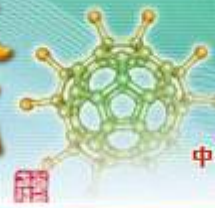




国家纳米科学中心
National Center for Nanoscience and Technology

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白春禮書



中国科学院
中华人民共和国教育部
共同組建

Center for Nanoscience and Technology

Understanding the Interactions of Nanoscale Materials with Biological Systems by Integrated Techniques

Chunying CHEN

chenchy@nanoctr.cn

National Center for Nanoscience and Technology, China

CAS Key Lab for Biological Effects of Nanomaterials and Nanosafety





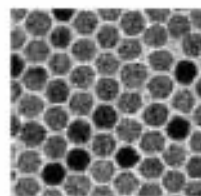
Various types of nanoparticles used in biomedical research



Inorganic Nanoparticle



Quantum dot



Iron oxide



Solid Lipid Nanoparticle



Liposome



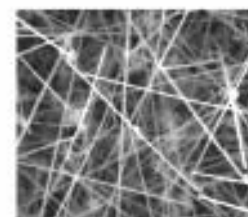
Nanocrystal



Nanotube



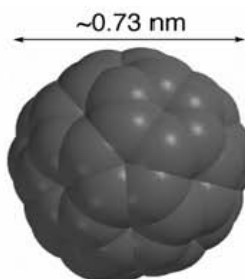
Gold



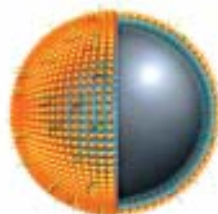
Nanowire



Polymeric Nanoparticle



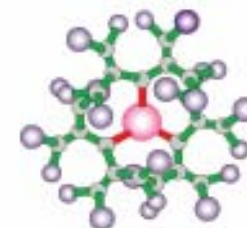
buckminsterfullerene



Perfluorocarbon



FeCo



Dendrimer



Dendrimer

How to design & screen the safe nanomaterials for the need of biomedical and other industrial application

The Janus Faces of Nanoparticles

Promising
advances in
nanomedicine

industrial
application

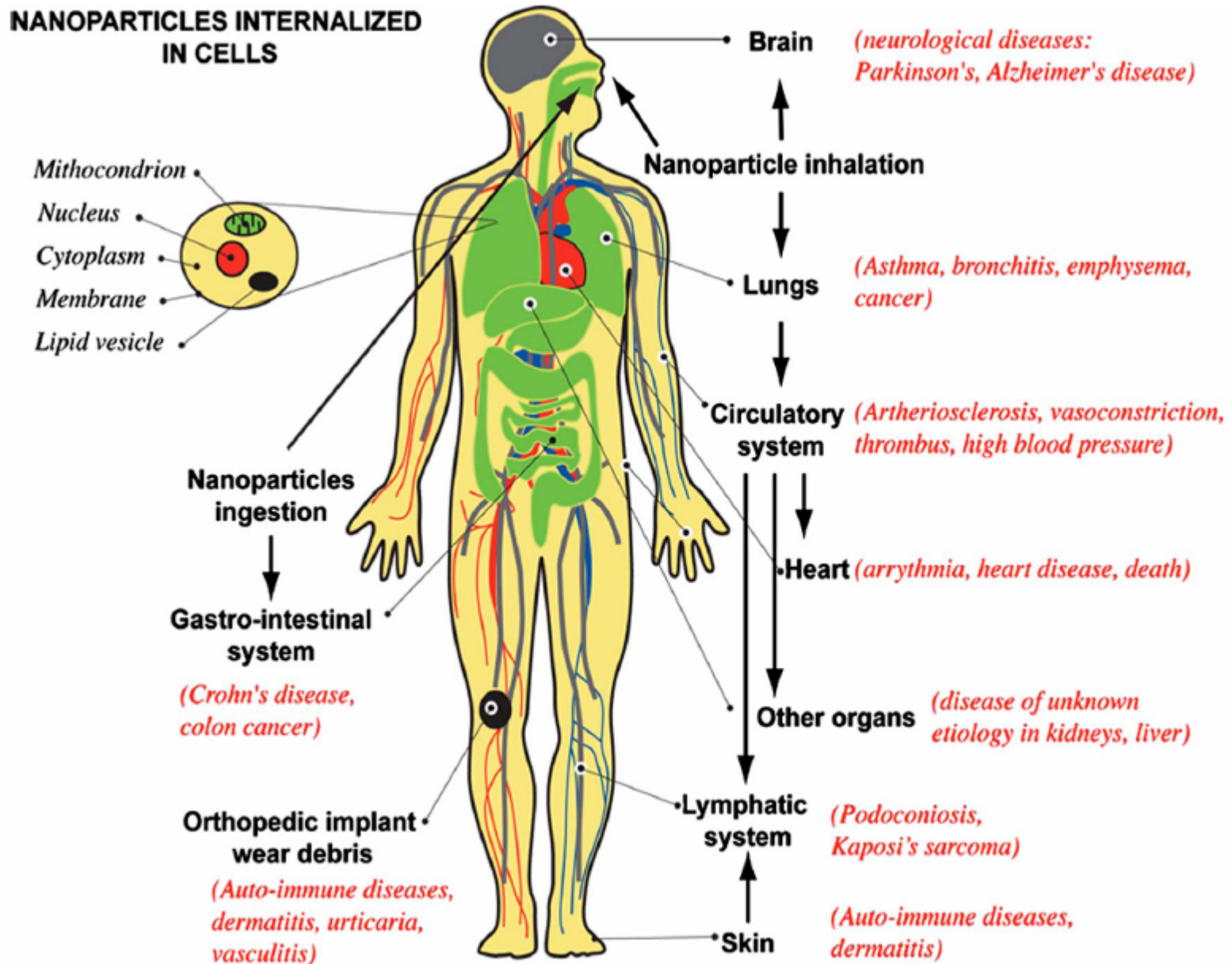


Toxic effects of nanoparticle exposure

- Cross blood-brain barrier –impair health
- Pulmonary toxicity
- privacy concerns
- limited understanding
-

Donaldson & Seaton, J Nanosci Nanotech 7(2007)4607-4611

Entry and target tissues for uptake of engineered nanoparticles





Outline

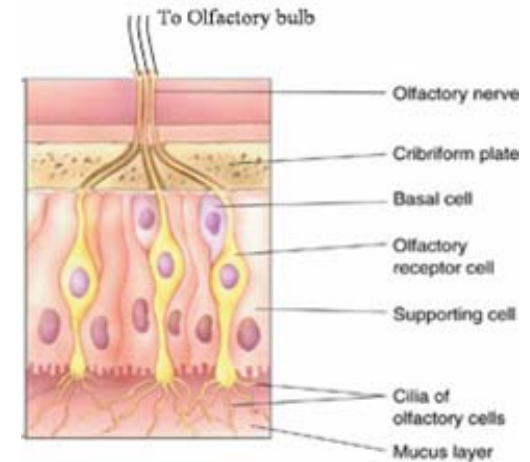
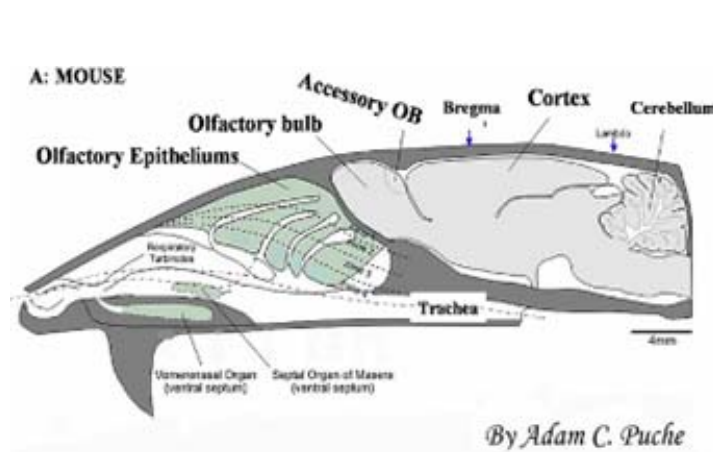
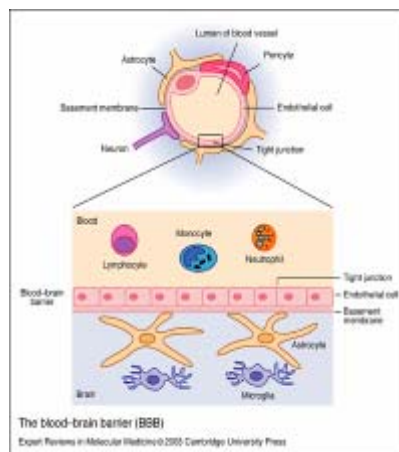
Understanding Interactions of Nanoscale Materials with Biological Systems

- The ability of NPs for biological barriers
- Pulmonary responses after Long-term retention of nanoparticles
- The Role of Nanoparticles During cell Mitosis
- Specific responses by different types of cells
- Key factors influence the nano-bio interactions.

Nose-to-Brain transfer?

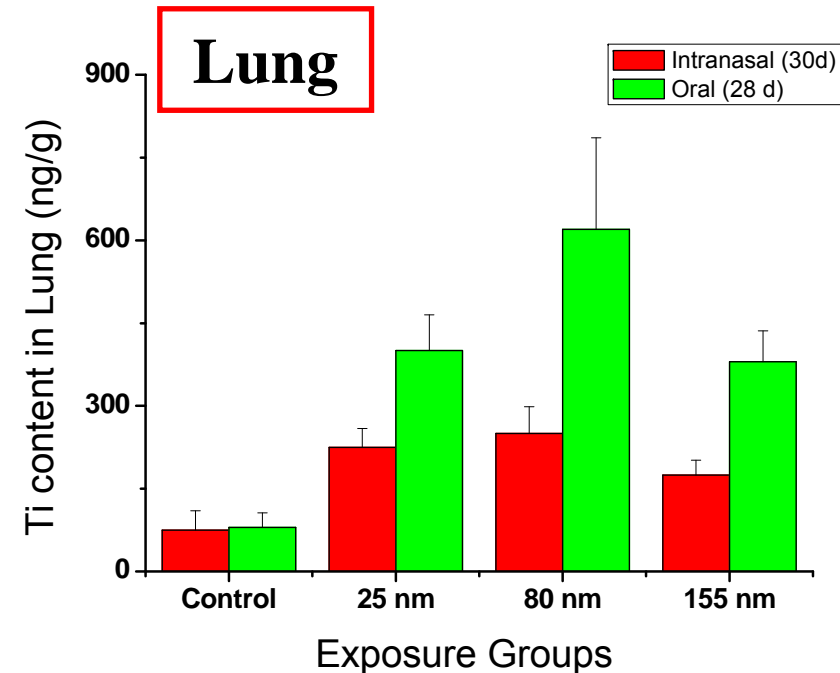
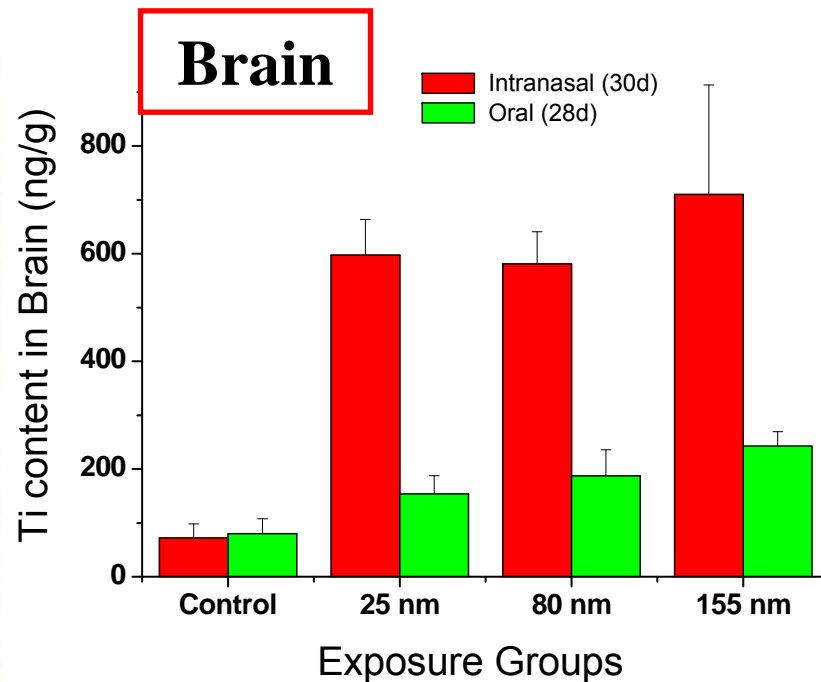
Blood Brain Barrier?

- Possibility and Ability of inhaled Nanoparticle Entering into Brain directly?
- Is the olfactory neuronal pathway efficient for translocating inhaled UFPs to the central nervous system ?
- How do physico-chemical characteristics of NPs influence uptake and translocation?
- Are there any toxicological consequences?



Ti retention in Brain and Lung tissues

Nasal Instillation vs. Oral administration



TiO₂ Accumulation:

Nasal Instillation: Brain > Lung

Oral Administration: Brain < Lung



Ti distribution in different brain areas

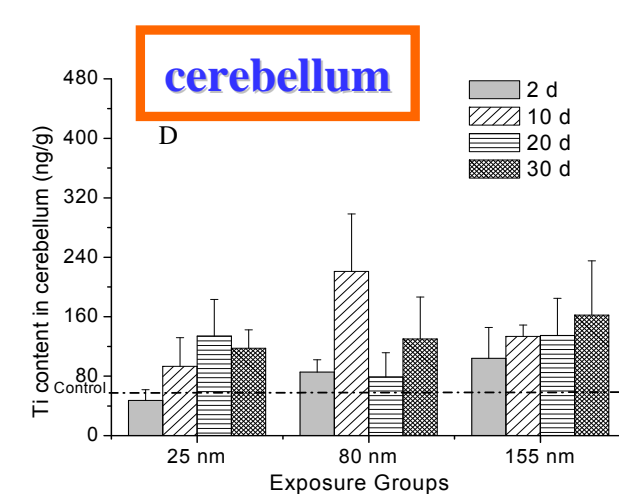
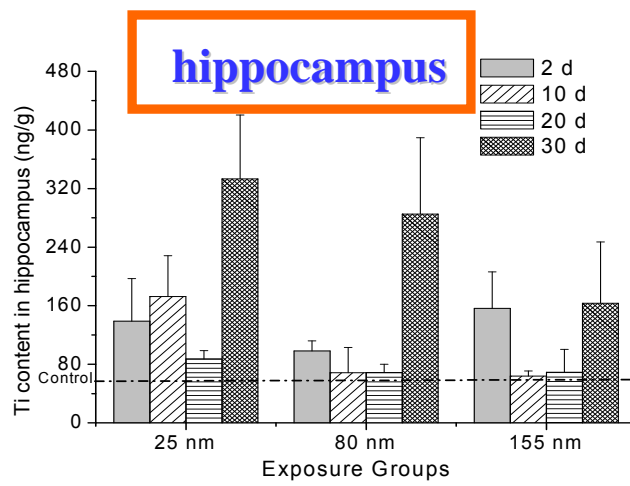
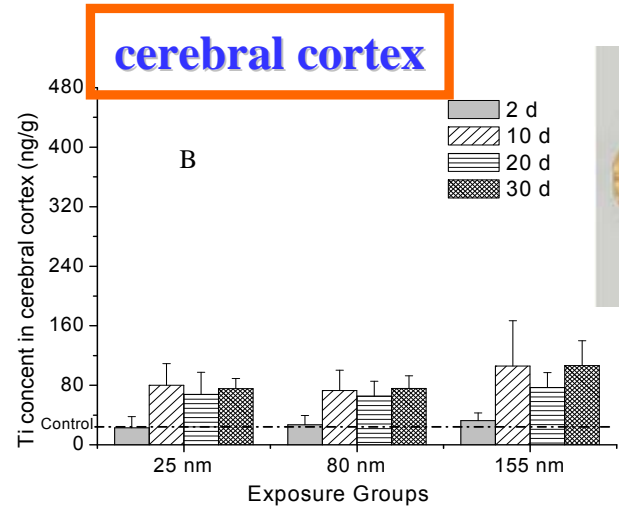
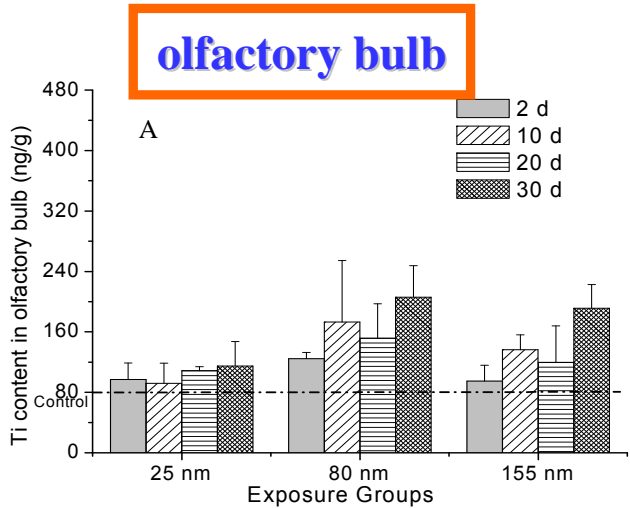
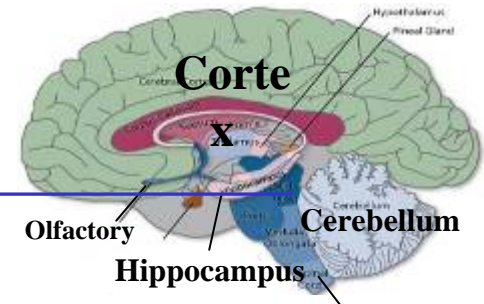


Fig. Titanium content in the olfactory bulb (A), cerebral cortex (B), hippocampus (C) and cerebellum (D) of mice (n=6) intranasally instilled 25 nm, 80 nm and 155 nm TiO₂ particles for 2, 10, 20 and 30 d.

