **Nanoparticles are being used in many industries**
  - **A variety of shapes and surface chemistry exist at this time- little is known about the effect of nanoparticles on the host**

    - Carbon nanotubes have shown asbestos-like health risks
    - Colloidal silver ingestion causes argyria
    - Pharmaceutical, biological research, medical applications
  - **"Wait and see" methodology not acceptable**
    - Exposure and fate both very important considerations

- **What do we want to know?**
  - If biomedically-relevant nano-materials can cause damage to cells, cell pathways or genomic DNA
  - If DNA damage correlates to organ specific effects or cytotoxicity
- **How are we investigating the concerns?**
  - Building on a suite of in vitro cellular assays for hazard assessment of nanoparticles
  - Prescreening biomarkers in vitro which might translate to in vivo biomarkers of hazard assessment
  - Predicatively modeling cellular effects of nanoparticles

Need for Concern?

Industrial Problem:

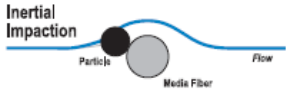
Nanoparticles May Not Be Eliminated by HEPA Filters

How HEPA filters work:

Filtration Mechanisms

There are four basic ways media captures particles:

Inertial Impaction

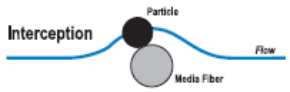


Inertia works on large, heavy particles suspended in the flow stream. These particles are heavier than the fluid surrounding them. As the fluid changes direction to enter the fiber space, the particle continues in a straight line and collides with the media fibers where it is trapped and held.

Diffusion

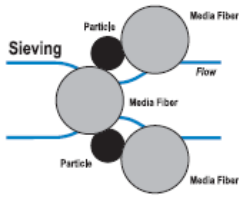
Diffusion works on the smallest particles. Small particles are not held in place by the viscous fluid and diffuse within the flow stream. As the particles traverse the flow stream, they collide with the fiber and are collected.

Interception



Direct interception works on particles in the mid-range size that are not quite large enough to have inertia and not small enough to diffuse within the flow stream. These mid-sized particles follow the flow stream as it bends through the fiber spaces. Particles are intercepted or captured when they touch a fiber.

Sieving



Sieving, the most common mechanism in filtration, occurs when the particle is too large to fit between the fiber spaces.

High Efficiency Particulate Air Filter

99.9% efficient at 0.3 micron

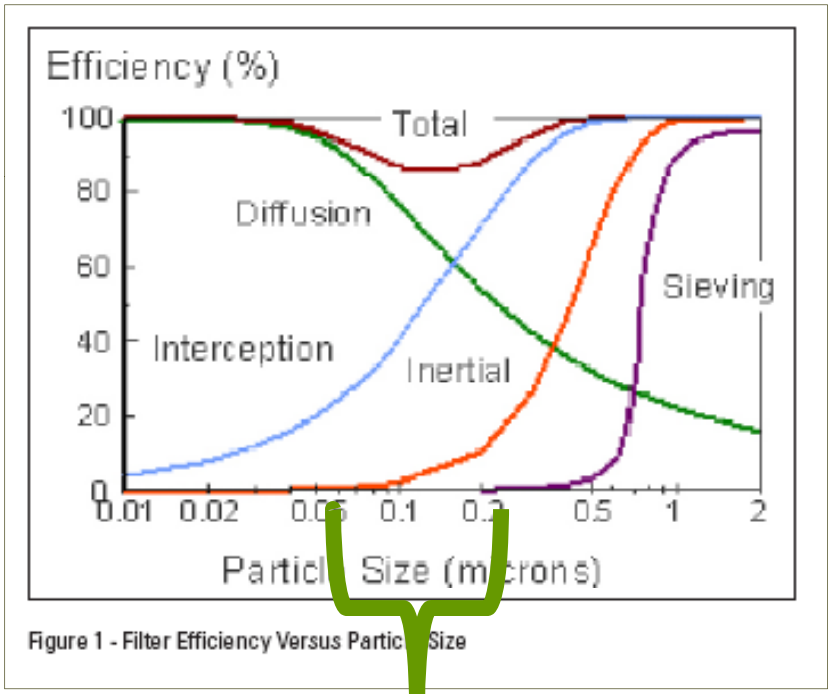


Figure 1 - Filter Efficiency Versus Partic Size

CRITICAL ISSUE

NOTE: Efficiency drops in this size range and …

Need for Concern?

Nanoparticles: Protection? Fate? Elimination?

Nanoparticle size and respiratory disposition
(Maynard and Kuempel, 2005)

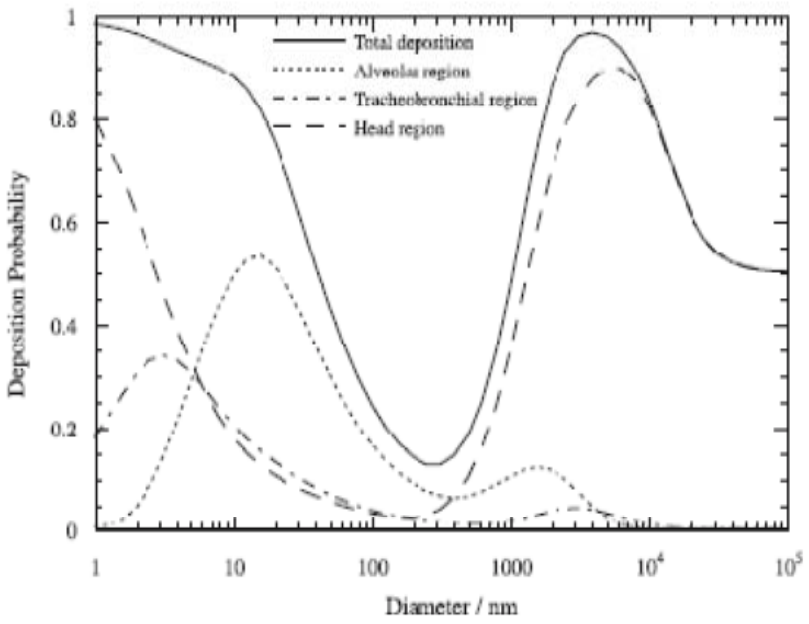


Table 1. Biodistribution and renal filtration as a function of hydrodynamic diameter

▲ Figures and tables index

Molecule	MW (kDa)	HD (nm)	Urine/blood filterability (%)	Blood half-life (min)	Whole body half-life (h)	Ref.
Inulin	5	3.0	100	9	1.9	18,19
Lysozyme	15	3.4	80	12	1.3	20,21
Myoglobin	17	3.8	75	9	2.0	20,22
ScFv	30	5.3[a]	74	11	1.4	5
Bence-Jones	44	6.1[a]	10	-	3.0	23,24
Fab'	50	6.0	9	28	4.0	4,20
$Sc(Fv)_2$	60	7.0[a]	-	78	5.1	5
Has	67	7.3[a]	0.3	110	16.0	22
$[sc(Fv)_2]_2$	120	9.3[a]	-	170	8.9	5
IgG	152	11.0	<0.1	330	730.0	5,20

[a] Unknown HDs were calculated using the following power law fit to literature values: $HD = A \times MW^B + C \times MW^D$, where $A = -0.000000002614$, $B = 3.326$, $C = 0.9482$ and $D = 0.5001$; $R^2 = 0.999$. HD, hydrodynamic diameter; MW, molecular weight.