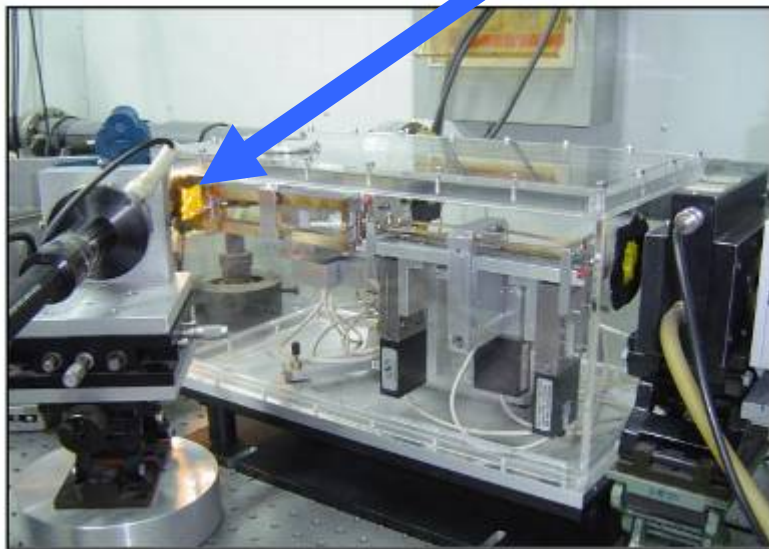
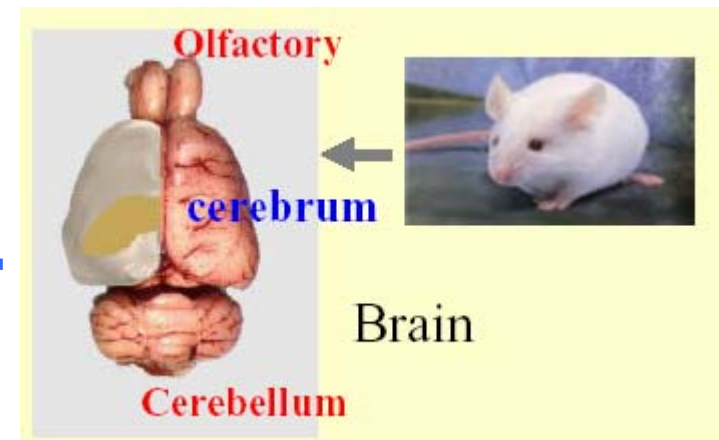
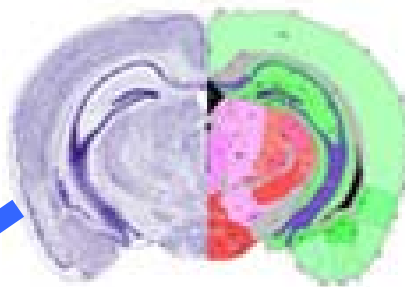
National Center for Nanoscience and Technology, China

Microbeam SR-XRF mapping techniques

Beamsize: $20 \times 20 \mu\text{m}^2$

$3 \times 5 \mu\text{m}^2$

Synchrotron radiation

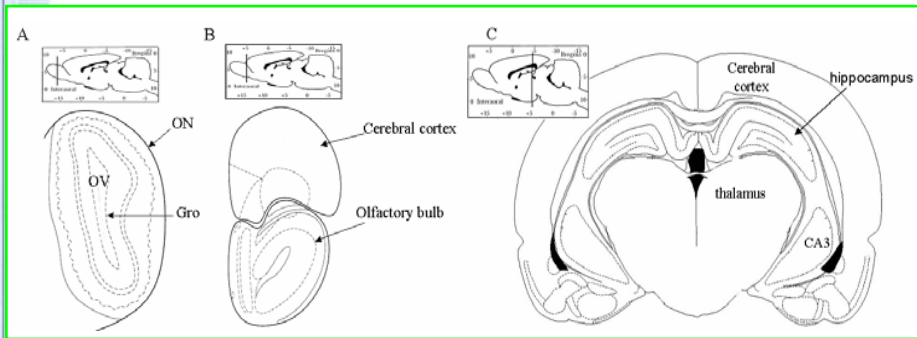


μ SRXRF mapping facility
Institute of High Energy Physics

Advantages:

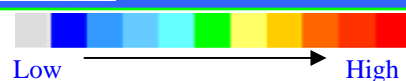
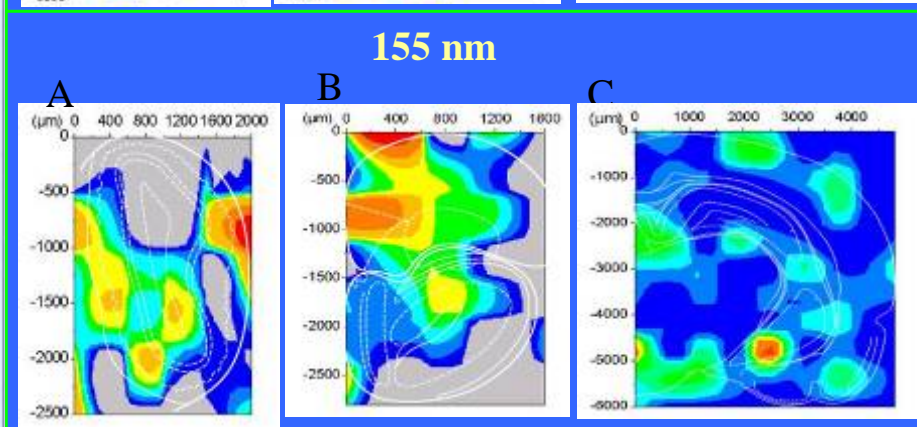
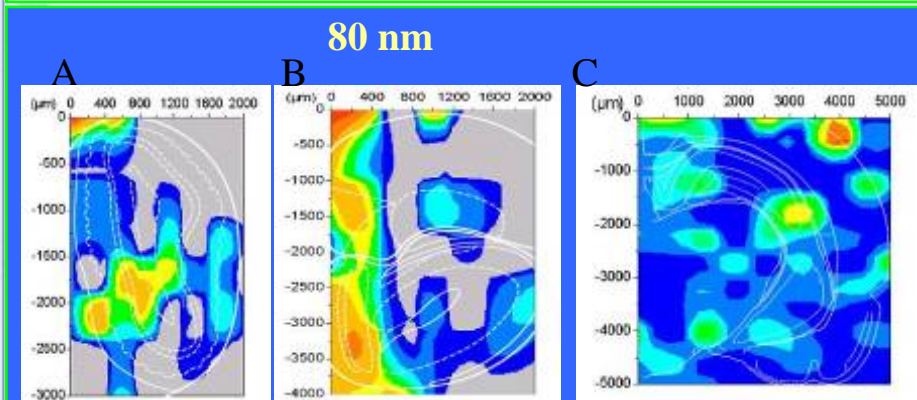
- ◆ Simultaneous multi-element determination
- ◆ the information in tiny areas and thin slices.
- ◆ improve the sensitivity and space resolution
- ◆ Non-destructive

Accumulation of nano-TiO₂ in mice olfactory bulb and brain following intranasal administration by SR-XRF mapping.



Olfactory Nerve Translocation pathways

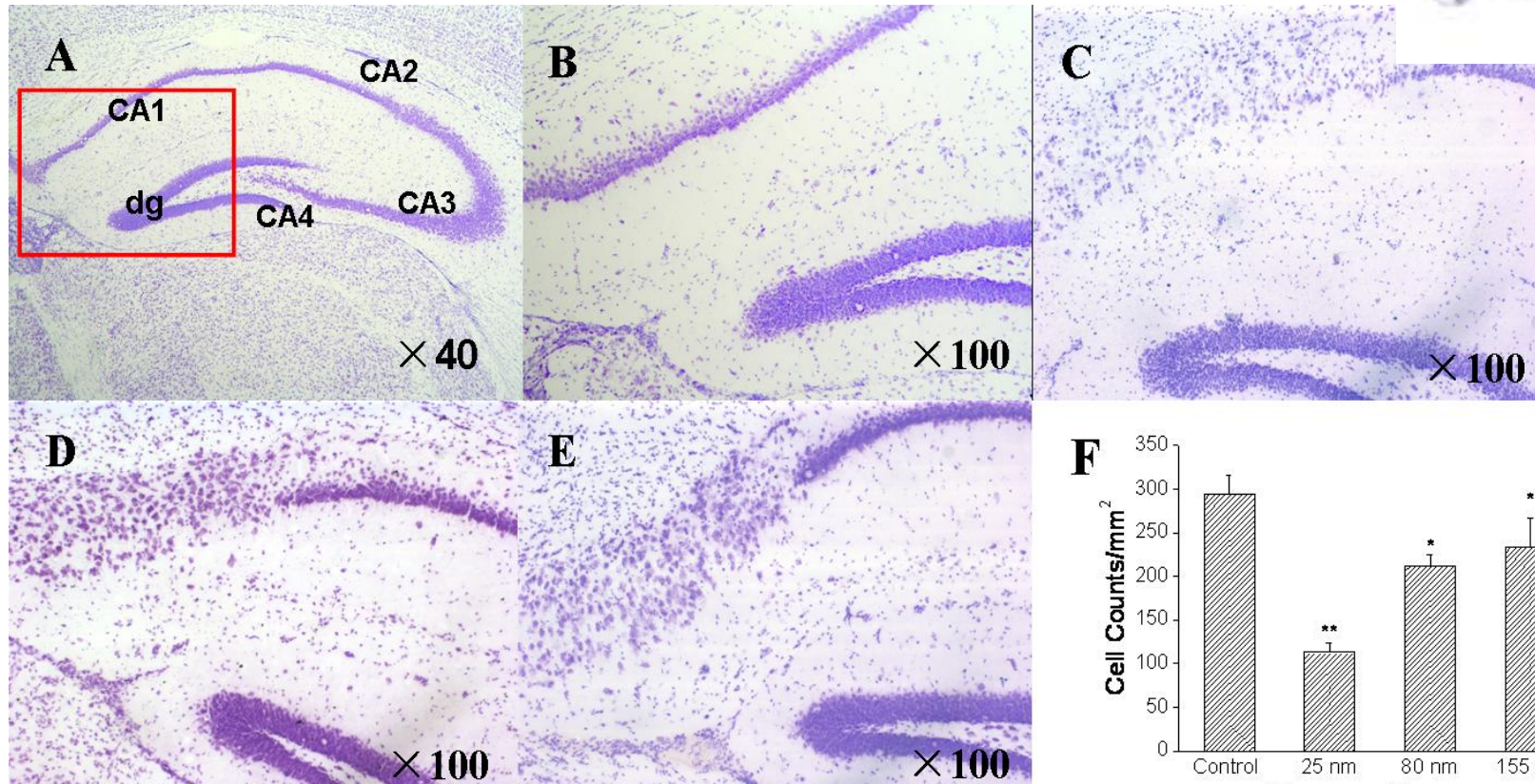
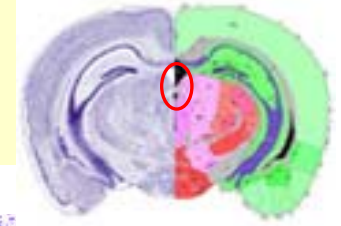
TiO₂ nanoparticles could be transferred via the secondary and tertiary olfactory pathways to reach most parts of brain.



Wang JX et al, High Energy and Nucl Physics, 2005
Wang JX et al, JRNC, 2007, Wang et al, 2008

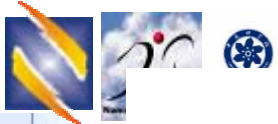


Histopathological examination Hippocampus



A control group; B 25nm group;
C 80nm group; D fine group

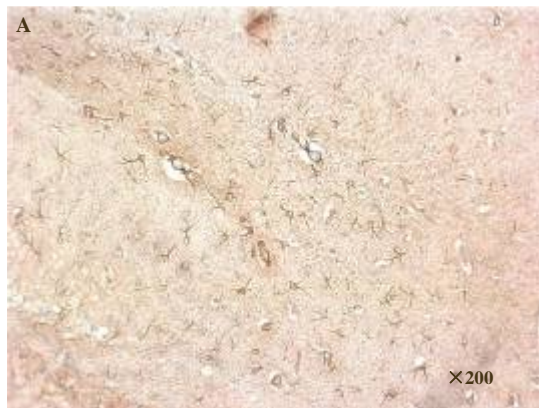
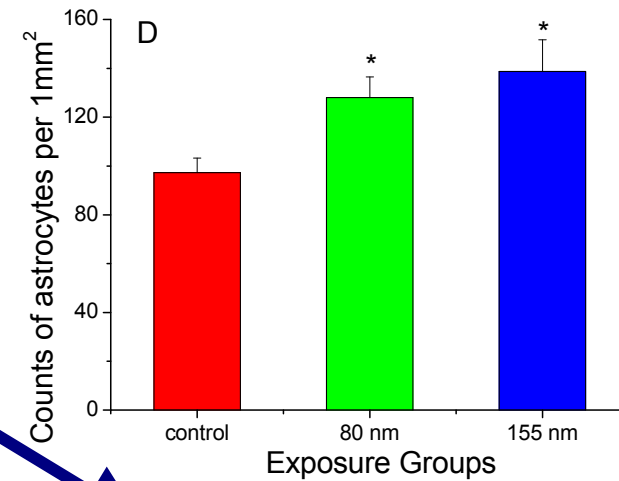
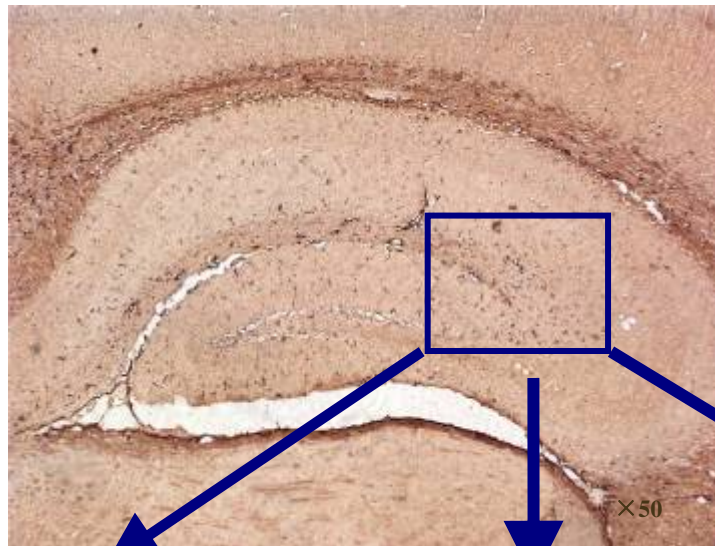
enlarged and elongated pyramidal cell soma
the stratum pyramidale was irregular
Nissl body decreased or disappeared.



Immunochemical examination

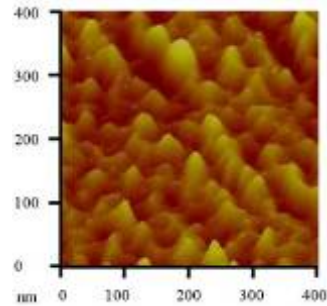
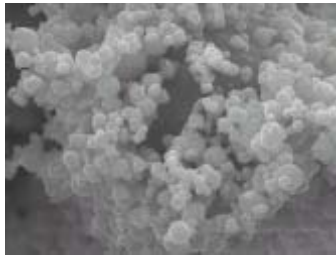
GFAP-positive glial cells in the hippocampus of murine brain

Activation of Astrocytes

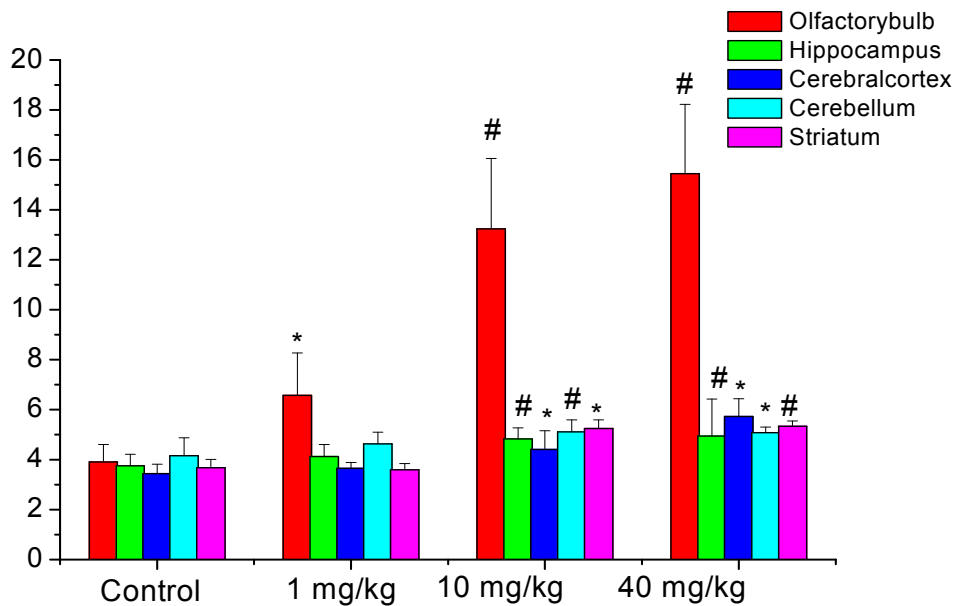


Wang JX, Chen CY, et al, Tox Lett, 2008

Cu nanoparticle

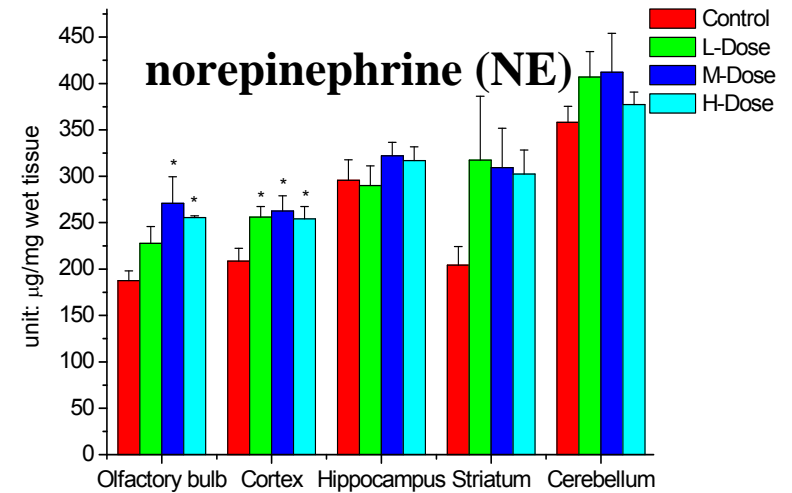
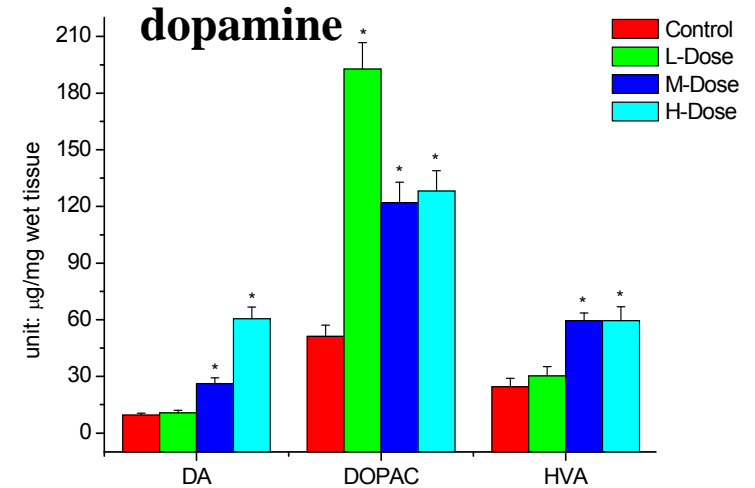


Cu accumulation in various brain regions



Concentrations of Cu in discrete murine brain regions after intranasally instilling copper nanoparticles 21 d.

Monoamine neurotransmitters changes in the brain

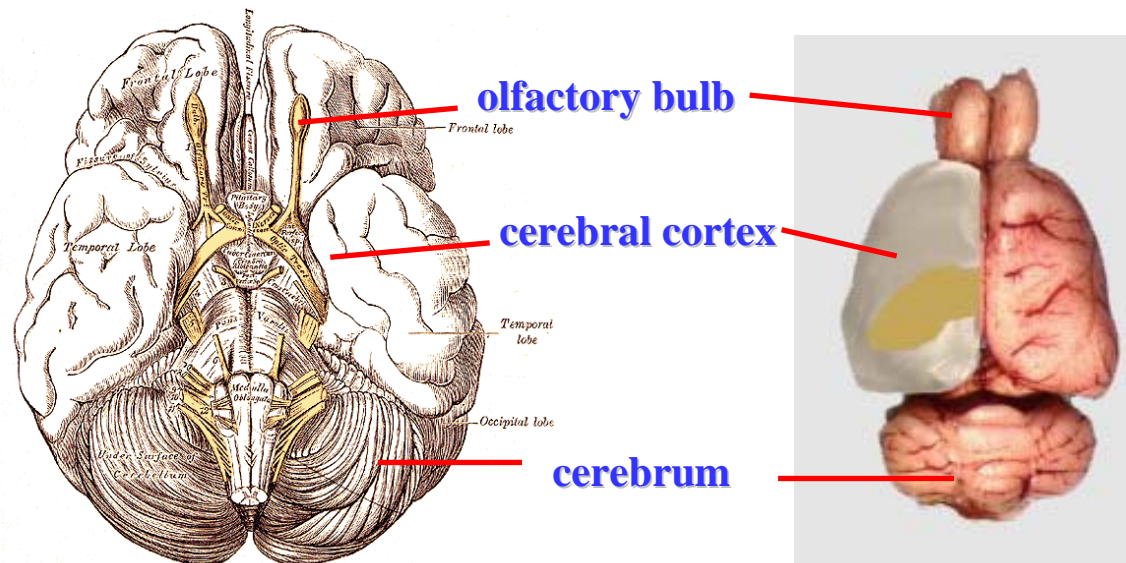


Question: What are differences of species?

The significance for humans still needs to be established.

Area: Rodents, the olfactory mucosa comprises **50%** of total nasal mucosal surface

Human, **5%** of the total nasal mucosal surface



Human brain, ventral view

Rat brain, Dorsal view

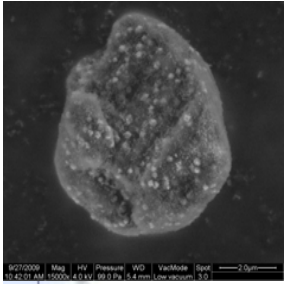
Can nasal administration be a new way for pharmaceutical treating neural diseases ?



Outline

Understanding Interactions of Nanoscale Material with Biological Systems

- The ability of NPs for biological barriers
- **Pulmonary responses after Long-term retention**
- The Role of Nanoparticles During cell Mitosis
- Specific responses by different types of cells
- Key factors influence the nano-bio interaction



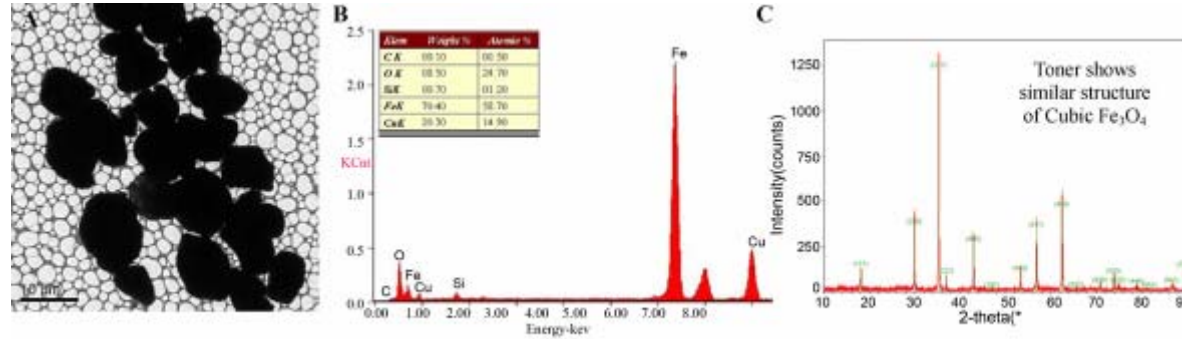
Pulmonary responses after Long-term retention of inhaled Toner particles

The Concentrations of PM_{2.5} and PM₁₀ in collecting particles from different indoor environments.

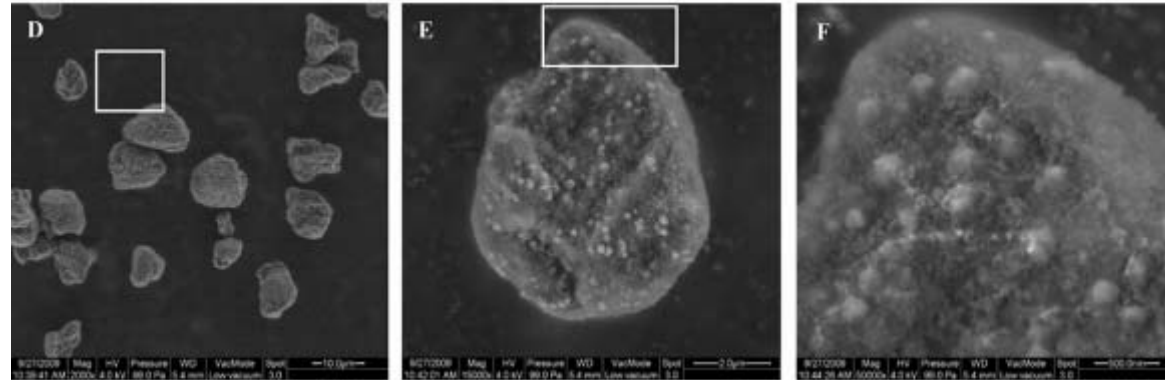
The Name of Experiment	Concentration of PM _{2.5} (μg/m ³)	Concentration of PM ₁₀ (μg/m ³)
The Background of particles in an office (No Printing)	19.6	18.3
Collecting particles in an office with intermittent printing	33.3	54.0
Continue collecting particles in a photocopy room (48h)	52.7	36.9
Continue collecting particles in a conference room (48h)	24.1	20.3



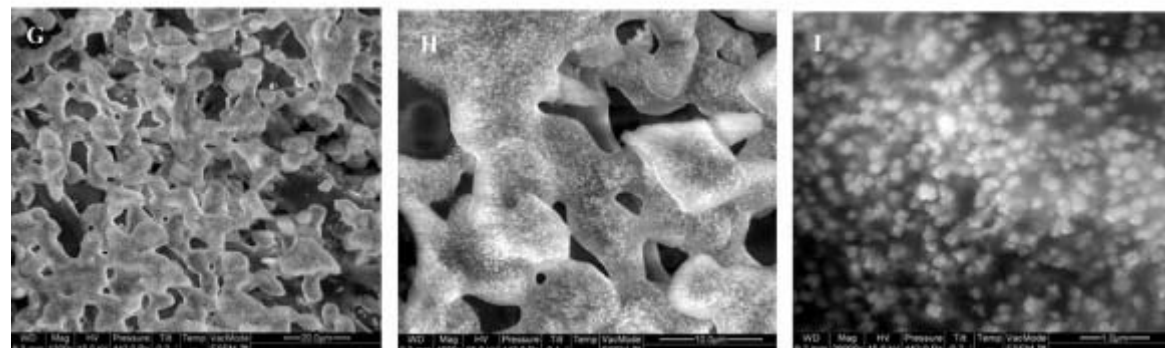
Characterization of toner particles

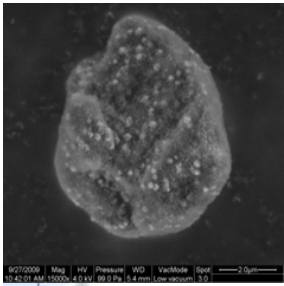


Original toner



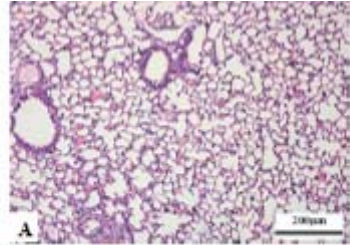
After printing



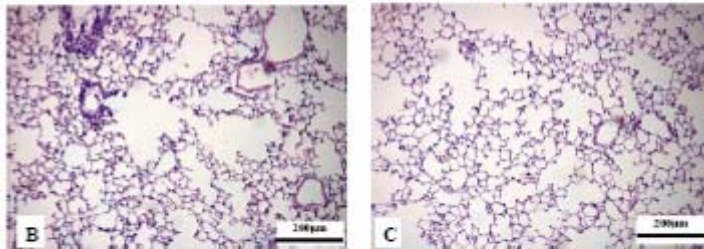


Long – term Retention of Inhaled Toner Particles in the Lung Tissues

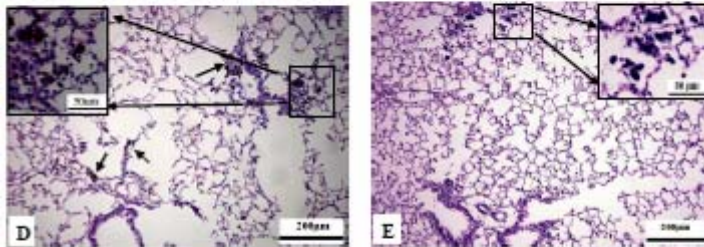
Saline control
day 9



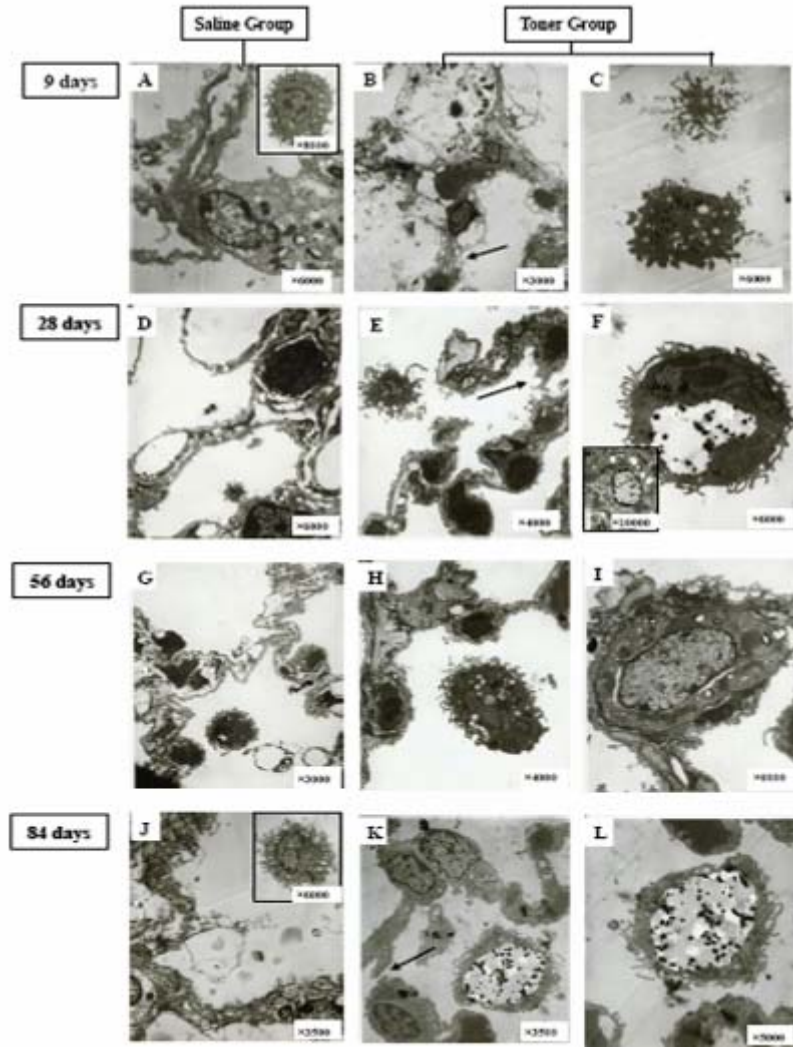
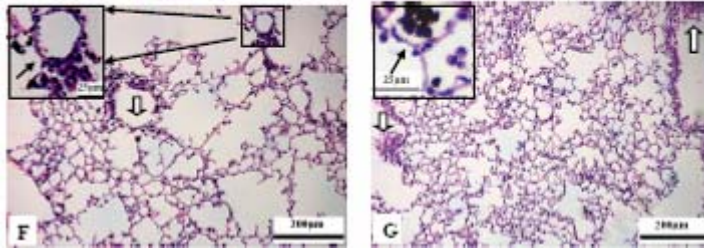
Saline control
days 28 and 84

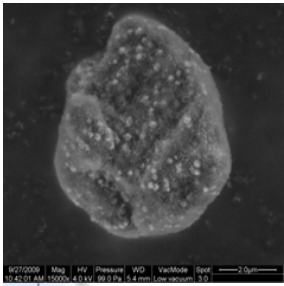


Toner exposure
day 9 and 28



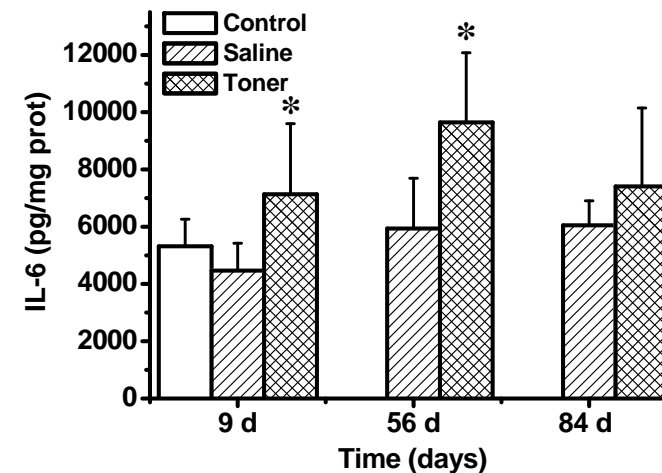
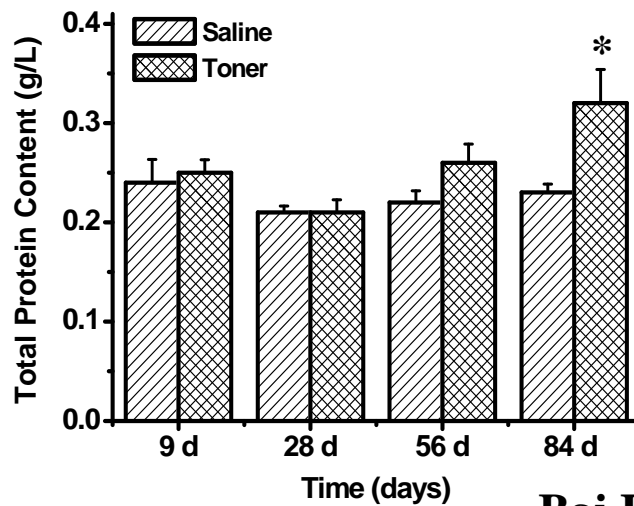
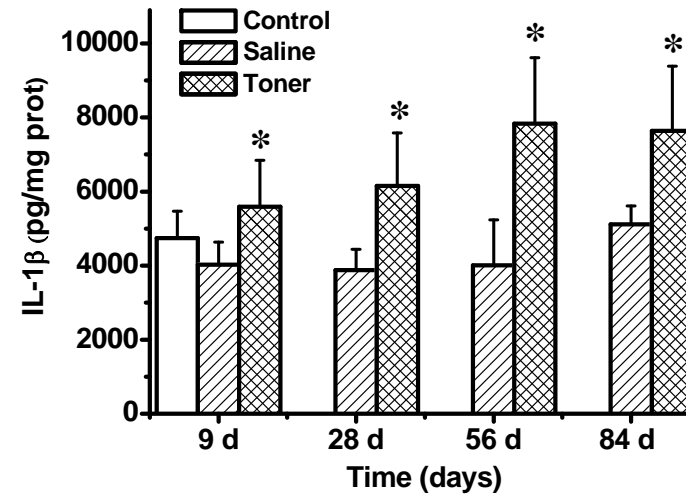
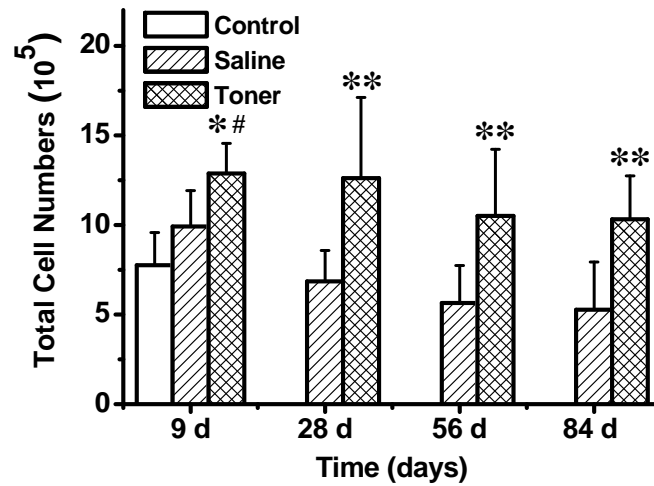
Toner exposure
day 56 and 84





Long – term Retention of Inhaled Toner Particles in the Lung Tissues induced the pulmonary inflammatory