…this is size where greatest depositi occurs in lungs

...and cannot be eliminated by filtration in the kidneys

Need for Concern?

Mec … d To Can…

Barrier Capacity of Human Placenta for Nanosized Materials

Peter Wick,[1,] Antoine Malek,[2,*] Pius Manser,[1] Danielle Meili,[2] Xenia Maeder-Althaus,[1] Liliane Diener,[1] Pierre-Andre Diener,[3] Andreas Zisch,[2] Harald F. Krug,[1] and Ursula von Mandach[2]*

[1]Empa, Swiss Federal Laboratories for Material Testing and Research, Laboratory for Materials–Biology Interactions, St. Gallen, Switzerland; [2]University Hospital Zurich, Department of Obstetrics, Zurich, Switzerland; [3]Institute of Pathology, Cantonal Hospital, St. Gallen, Switzerland

BACKGROUND: Humans have been exposed to fine and ultrafine particles throughout their history. Since the Industrial Revolution, sources, doses, and types of nanoparticles have changed dramatically. In the last decade, the rapidly developing field of nanotechnology has led to an increase of engineered nanoparticles with novel physical and chemical properties. Regardless of whether this exposure is unintended or not, a careful assessment of possible adverse effects is needed. A large number of projects have been carried out to assess the consequences of combustion-derived or engineered nanoparticle exposure on human health. In recent years there has been a growing concern about the possible health influence of exposure to air pollutants during pregnancy, hence an implicit concern about potential risk for nanoparticle exposure *in utero*. Previous work has not addressed the question of whether nanoparticles may cross the placenta.

OBJECTIVE: In this study we investigated whether particles can cross the placental barrier and affect the fetus.

METHODS: We used the *ex vivo* human placental perfusion model to investigate whether nanoparticles can cross this barrier and whether this process is size dependent. Fluorescently labeled polystyrene beads with diameters of 50, 80, 240, and 500 nm were chosen as model particles.

RESULTS: We showed that fluorescent polystyrene particles with diameter up to 240 nm were taken up by the placenta and were able to cross the placental barrier without affecting the viability of the placental explant.

CONCLUSIONS: The findings suggest that nanomaterials have the potential for transplacental transfer and underscore the need for further nanotoxicologic studies on this important organ system.

KEY WORDS: barrier tissue, *ex vivo* perfusion, human placenta, nanoparticles, nanotoxicity. *Environ Health Perspect* 118:432–436 (2010). doi:10.1289/ehp.0901200 available via *http://dx.doi.org/* [Online 12 November 2009]

cytotrophoblast cells and form a true … tium with no lateral cell membranes, … in rats or mice three trophoblast layers a… ent between maternal blood and fetal … capillaries (for comprehensive revie… Enders and Blankenship 1999; Takata… 1997). Thus, the transport efficiency fo… biotics or nanoparticles across the place… to be defined for humans explicitly.

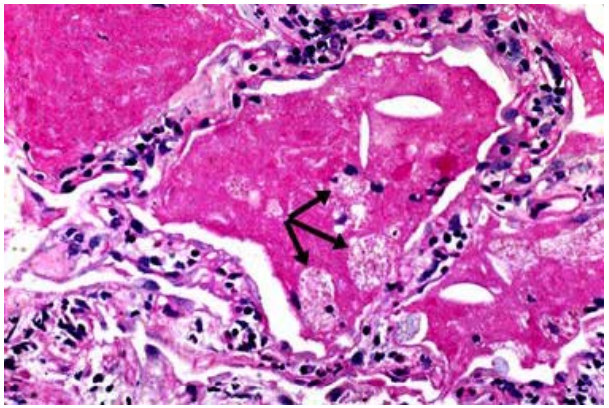
In detail, the cellular barrier betwe… of maternal and the fetal blood is form… the syncytiotrophoblast layer, which fa… al maternal environment, and the endo… e cell layer of the fetal microcapillaries. B… these two cell layers there are several s… cells such as cytotrophoblasts, fibroblas… Hofbaur cells (placental macrophages… layer is relatively thick in early pregnan… becomes progressively thinner with gest… ey age. This reduction in thickness togethe… an increase in the number of fetal ca… ies enhances the efficiency of materna… exchange during the development of th… (Enders and Blankenship 1999). The t…

Research Question: What is relationship of chemistry, nanoparticle size and shape to risk?

Need for Concern?

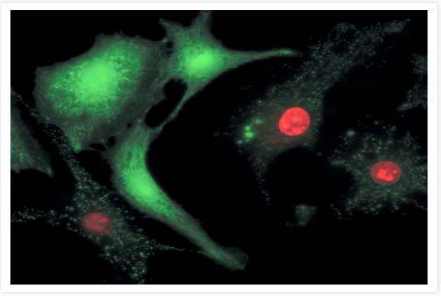
Hazards of non toxic dust: Effects may not be acute

- Pulmonary alveolar proteinosis is a rare disease in which a type of protein builds up in the air sacs (alveoli) of the lungs, making breathing difficult
- In some cases, the cause of pulmonary alveolar proteinosis is unknown
- In other cases it is associated with infection or an immune problem
- It also can occur with cancers of the blood system, and **after exposure to high levels of dust**
- This rare disorder generally affects people 30 - 50 years old and is seen in men more often than in women

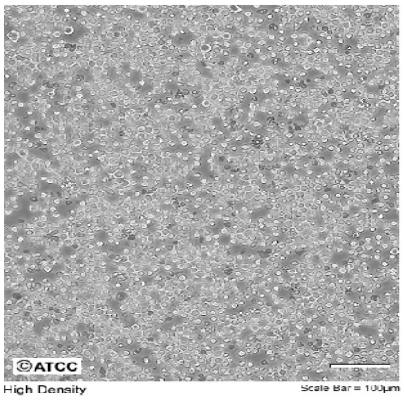


How are we investigating?
Suite of Assays and Cells

- Assays- Cellular Screen
  - Acute measures of toxicity
    - MTT Assay- cellular metabolic activity
    - Live/Dead Assay- cellular membrane integrity
  - Long-term measures
    - DNA Ladders; Flow Cytometry (Annexin-V)- apoptosis/ necrosis
    - Flow Cytometry- Cell cycle analysis/ growth kinetics
    - Cytokine Detection Assay- multiplex immunoassay for human cytokines

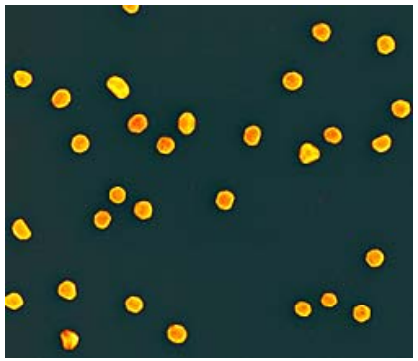


- Cells Lines
  - HL60- promyloblast- peripheral blood
  - THP1- monocytes- peripheral blood
  - HepG2- hepatocyes- liver
  - A549- alveolar epithelial-lung
  - MCF7- breast adenocarcinoma- mammary gland