National Center for Nanoscience and Technology



Bai R, Zhang L, Chen C, Tox lett, 2010



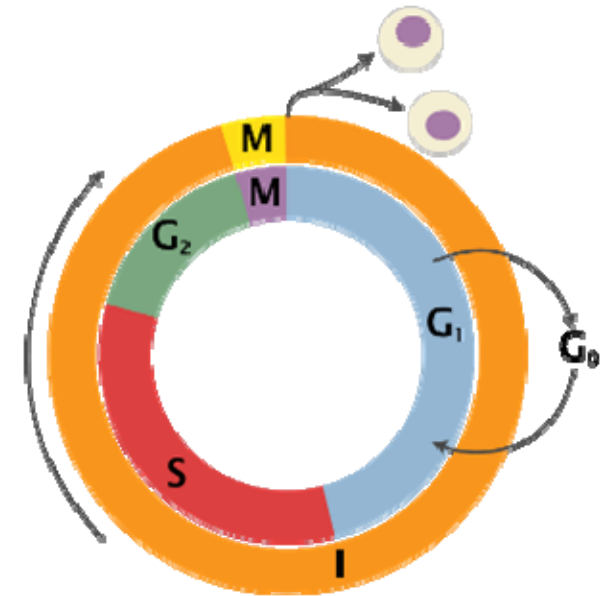
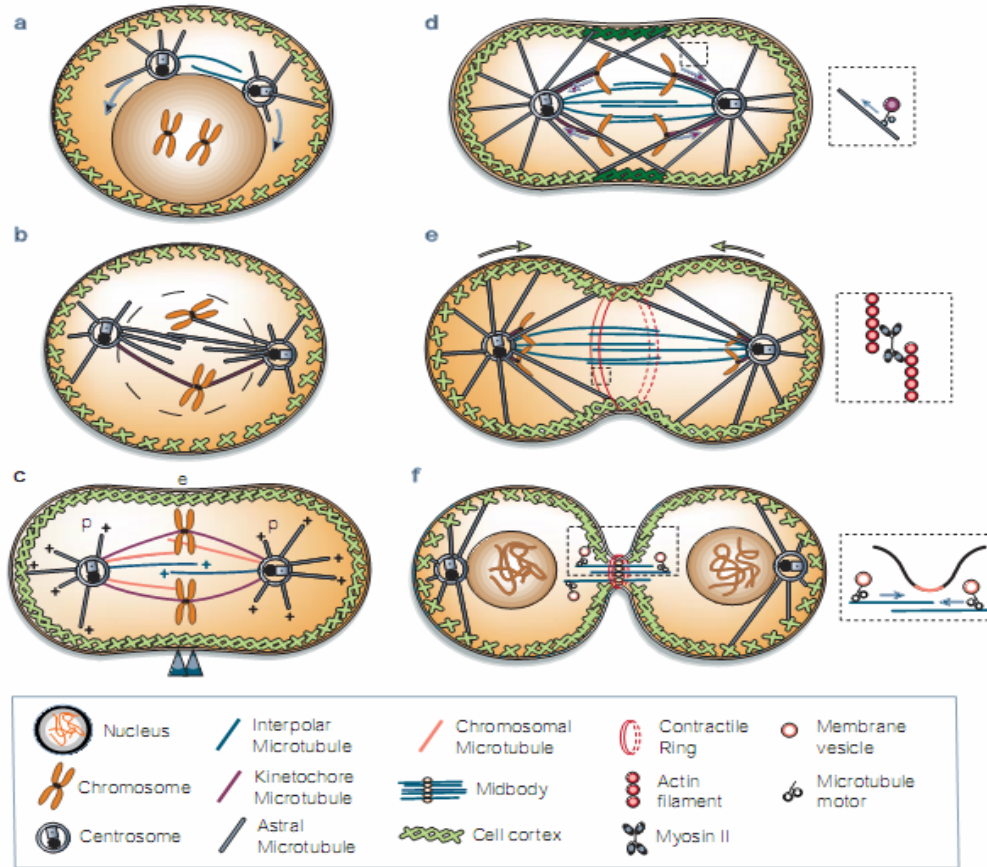
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- Specific responses by different types of cells
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The Role of Nanoparticles During the Mitotic Phase

Cell division



Interphase

Mitosis

prophase

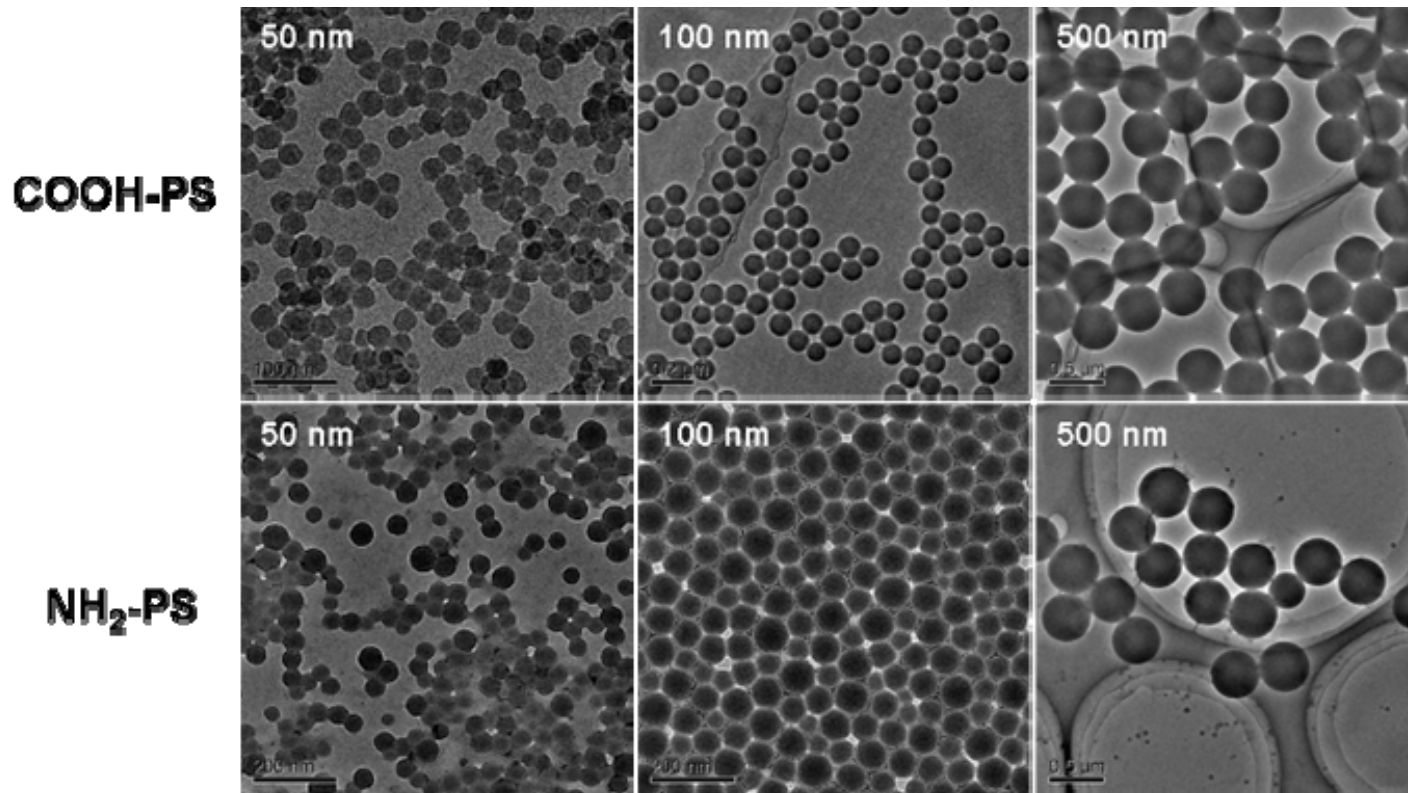
metaphase

anaphase

telephase

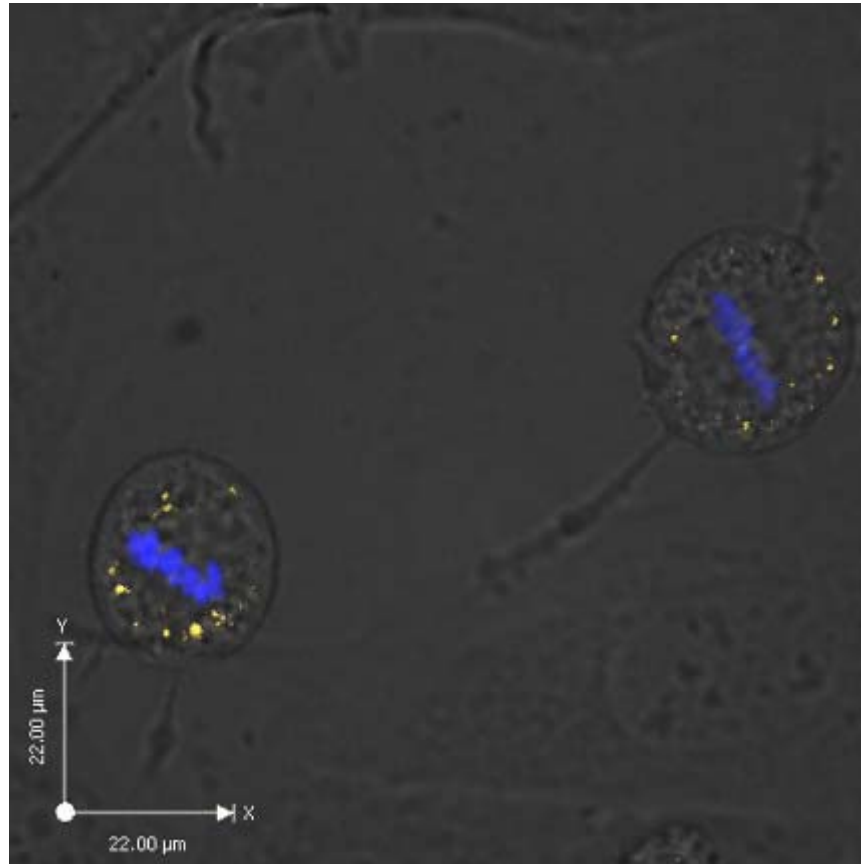
Nanoparticles

Carboxyl-modified (COOH-PS)
amino-modified (NH₂-PS) polystyrene particles
various sizes (50, 100, 500 nm in diameter)
fluorescence conjugation





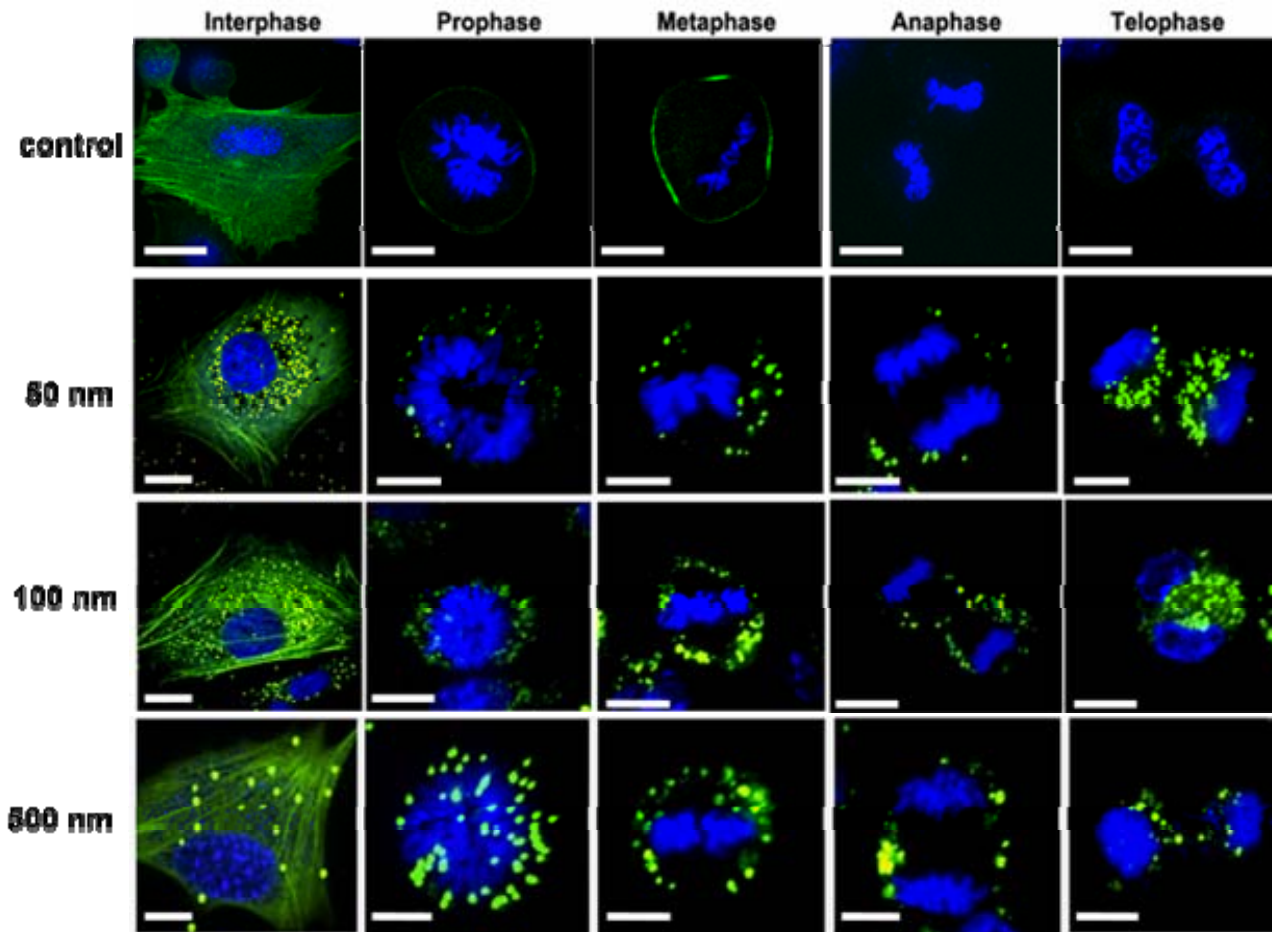
The dynamics and Intracellular Trafficking of PS Particles in live cells



Time-lapse observation of 100 nm COOH-PS nanoparticles in mitotic NIH 3T3 cells

Liu, Li, Zhao, Chen, *Biomaterials*, 2011

Spatial distribution of COOH-PS nanoparticles at different phase of mitosis in GFP-actin NIH 3T3 cells

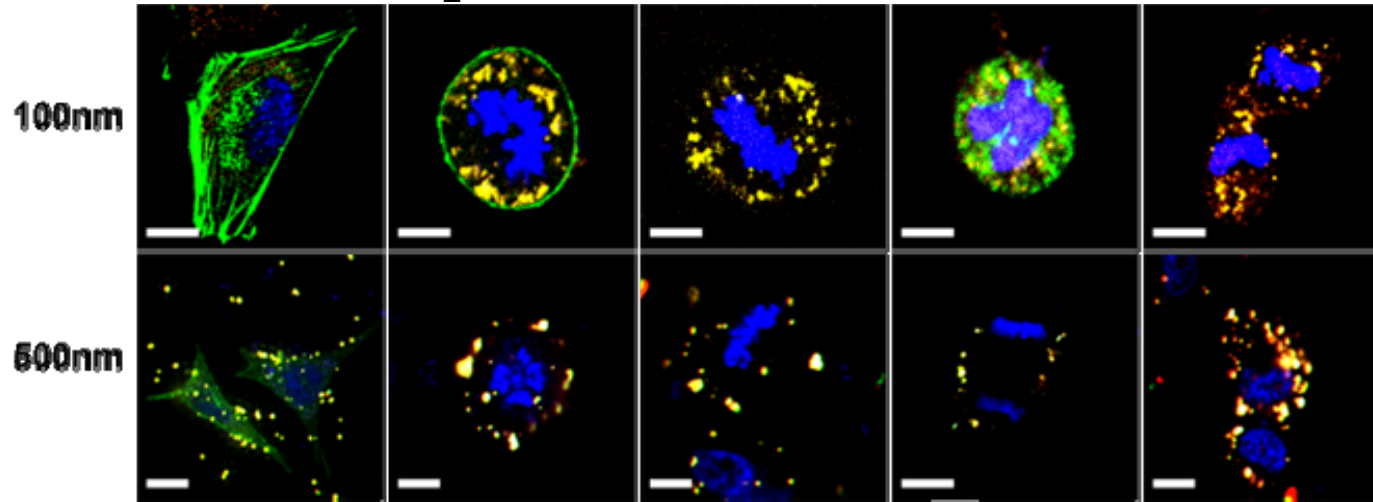


No effect on the reorganization of the chromosome and actin cytoskeleton

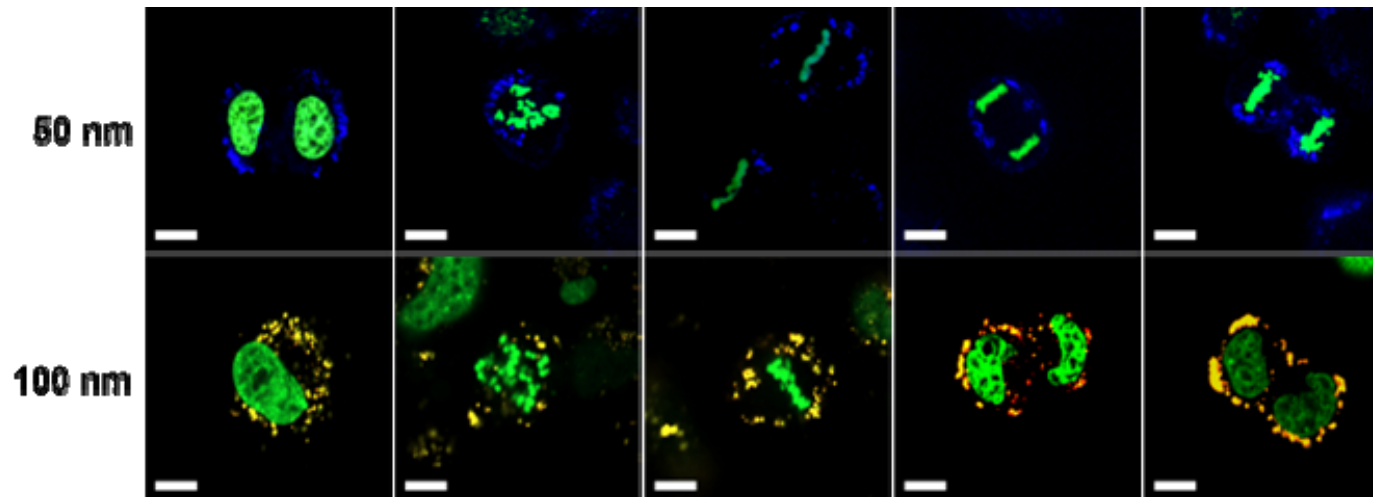
Liu, Li, Zhao, Chen, Biomaterials, 2011

Spatial distribution of NH₂-PS nanoparticles at different phase of mitosis in GFP-actin NIH 3T3 cells

Localization of NH₂-PS nanoparticles in GFP-actin NIH 3T3 cells



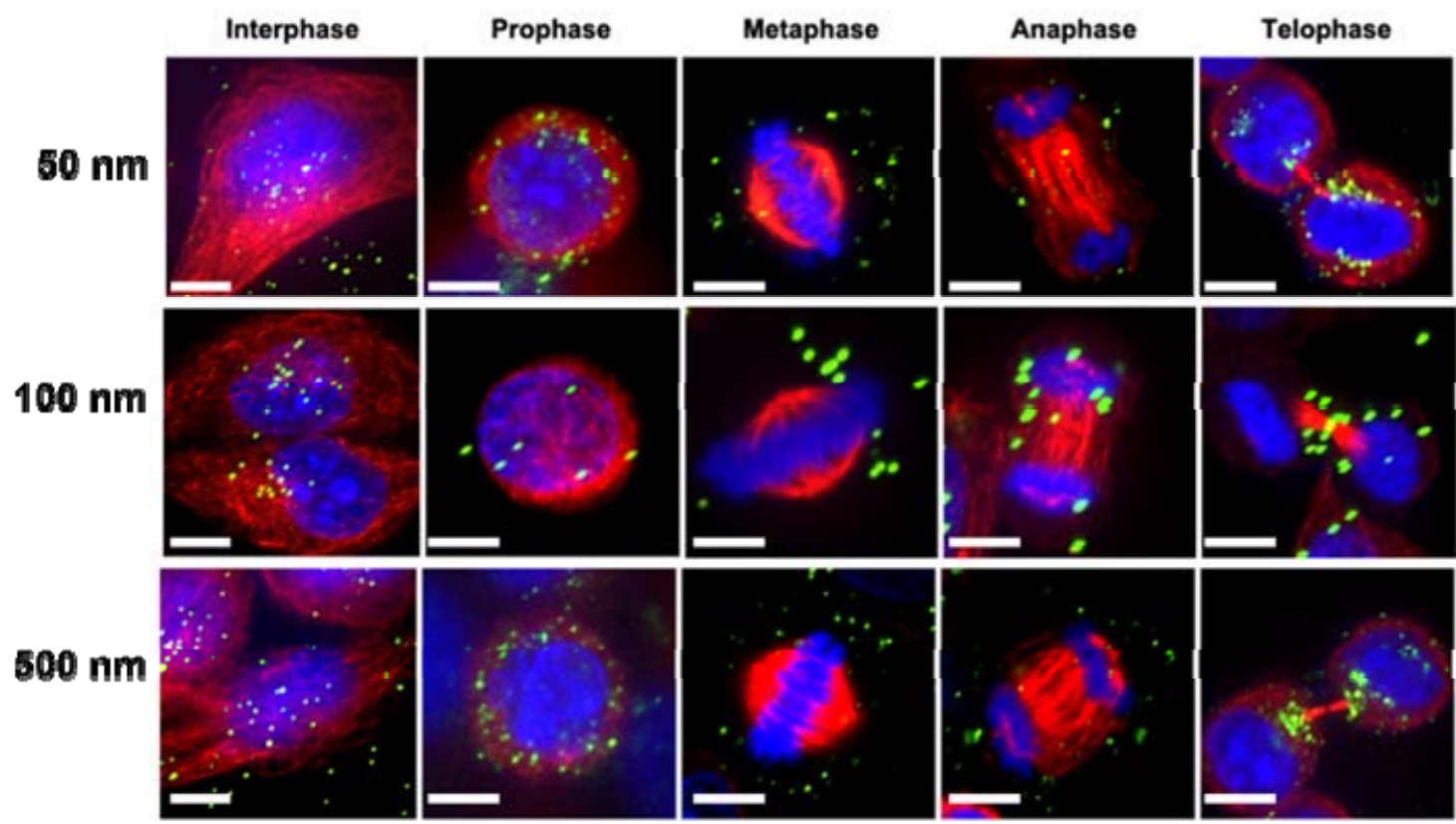
Localization of NH₂-PS nanoparticles in GFP-histone HeLa cells





Effect on the Organization of Mitotic Spindle and whole cell cycle

Localization of COOH-PS Nanoparticles and tubulin in fixed HeLa cells



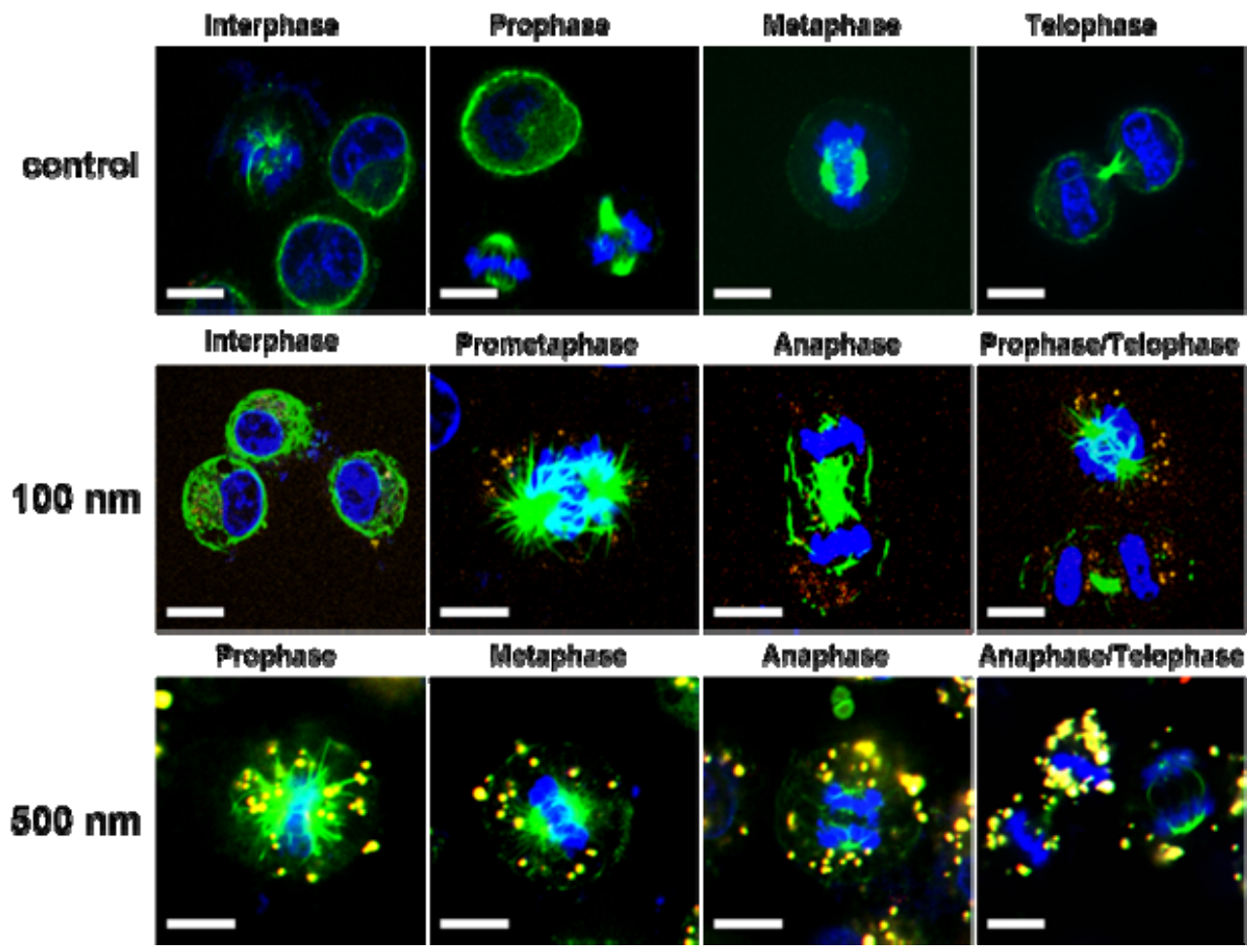
HeLa cells, tubulin tracker red, PS NPs Orange, Chromosome Green

Liu, Li, Zhao, Chen, Biomaterials, 2011



Effect on the Organization of Mitotic Spindle and whole cell cycle

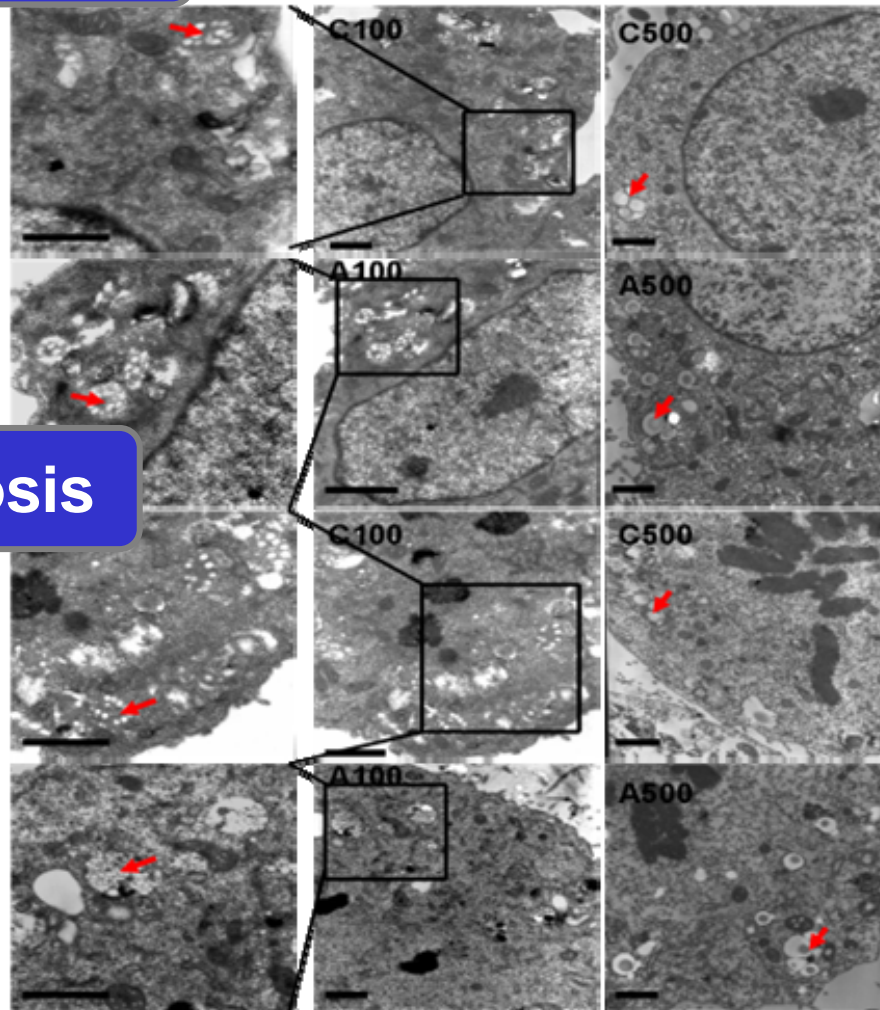
Localization of NH₂-PS Nanoparticles and tubulin in live HeLa cells



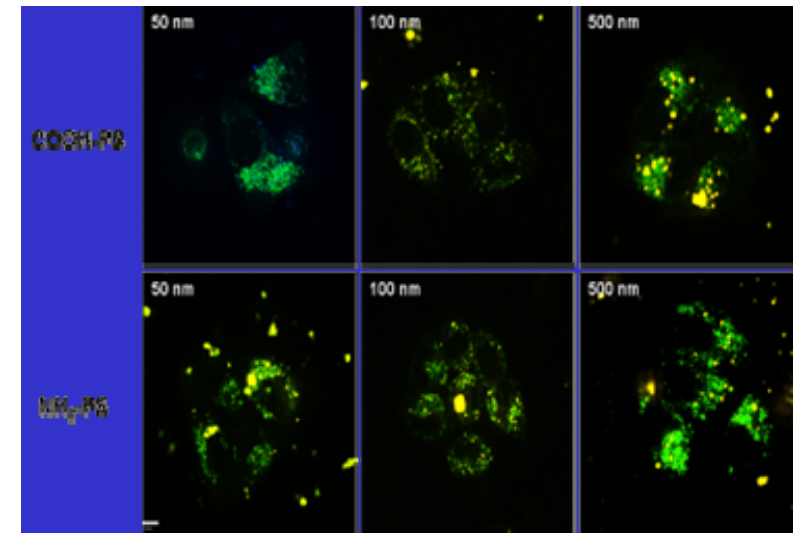


Intercellular localization of PS Nanoparticles

Interphase

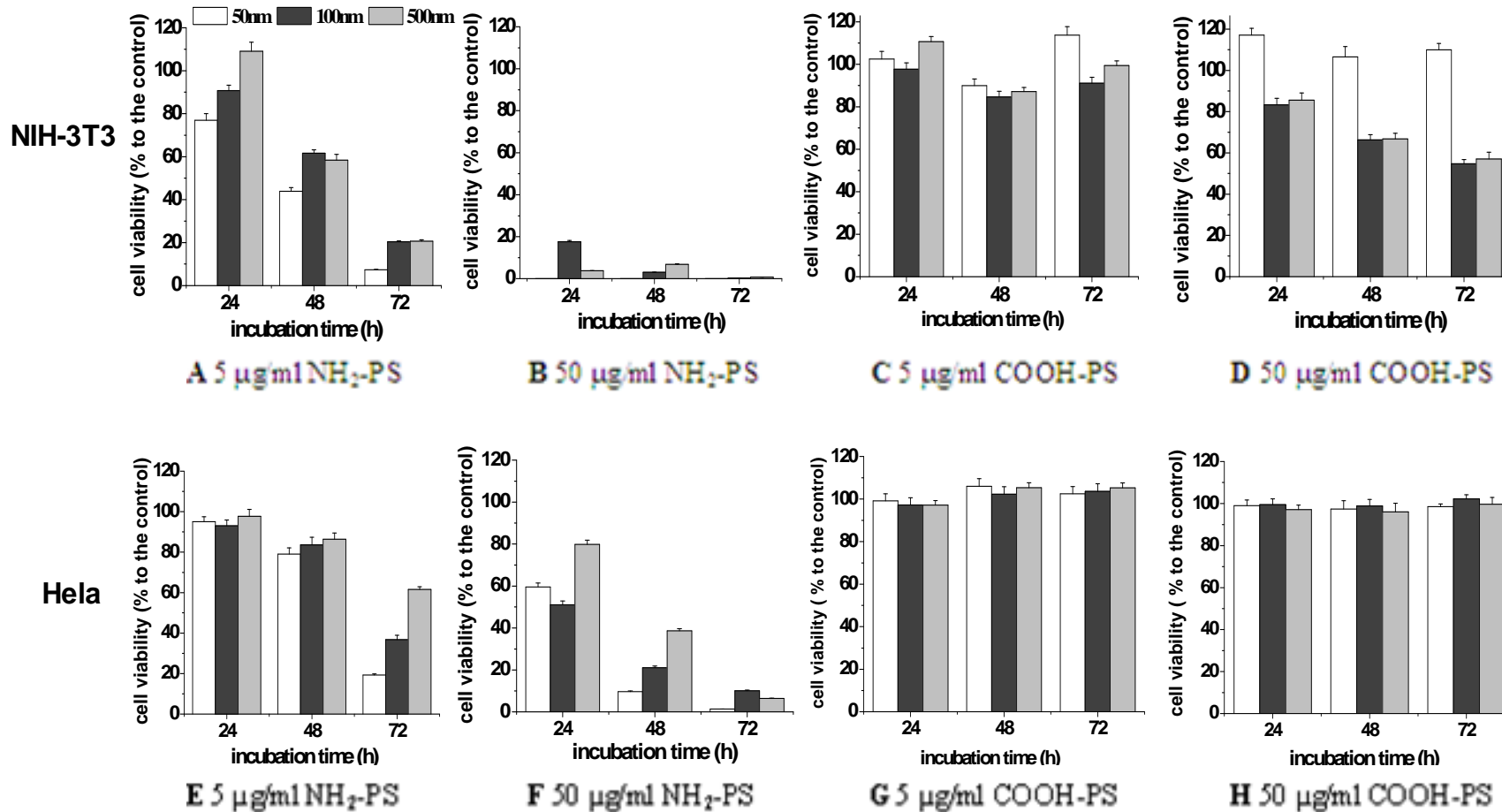


Mitosis



Lysosome

Time-Course and Size-Dependent Cytotoxicity



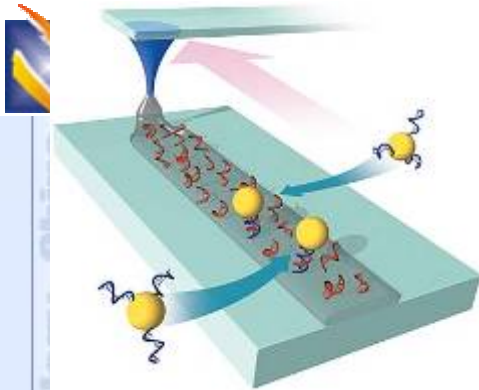
Cytotoxicity: $\text{COOH-PS} \ll \text{NH}_2\text{-PS}$



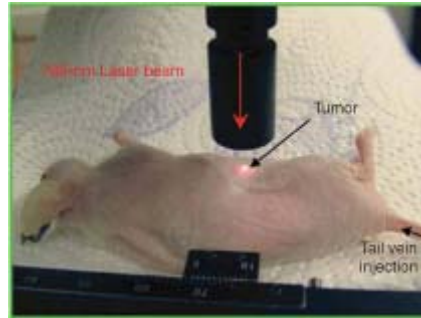
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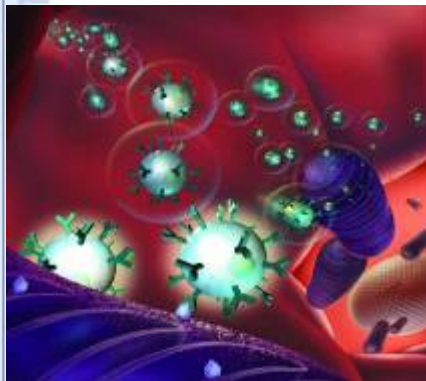
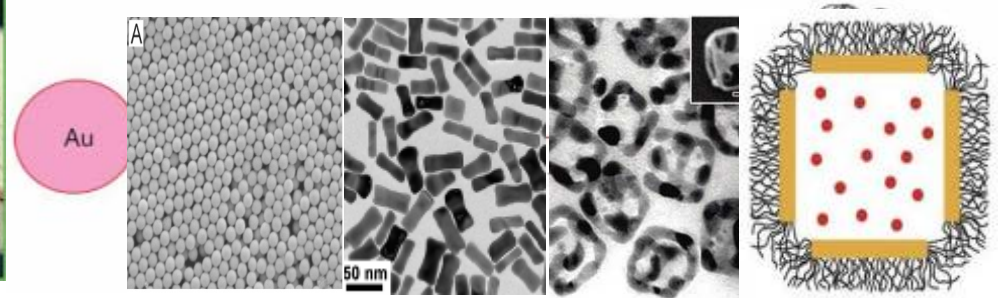
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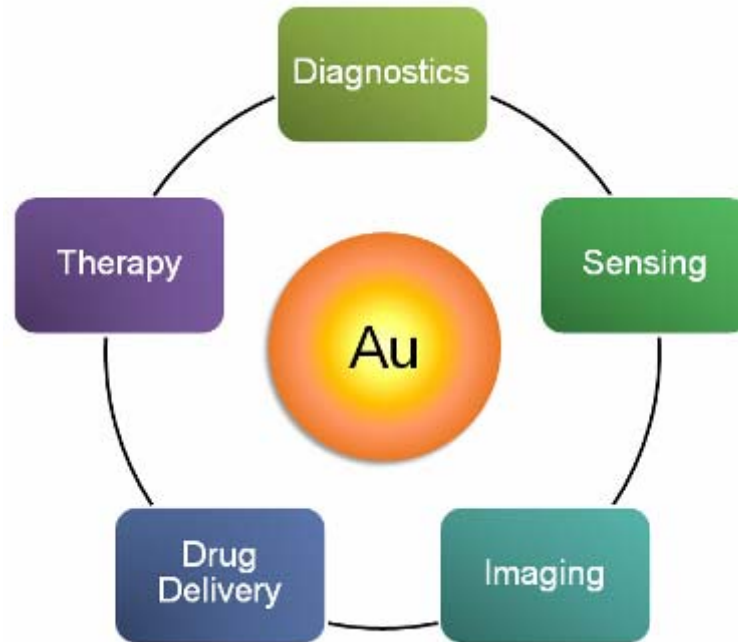
SERS: Disease diagnosis and detection