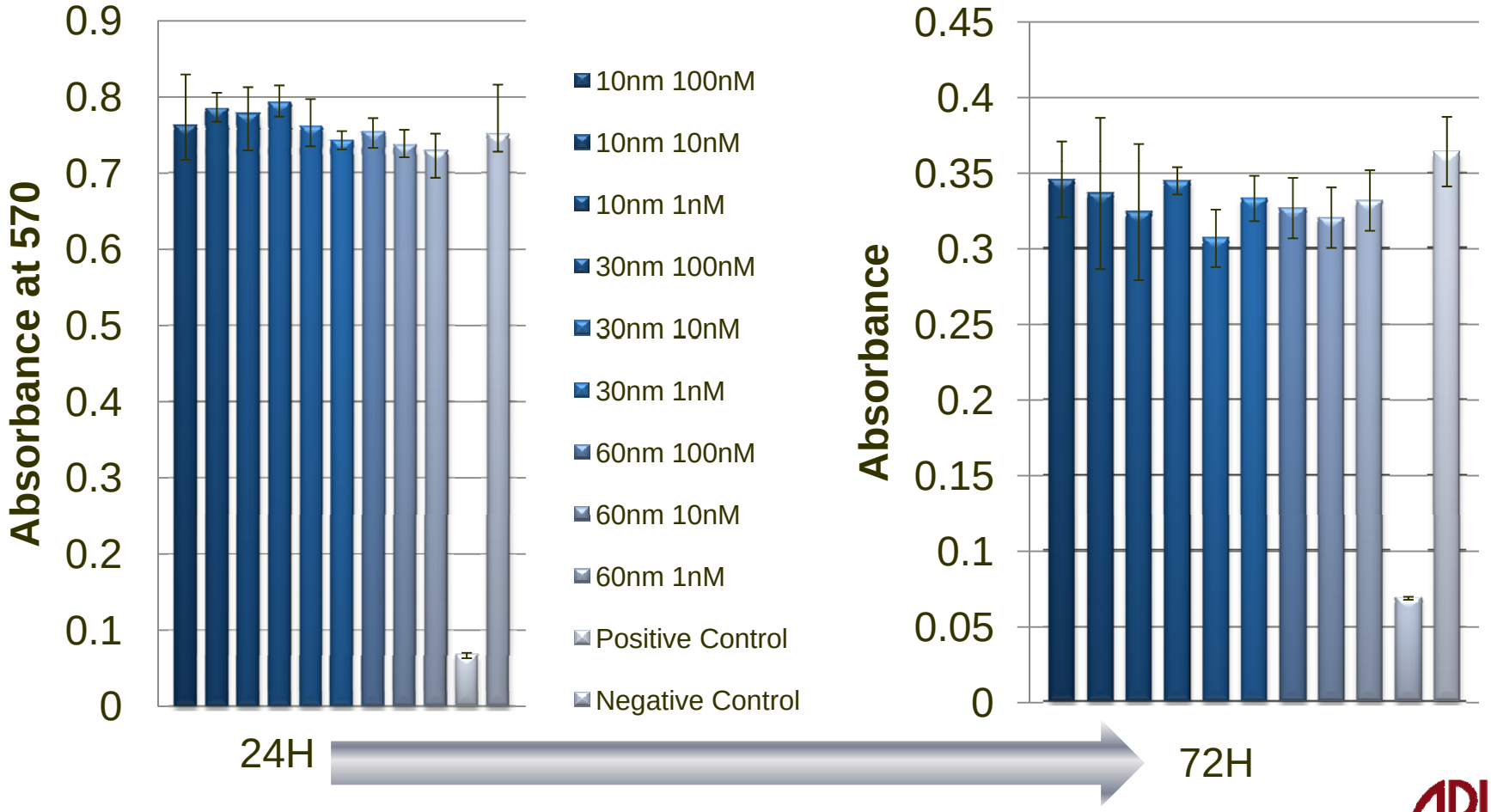
Nanoparticle Variations

- Nanoparticles are significantly different in all aspects
- Our studies will consider the following distinctions in particles:
  - Composition
  - Size
  - Shape (aspect ratio)
  - Surface charge
- Initial Studies:
  - Gold nanoparticles characterized by NIST
  - 2nm,10nm, 30nm, 60nm
  - Results shown for HepG2 and A549 cell lines
- Follow up Experiments:
  - Comparison of Carbon nanotubes and Fullerenes
  - Immune cell responses to incubations
  - ROS Evaluations



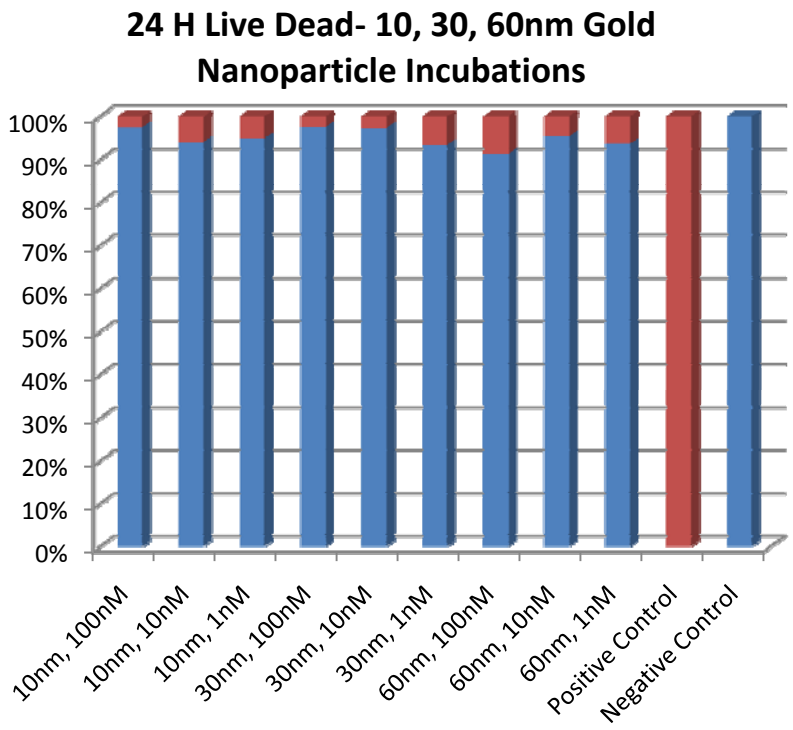
Results:
MTT ASSAY 24H-72H

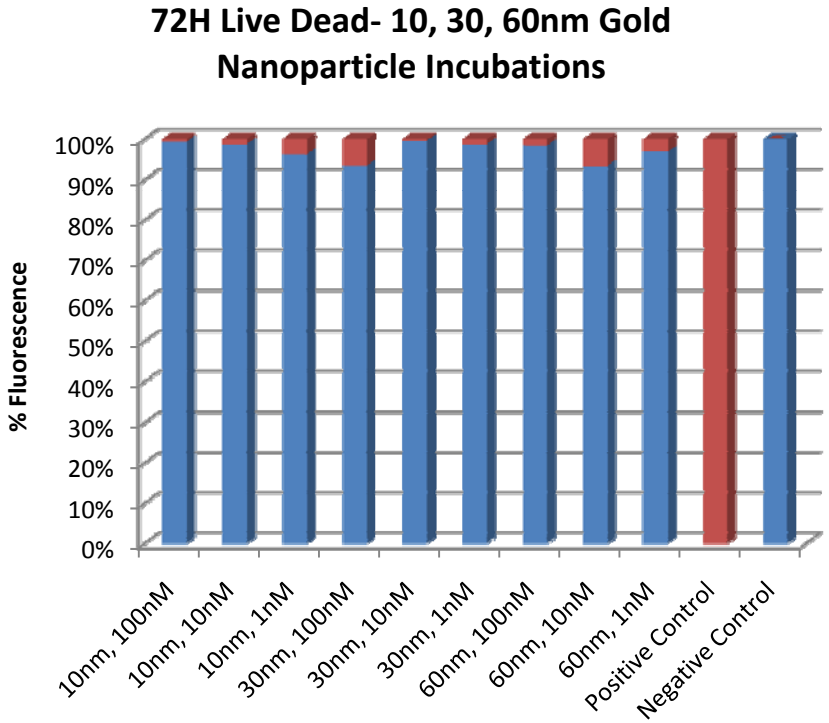
Assay results shown up to 72H of incubation with nanoparticles- little death observed compared to positive control (cell death induced by H2O2)