Immunological Labeling

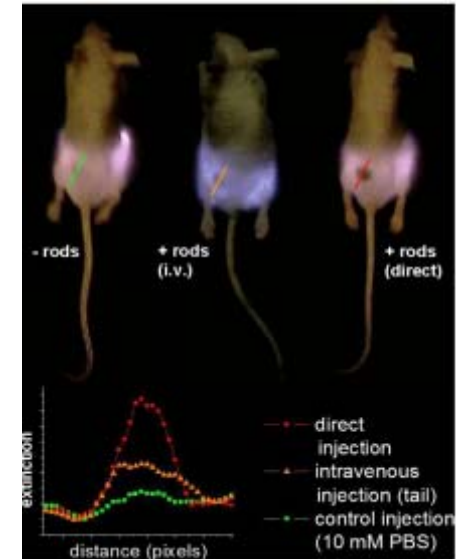


Drug and gene carriers

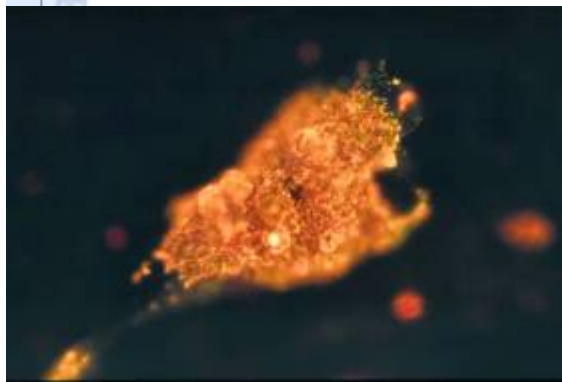


Application of Gold NPs in Biomedicine

Optical extinction



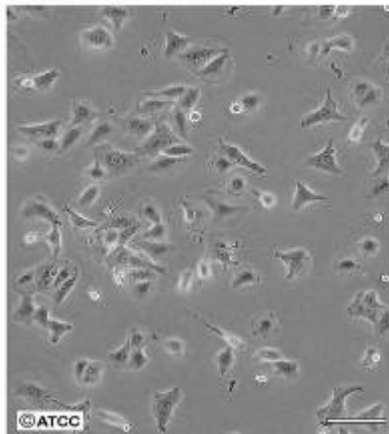
Labeling and tracking for Tumor (NIR, X-ray CT imaging, SERS)



Thermotherapy agents and temperature sensitive container

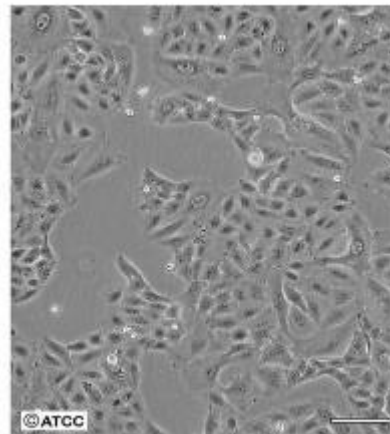


ATCC Number: CCL-185
Designation: A-549



Low Density

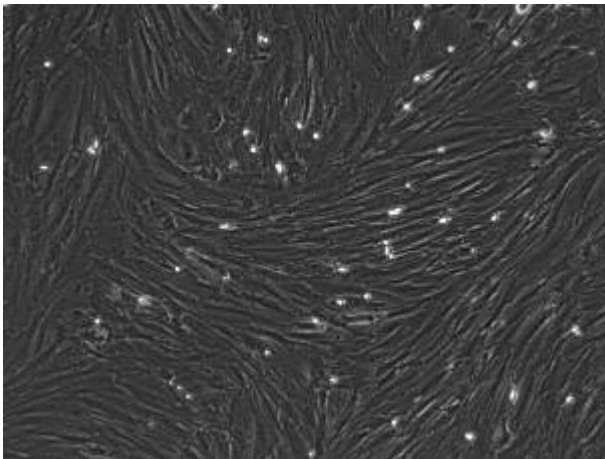
Scale Bar = 100µm



High Density

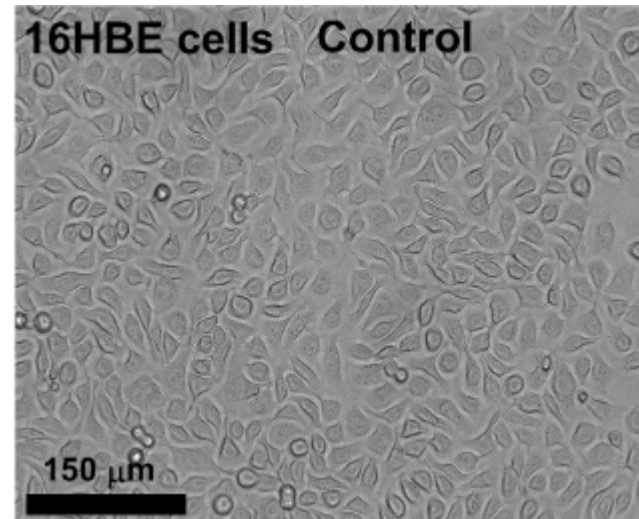
Scale Bar = 100µm

Human pulmonary adenocarcinoma cell (A549 cells)



Rat Bone marrow mesenchymal stem cells (MSC cells)

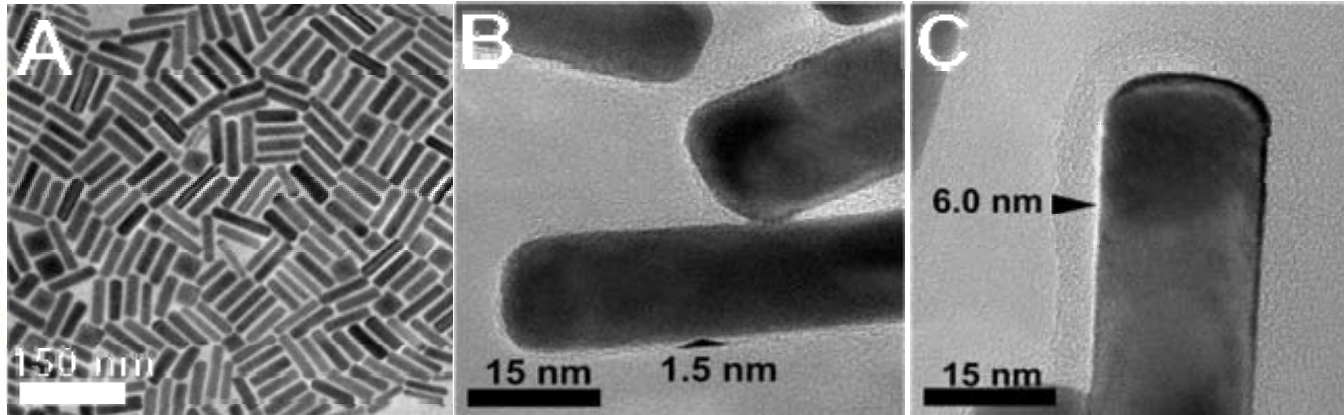
Cell types



Normal human bronchial epithelial cell(16HBE cells)

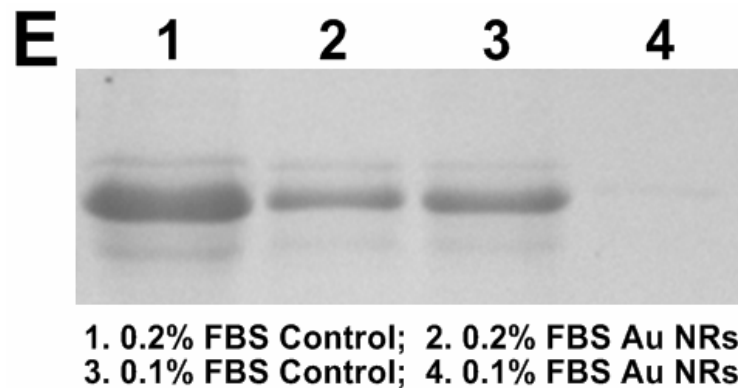
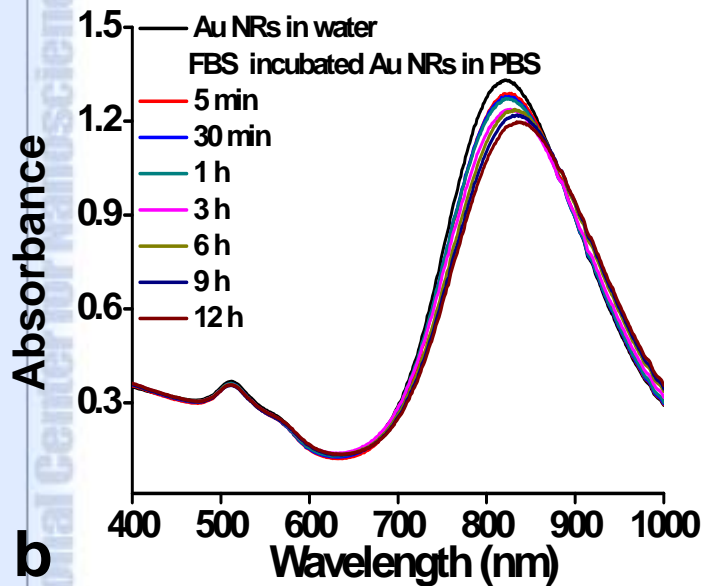


Fast Adsorption of serum proteins to Au NRs



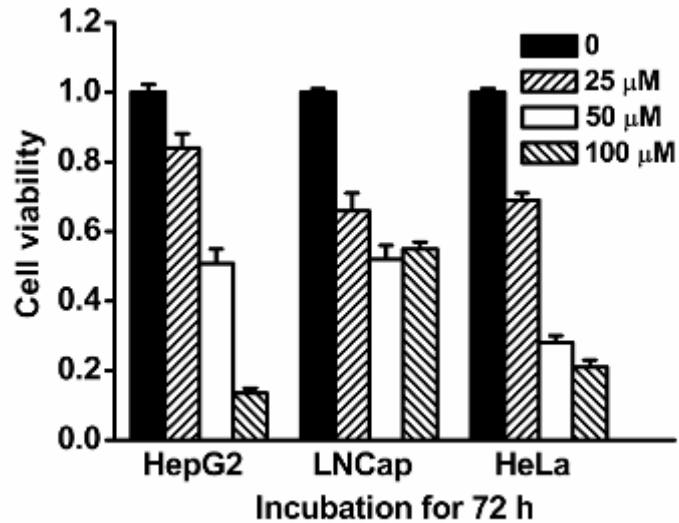
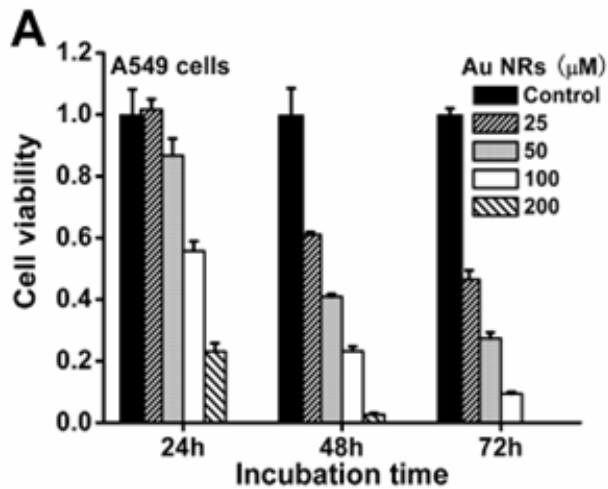
$(55.6 \pm 7.8) * (13.3 \pm 1.8) \text{ nm}$

A thicker layer around nanorod

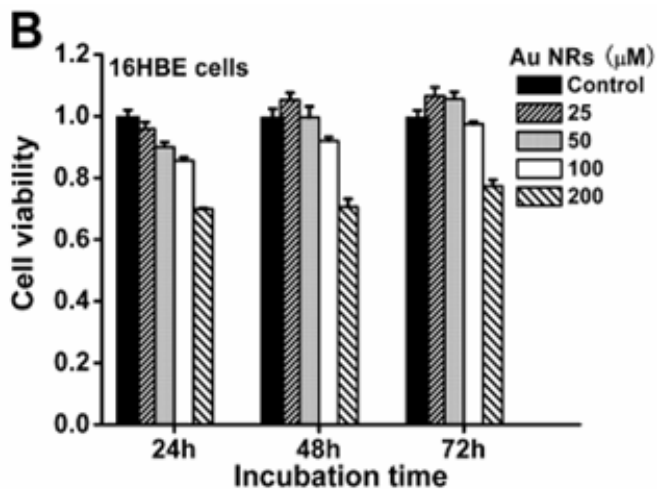


Serum protein adsorption can facilitate the internalization

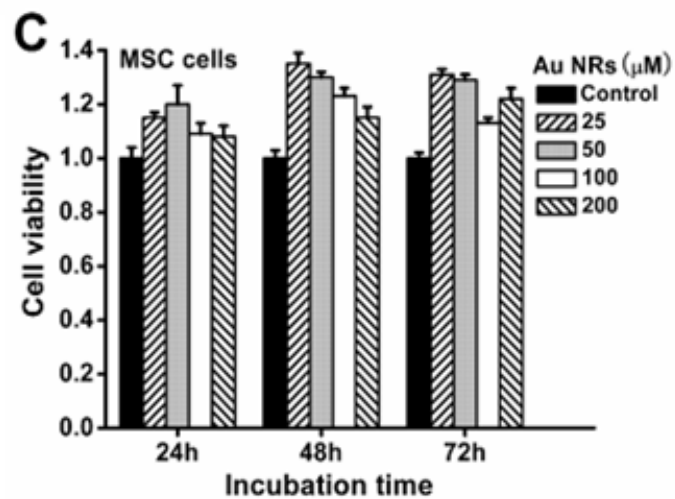
Specific responses to cancer cells



Cancer cells

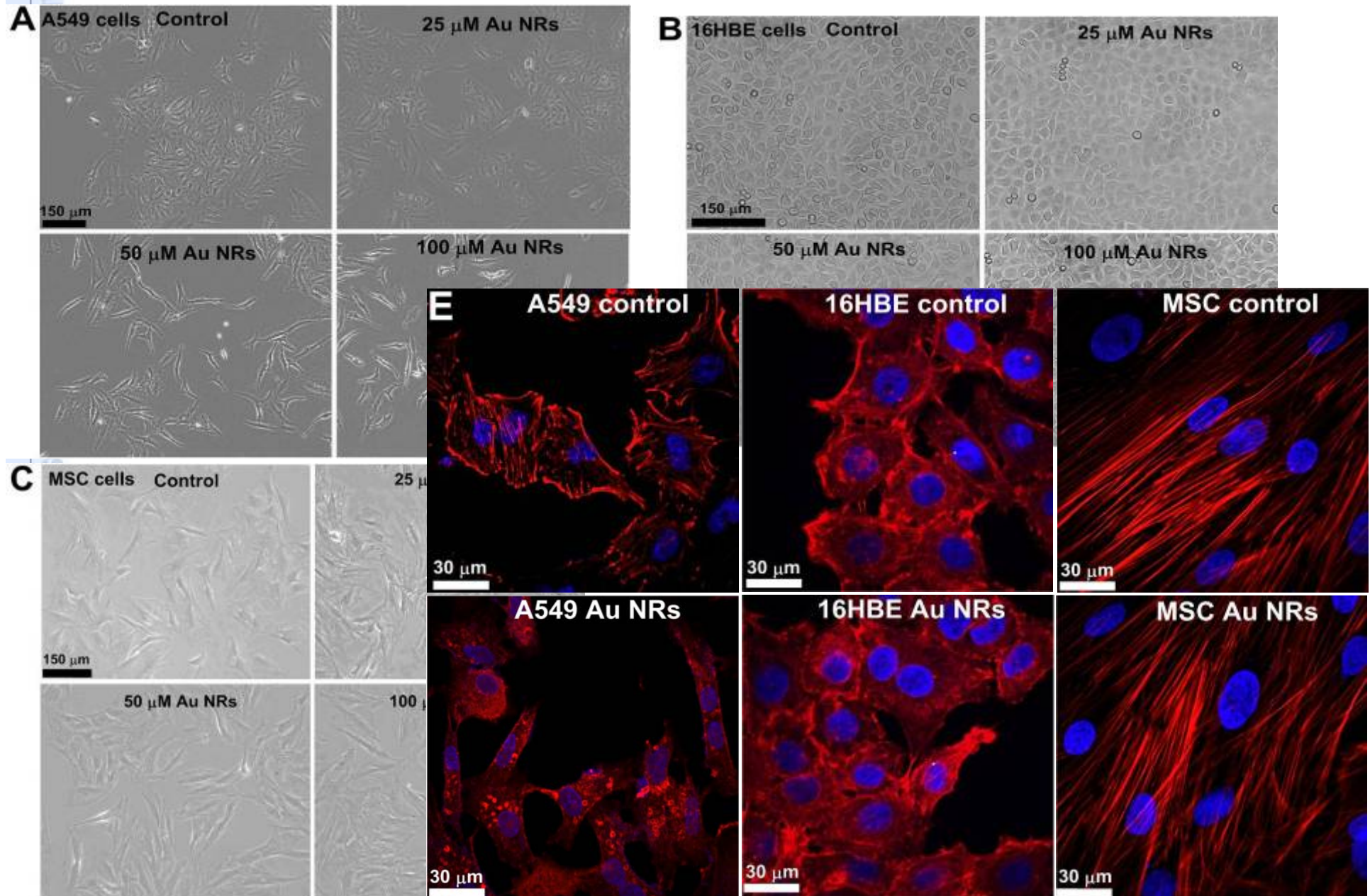


Normal cells



Stem cells

Changes in cell shape

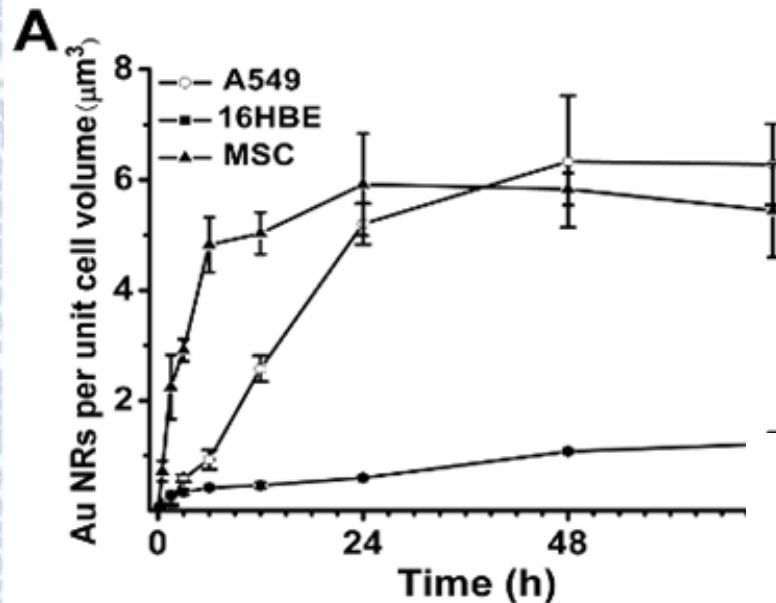




Why cell-specific responses?