Results:
LIVE / DEAD ASSAY 24H-72H

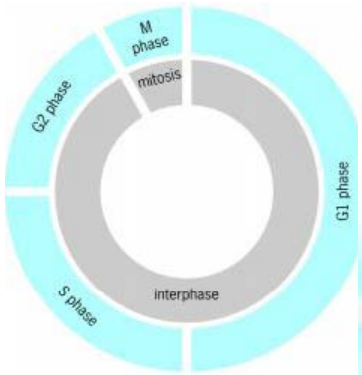
Assay results shown up to 72H of incubation with nanoparticles- little death observed compared to positive control (cell death induced by H2O2)

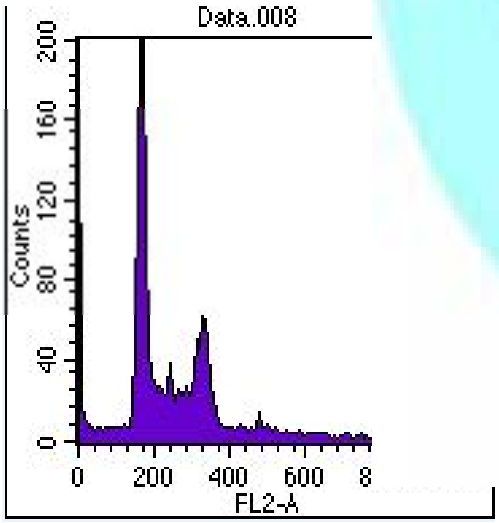




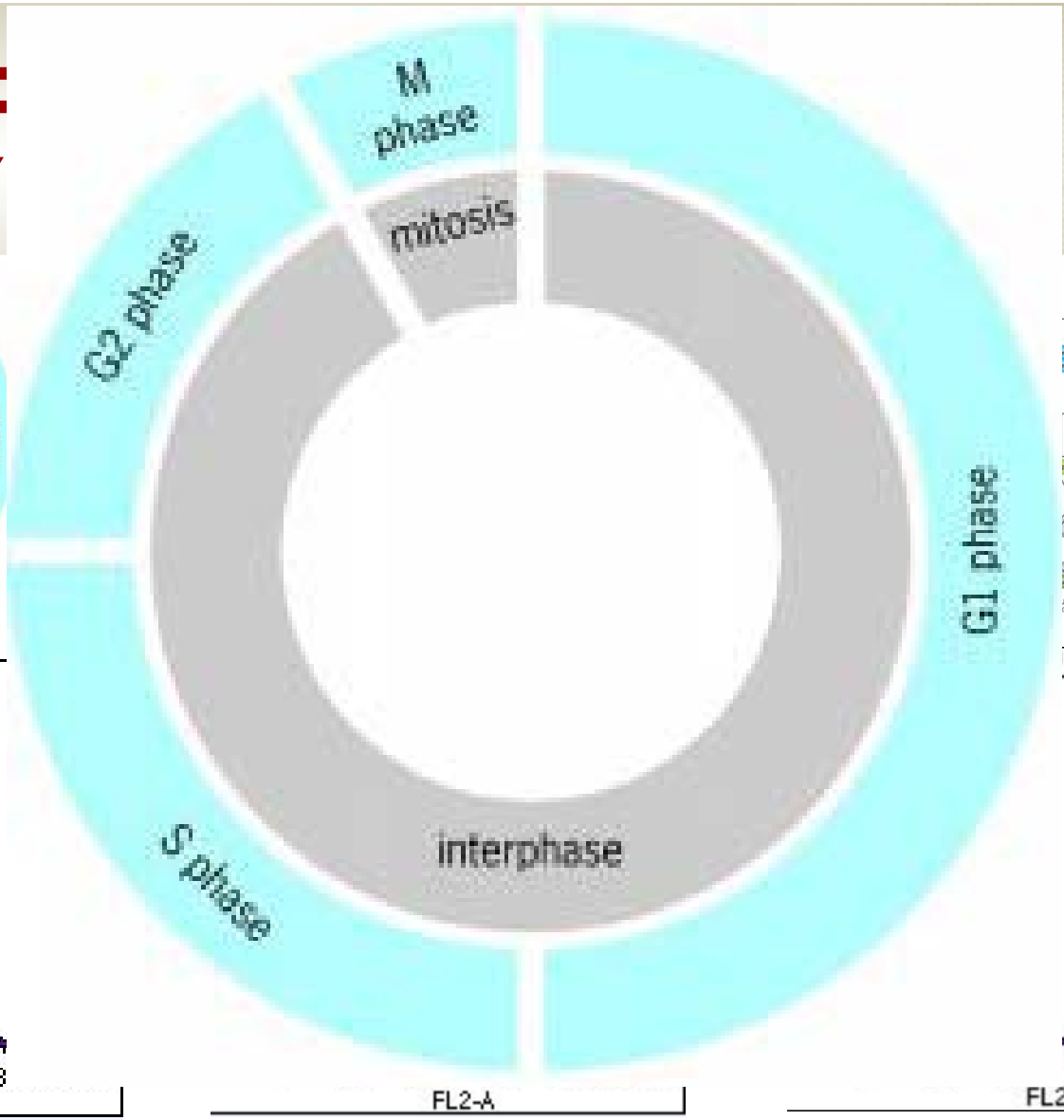
24H → 72H

Results: CELL CY

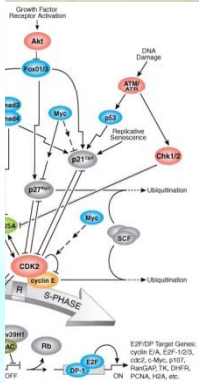




Normal Cell Cycle

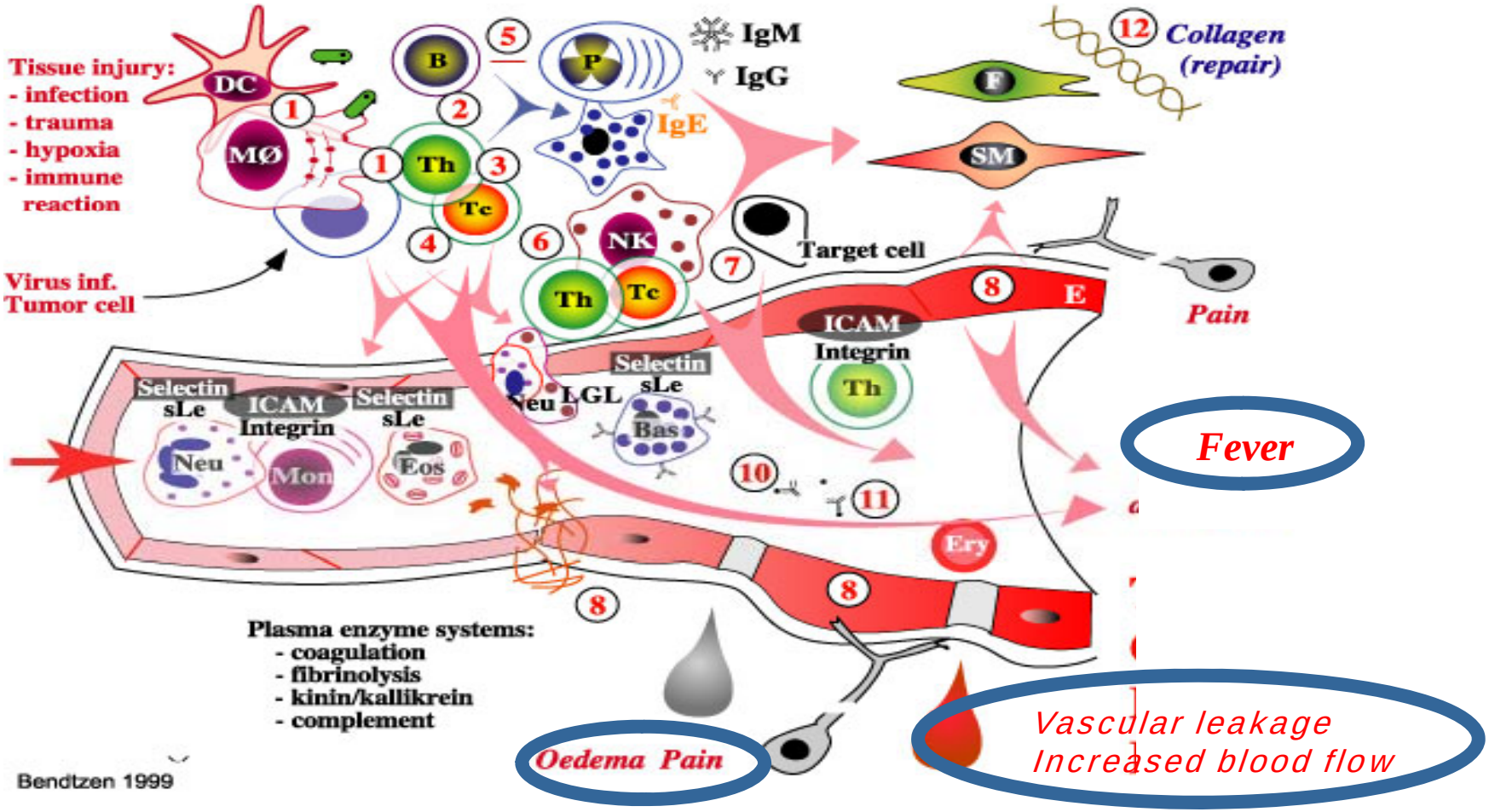


G2 phase Block

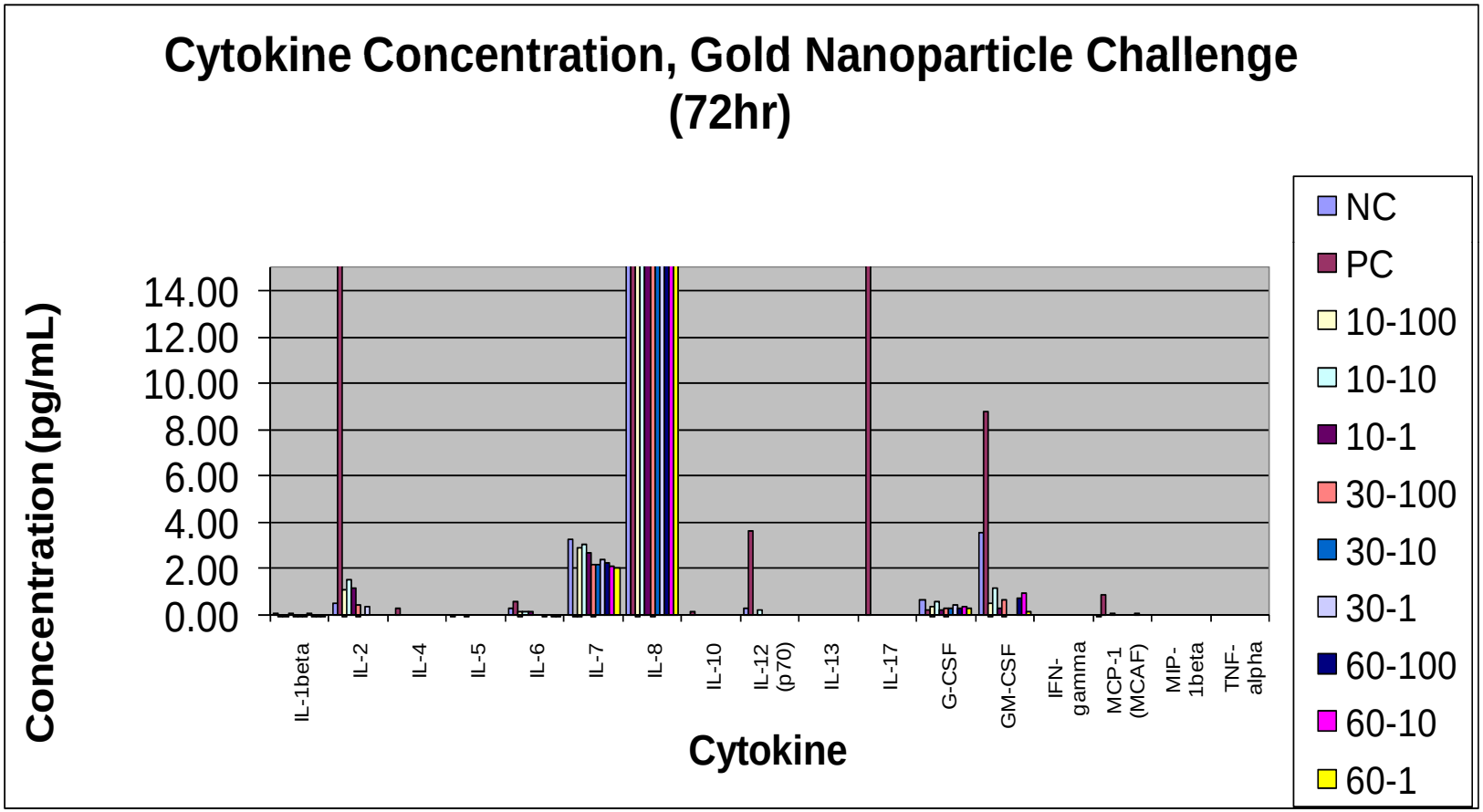


G1 Phase Block*

The Inflammatory Response



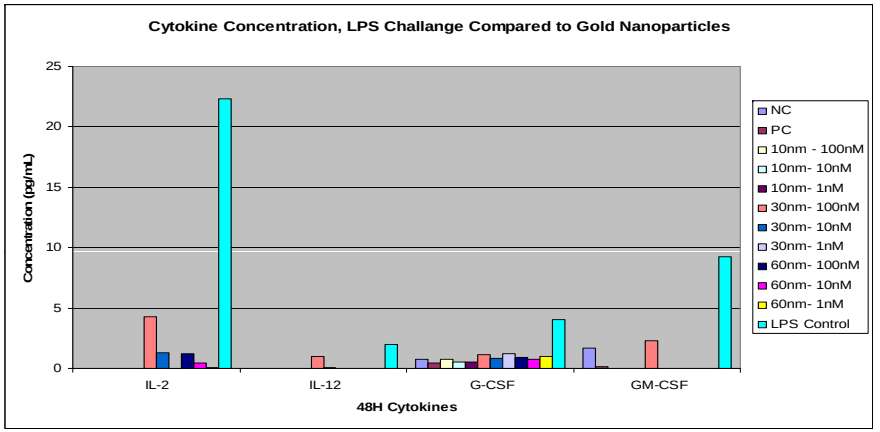
Results:
17-Plex Cytokine Assay