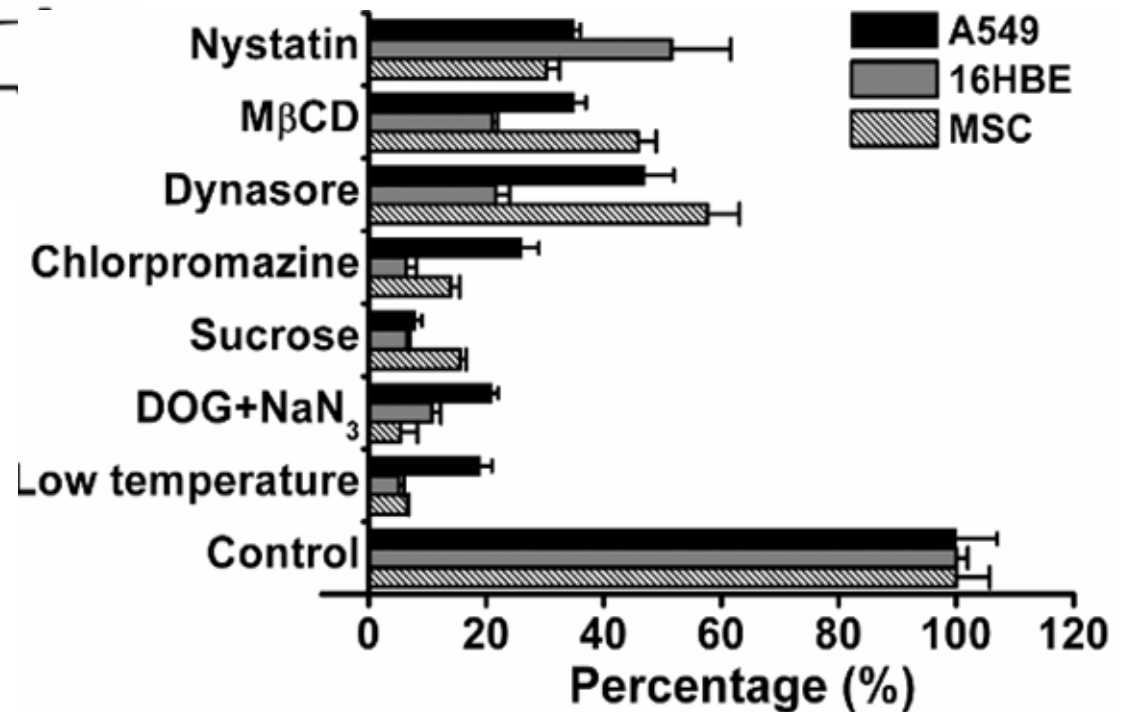
1. Internalized amounts of Au NRs?
2. Uptake pathways?
3. Intracellular trafficking?

Different Internalization of Au NRs

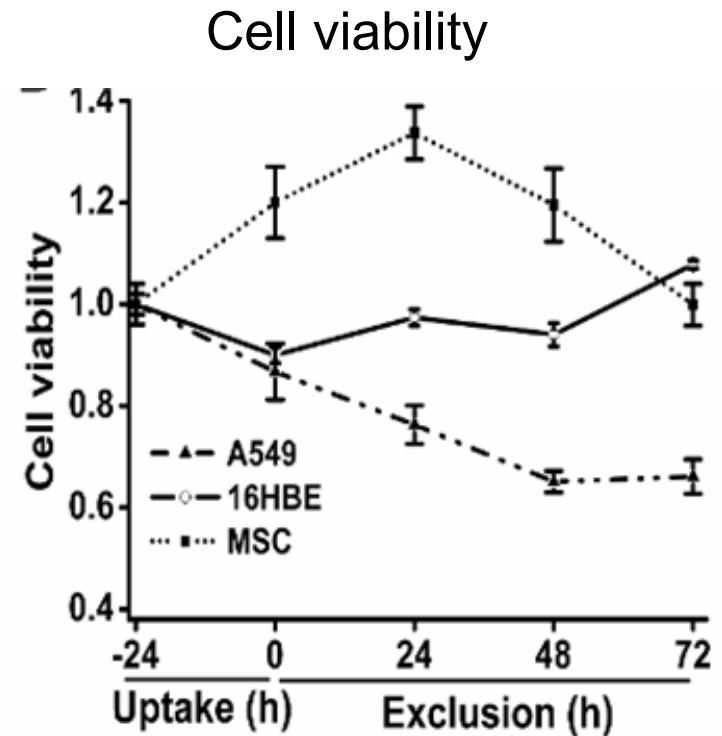
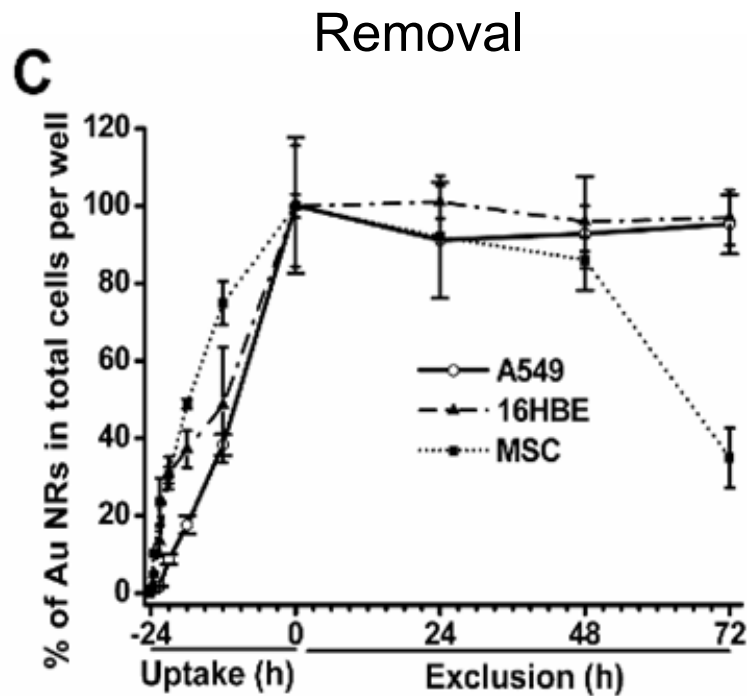


16HBE < A549 = MSC (cell volume)

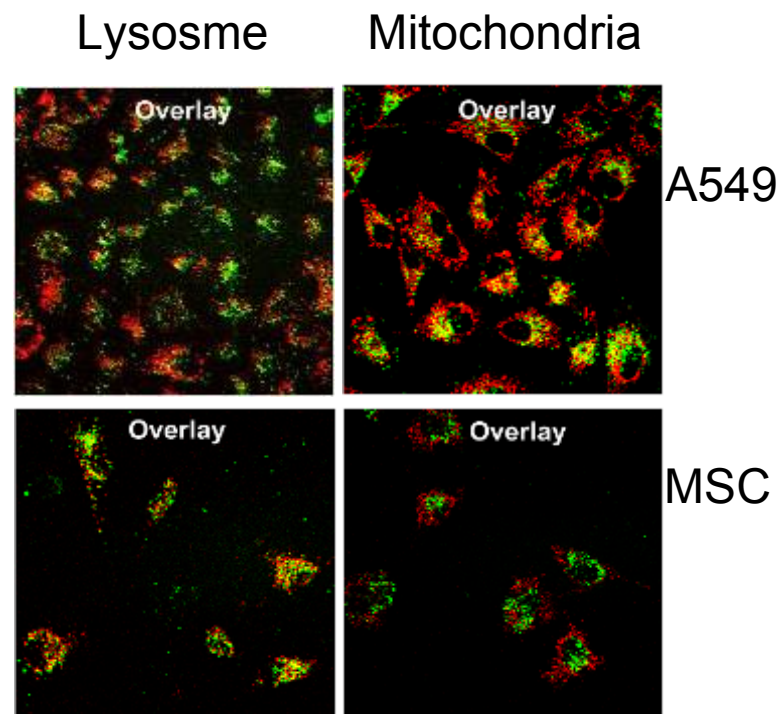
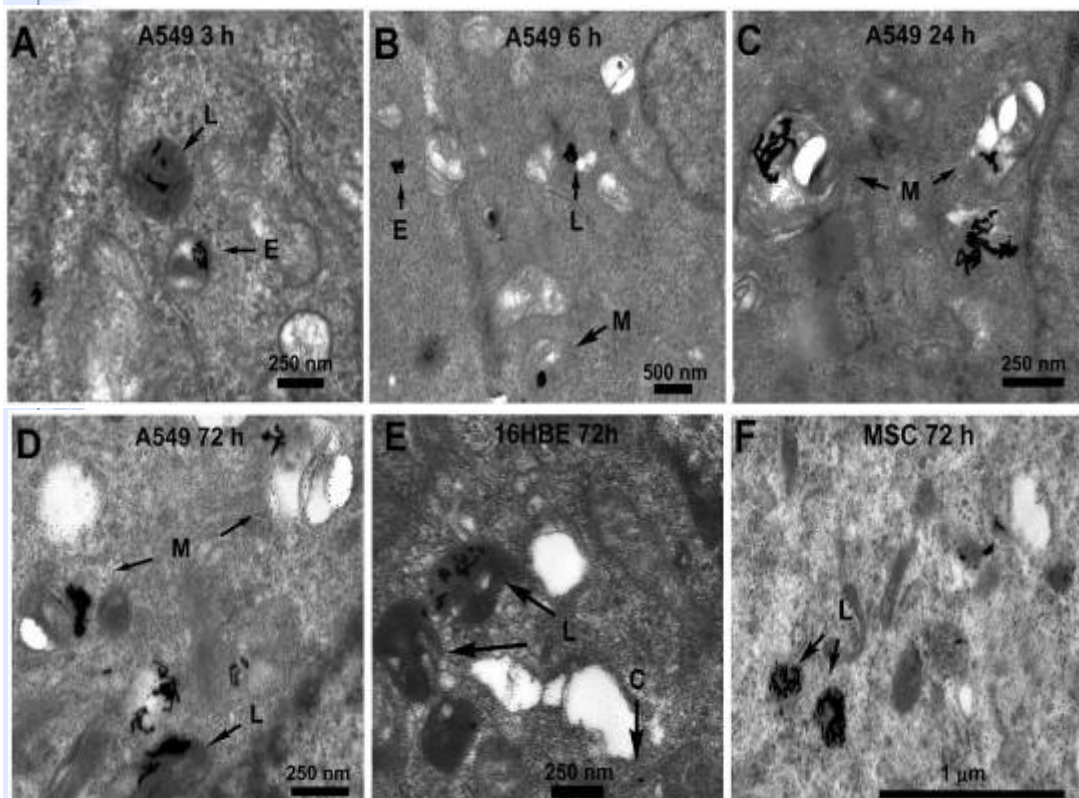
**Caveolin, Clathrin
and energy-dependent
endocytosis**



Different Removal of Au NRs



Intracellular localization



A549: mitochondria, lysosomes/endosome

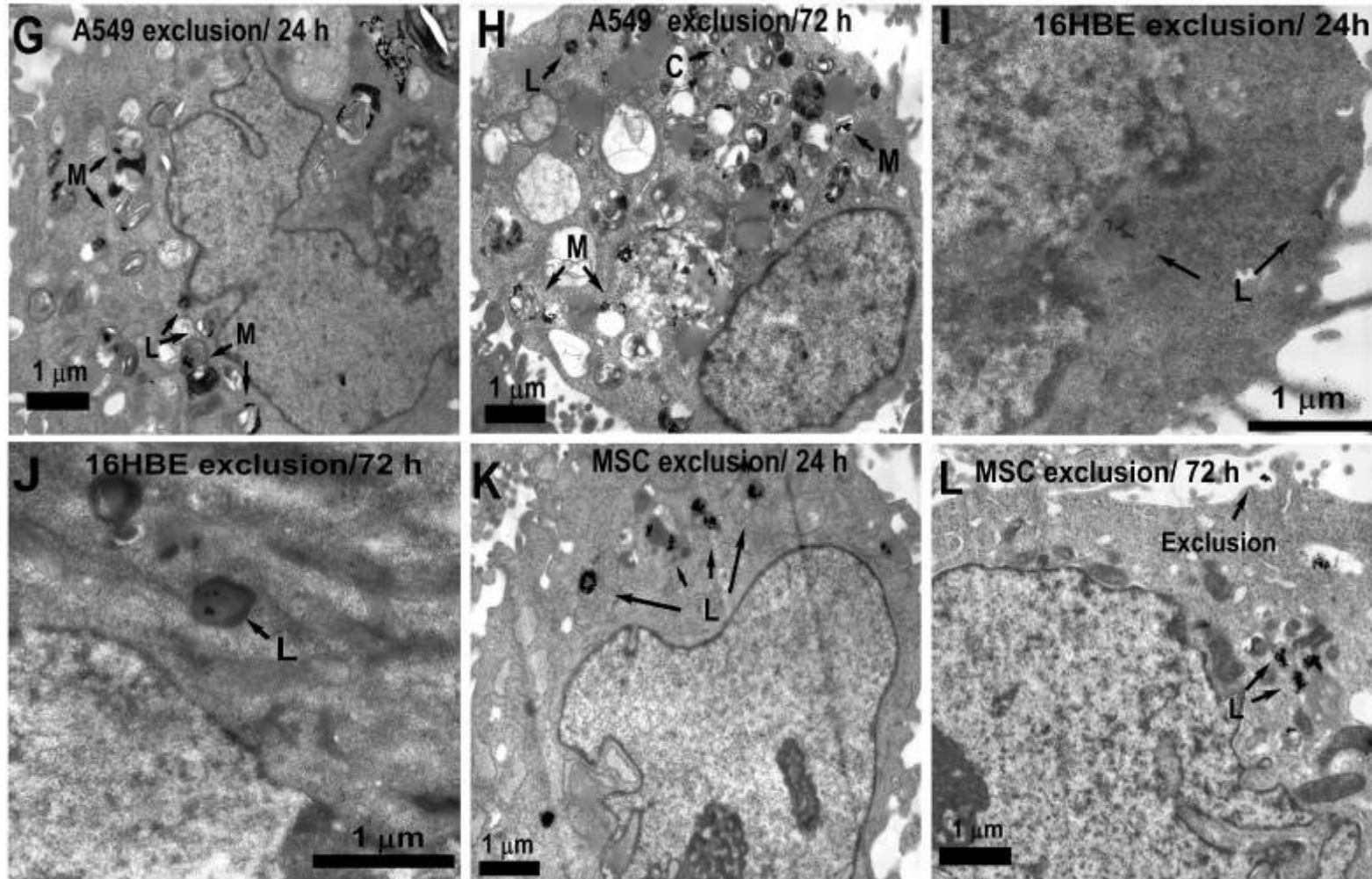
16HBE: lysosomes/endosome

MSC: lysosomes/endosome

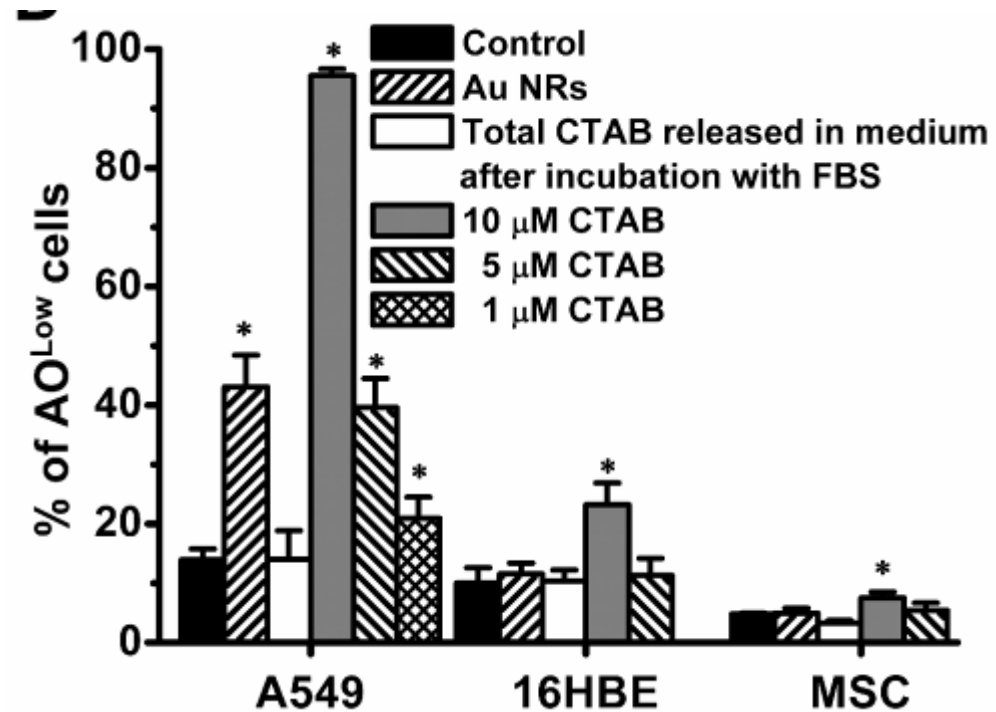
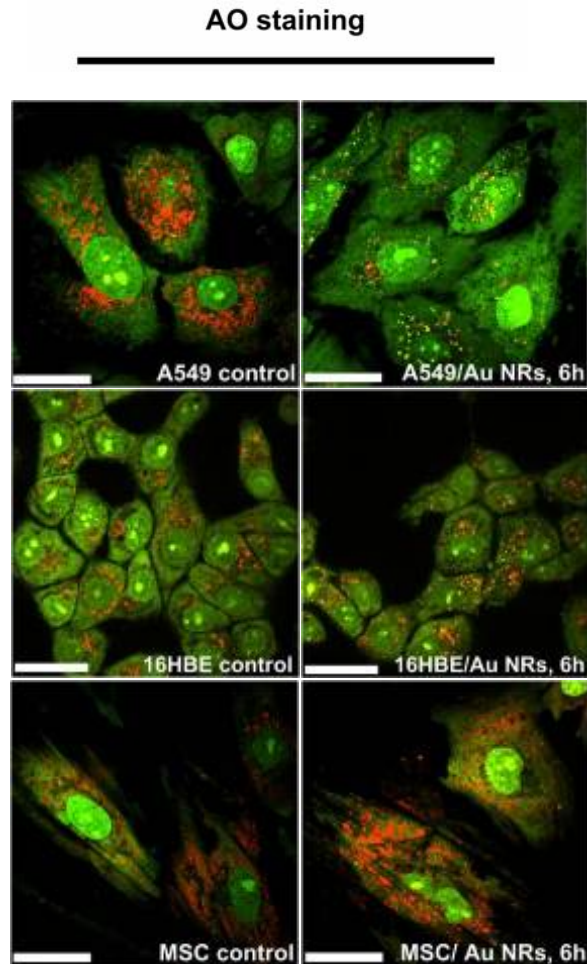
Wang, Chen, et al, Nano Letters, 2011, 11, 772–780