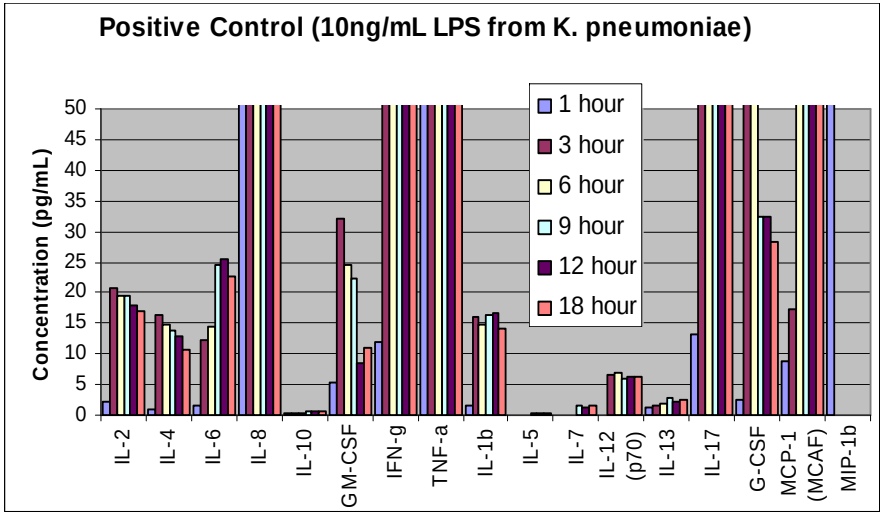
Cytokines appear to have been induced by Nanoparticles…

Results:
17-Plex Cytokine Assay

- Comparison to LPS induced cells shows higher induction in positive controls



- Cytokine induction in immune cells is much higher than in hepatocytes



Results:

Prescreening Biomarkers *in vitro* and Predicative Modeling

- **Investigation of cytokine responses using modeling is necessary for prescreening of potential biomarkers**
- **When placed into model**
  - **Analyzing cytokine assay data- noticed that response values for 2nm and 30nm particles were less then for 10nm and for 60nm**
  - **This was noticed for almost all cytokine assays**
  - **Selected IL-7 assays have the same distribution of values (expected that the response value should increase with decreasing or increasing nanoparticle size)**
  - **Wanted to find any rule for experimental data distribution and then find parameters that change noticeably with the cluster size increasing**
  - **By analyzing the data for IL-7 assay -found some interesting distribution of data**

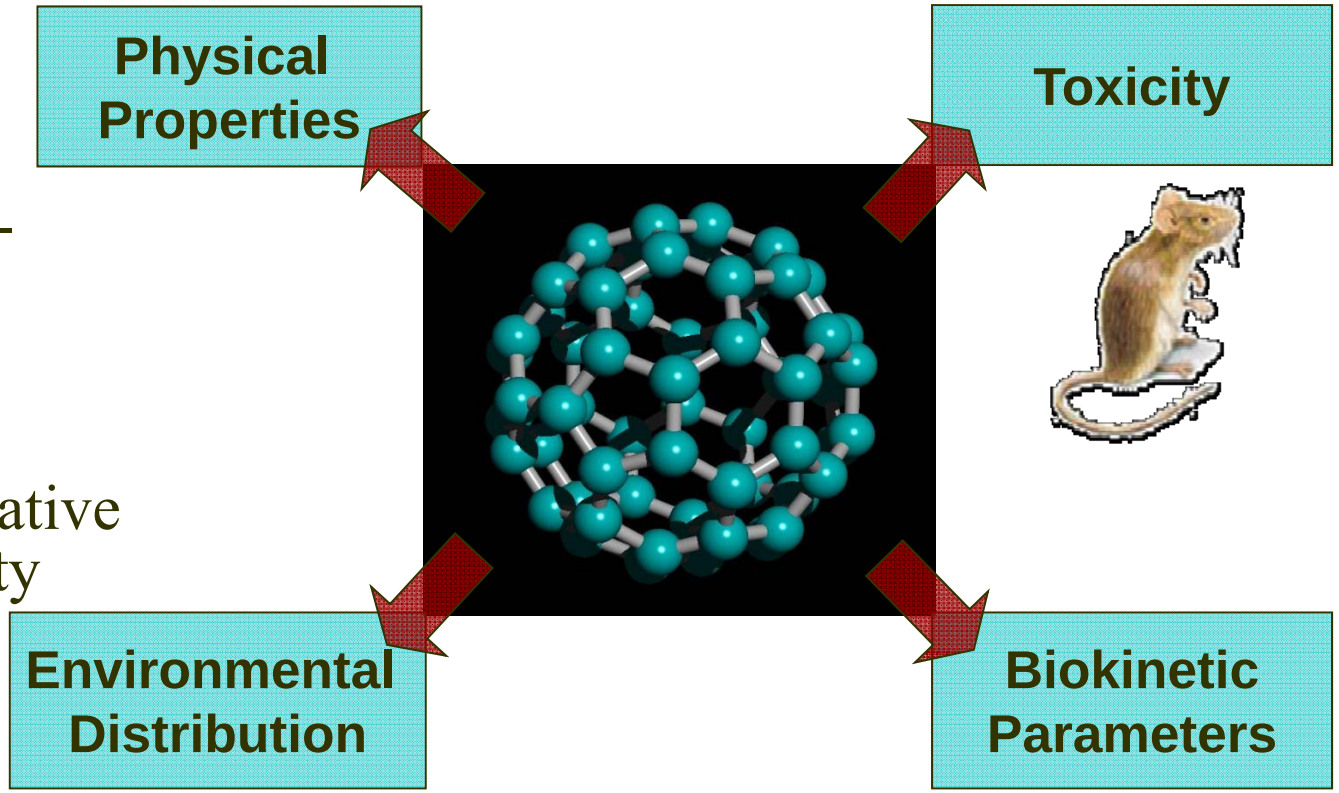
Modeling Effort Conducted by:

Interdisciplinary Center for Nanotoxicity ~ Jackson State University

Results:

Prescreening Biomarkers *in vitro* and Predicative Modeling- In silico approaches

- SARs: Structure-Activity Relationships
- QSARs: Quantitative Structure-Activity Relationships

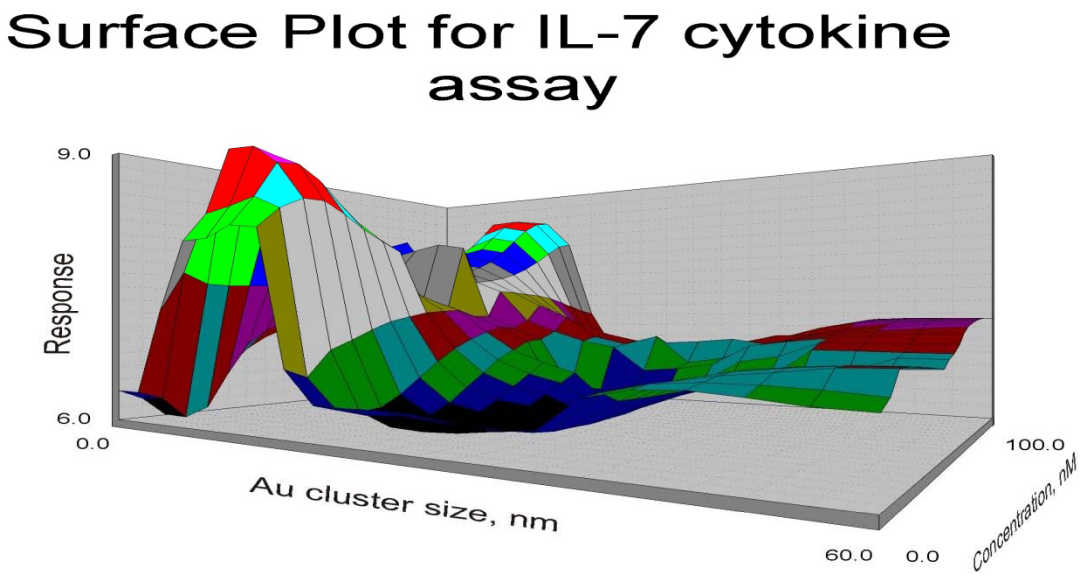


Modeling Effort Conducted by:
Interdisciplinary Center for Nanotoxicity ~ Jackson State University

Results:

Prescreening Biomarkers *in vitro* and Predicative Modeling

- **3-dimensional plot of data- difficult to see regularity**



Modeling Effort Conducted by:
Interdisciplinary Center for Nanotoxicity ~ Jackson State University

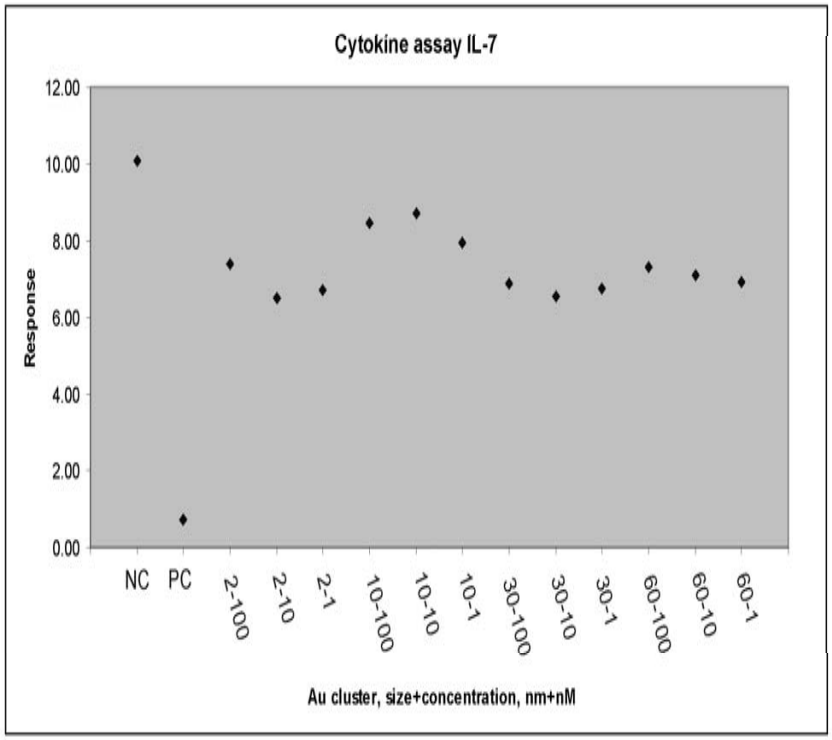
Results:

Prescreening Biomarkers *in vitro* and Predicative Modeling

- **When plotted on one axis, can see a sinusoidal curve, which is gradually damping**
- **Looked for equation to fit the curve**
- **Among several thousands curve fitting functions we found the following:**

$$Y(IL7)=7.88E\text{-}03\cdot(X_1^2\cdot ATAN(X_2))+3.64\cdot(COS(X_1)\ ATAN(X_2))+3.64$$

- **where Y(IL7) is response variable, IL-7 cytokine assay values, X_1 – gold nanoparticle size in nm, X_2 – concentration in nM**
- **Need to check robustness of this equation with experimental data for other sizes of gold nanoparticles**

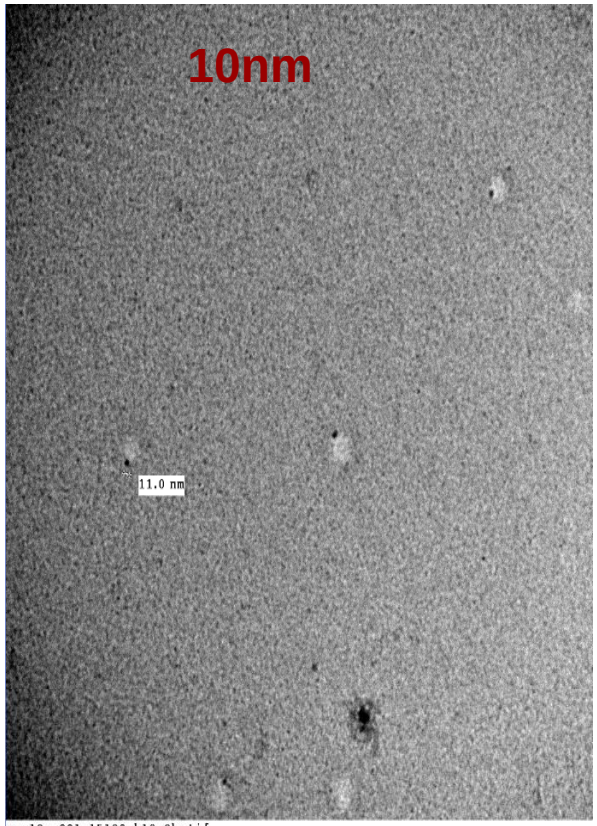


Modeling Effort Conducted by:

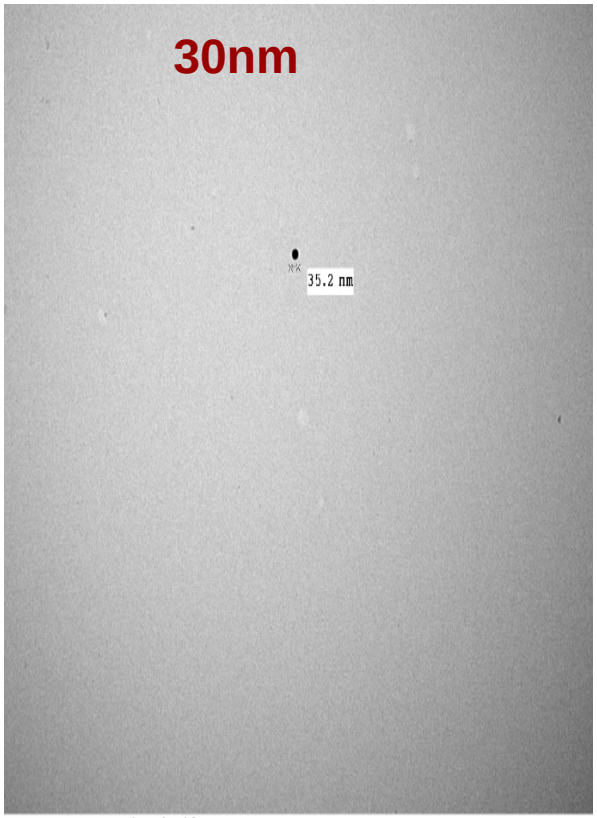
Interdisciplinary Center for Nanotoxicity ~ Jackson State University

TEM Images of Gold Nanoparticles

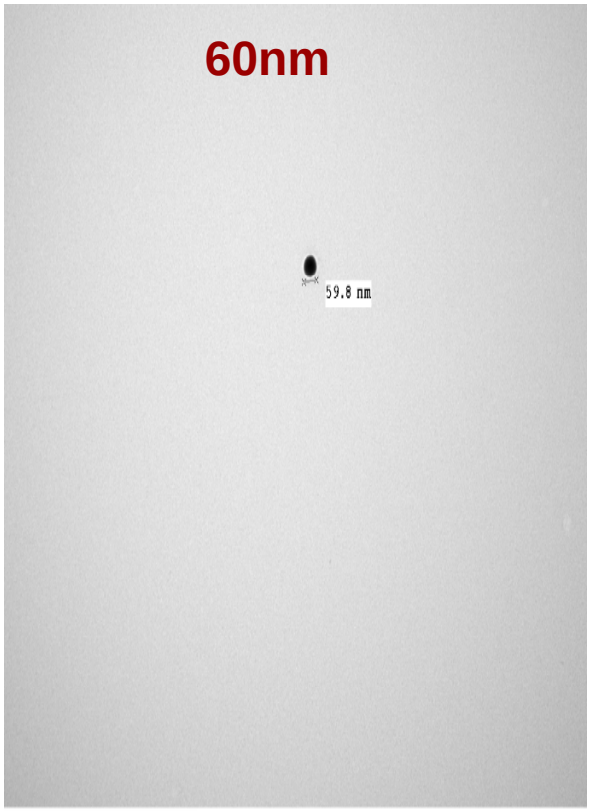
10nm



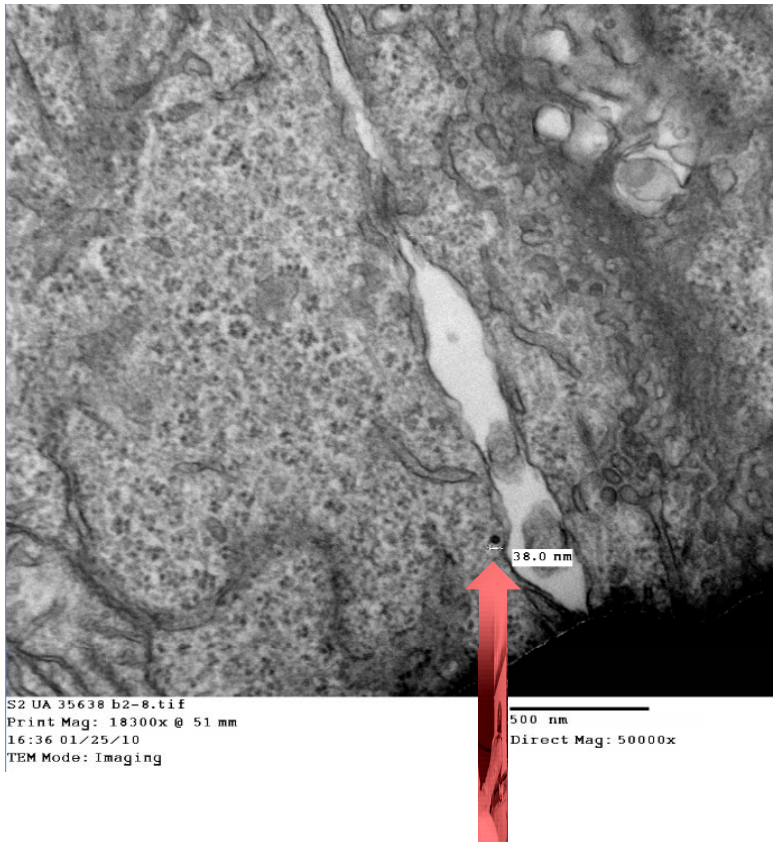
30nm



60nm



TEM Images of Gold Nanoparticles: Incubated with cells



Potential uptake of nanoparticles into cells

Conclusions:

- Well characterized gold nanoparticles have shown little/ no effect in acute toxicity assays
- Further testing is scheduled for more cell lines and various types of nanoparticles
- Data from follow on experiments will be compiled into the database for continued modeling for prediction of relative risk of well characterized materials
- Disruption of cellular pathways and biomarkers of exposure will be used to develop predictive monitoring tools

- **Acknowledgements:**

  APL: Melissa Betz

  NIST: Dr. Bryant Nelson, Dr. John Elliot

  Johns Hopkins University: Microscopy Facility

References:

- Atsuya Takagi, Akihiko Hirose, Tetsuji Nishimura, Nobutaka Fukumori, Akio Ogata, Norio Ohashi, Satoshi Kitajima and Jun Kanno: “Induction of mesothelioma in p53+/− mouse by intraperitoneal application of multi-wall carbon nanotube”: J. Toxicol. Sci., Vol. 33: No. 1, 105-116. (2008)
- HEPA Filtration Facts. D. F. Solutions. Minneapolis: 1-2
- Maynard, A. and E. Kuempel (2005). "Airborne Nanostructured Particles and Occupational Health." Journal of Nanoparticle Research 7(6): 587-614
- Poland, C. A., R. Duffin, et al. (2008). "Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study." Nat Nano 3(7): 423-428
- Song, Y., X. Li, et al. (2009). "Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma." European Respiratory Journal 34(3): 559-567
- Wick, P., A. Malek, et al. (2009). "Barrier Capacity of Human Placenta for Nanosized Materials." Environ Health Perspect 118(3)

Image Sources

- http://www.atcc.org/Attachments/1761.jpg
- http://losangelespublicrelations.com/colloidal-silver-blue-man/0781
- http://www.pharmacology2000.com/respiratory_anesthesiology/pulmonary_assessment/pulmonary_assessment1.htm
- https://tools.invitrogen.com/content/sfs/gallery/low/g001619.jpg