Different localization during exclusion in vitro



Increased lysosomal permeation by Au NRs in cancer cells

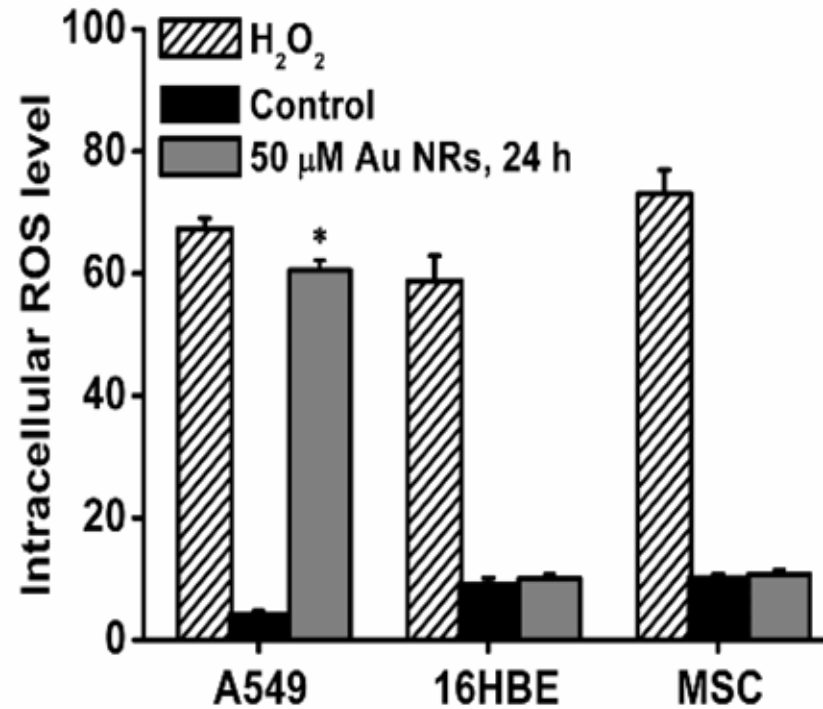
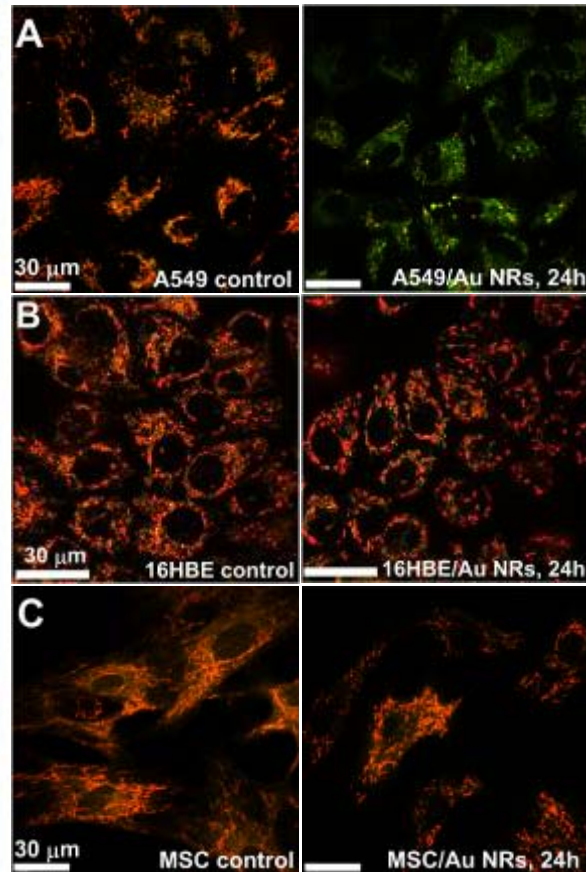


The integrity of lysosome

damage to the lysosomal membrane lead to further translocation of the Au NRs to other organelles

What is next for Au NRs?

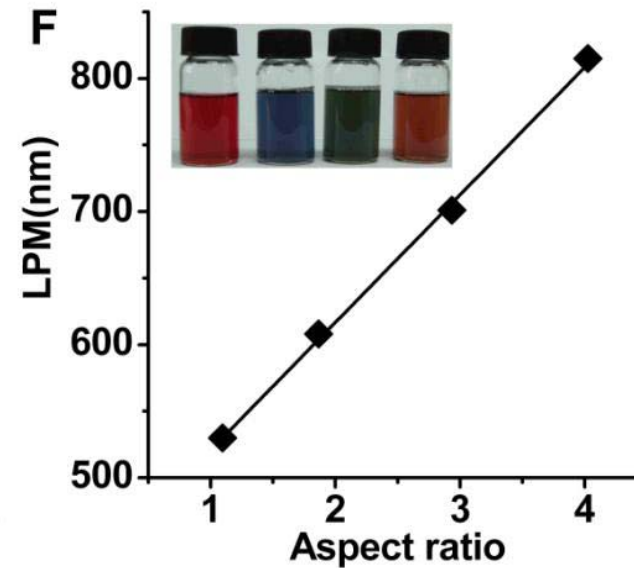
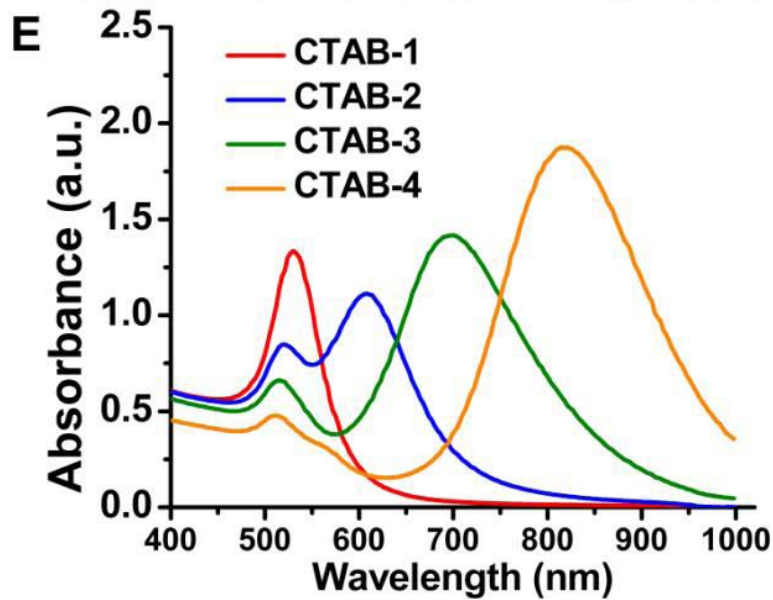
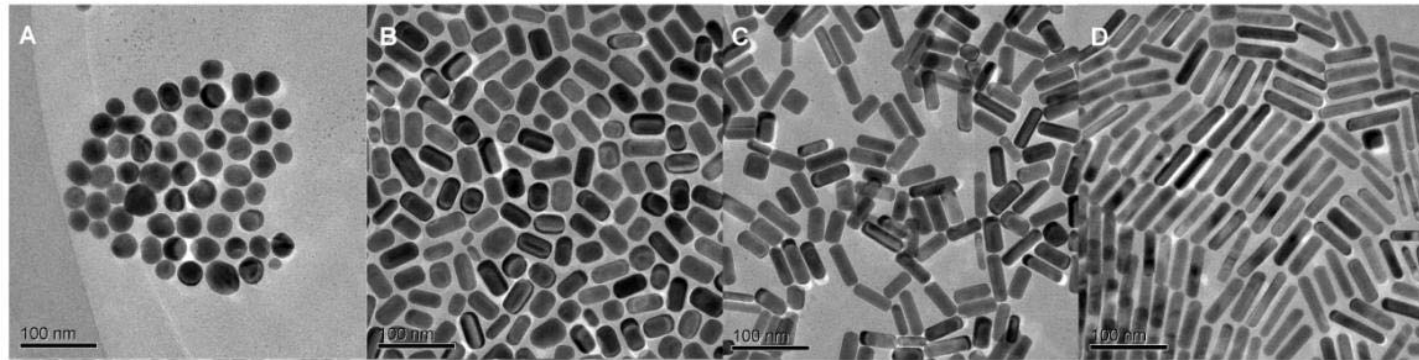
JC-1 staining



**For A549 cells,
Decrease in mitochondrial membrane potentials.
Increased intracellular ROS level**



Cellular uptake and cytotoxicity of Au nanorods: The influence of surface chemistry and aspect ratio



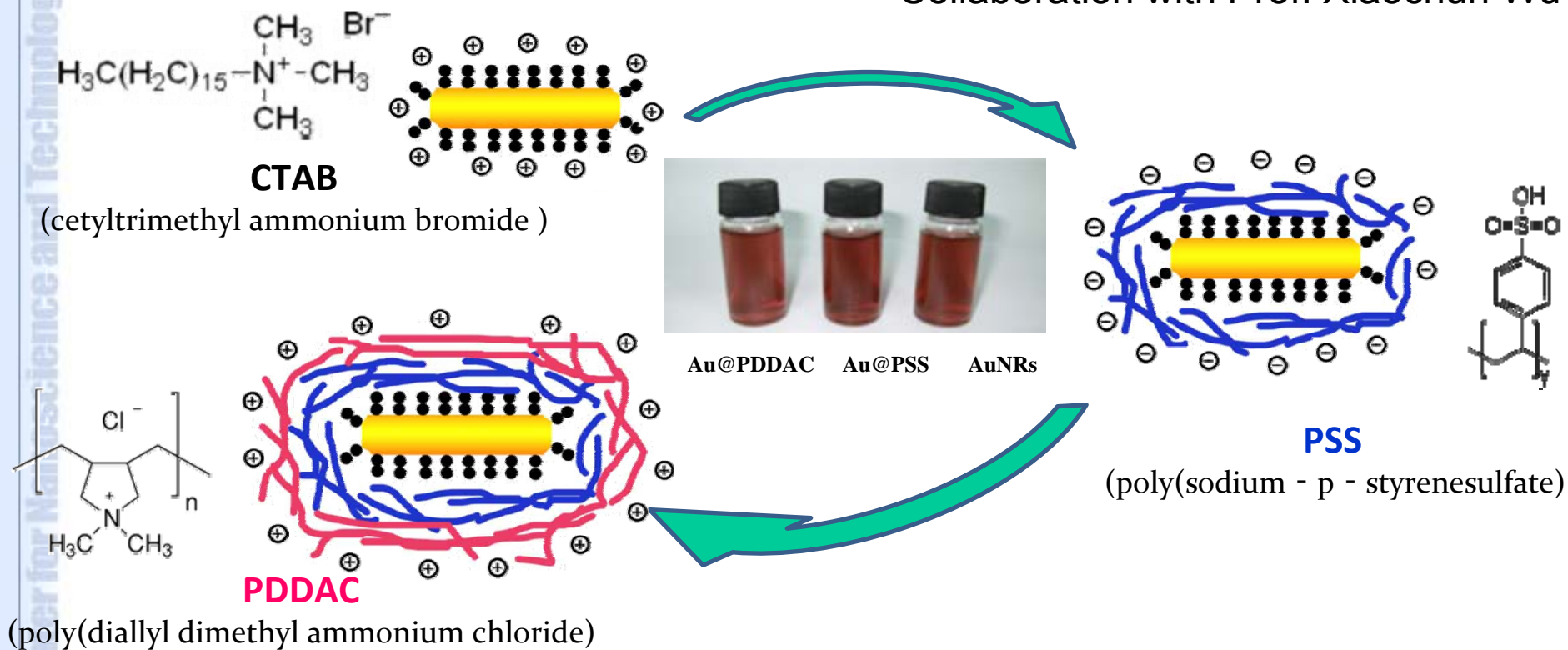
Calculated aspect ratio: 1.2, 2.0, 3.0, 4.0

The linear fitting of longitudinal plasmonic maximum to aspect ratio calculated from data based on TEM images.



Au NRs of different coatings and shape

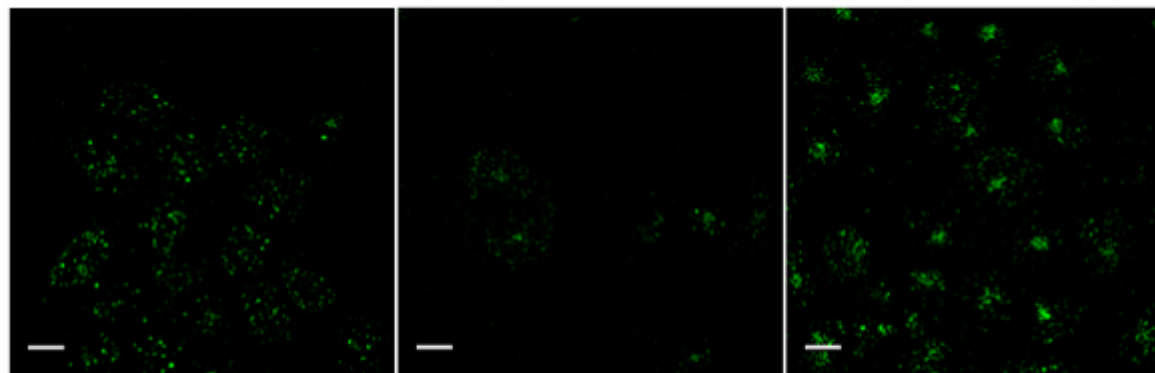
Collaboration with Prof. Xiaochun Wu



Aspect Ratio	1	2	3	4
Au NRs	CTAB-1	CTAB-2	CTAB-3	CTAB-4
	PSS-1	-	-	PSS-4
	PDDAC -1	-	-	PDDAC-4

Coating and Shape dependent cellular uptake

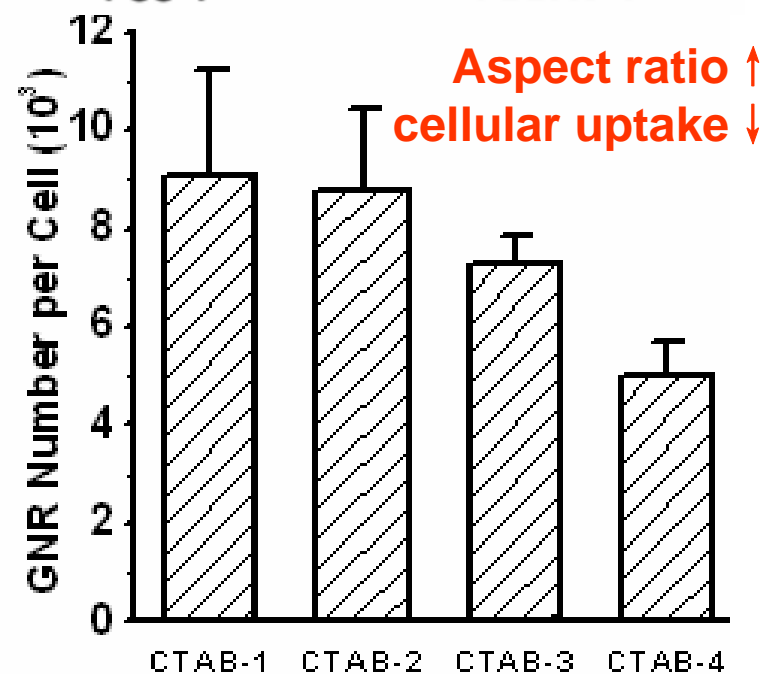
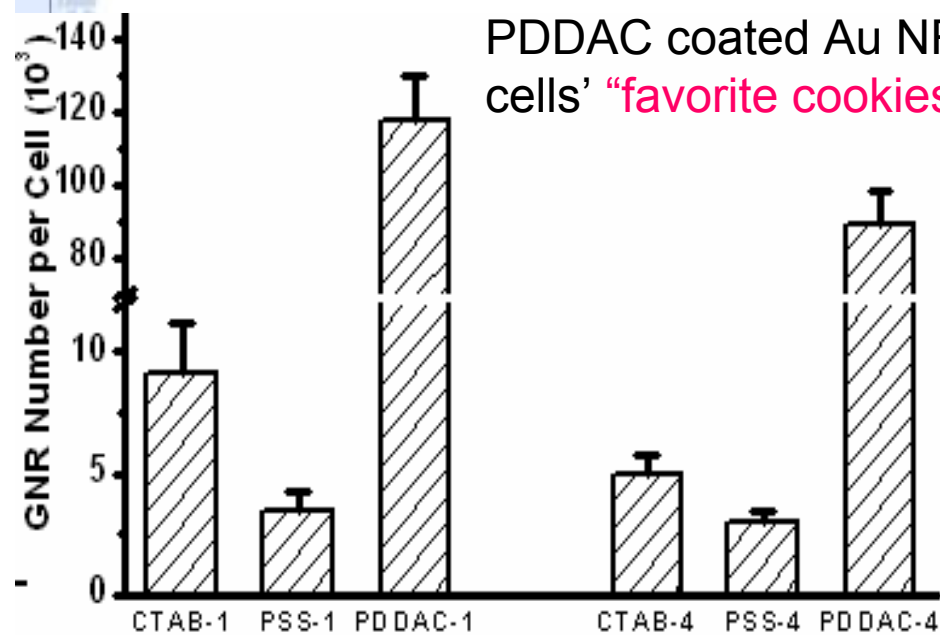
Two photonluminescence (TPL) images of Au NRs with different surface coatings in MCF-7 cells.



CTAB-4

PSS-4

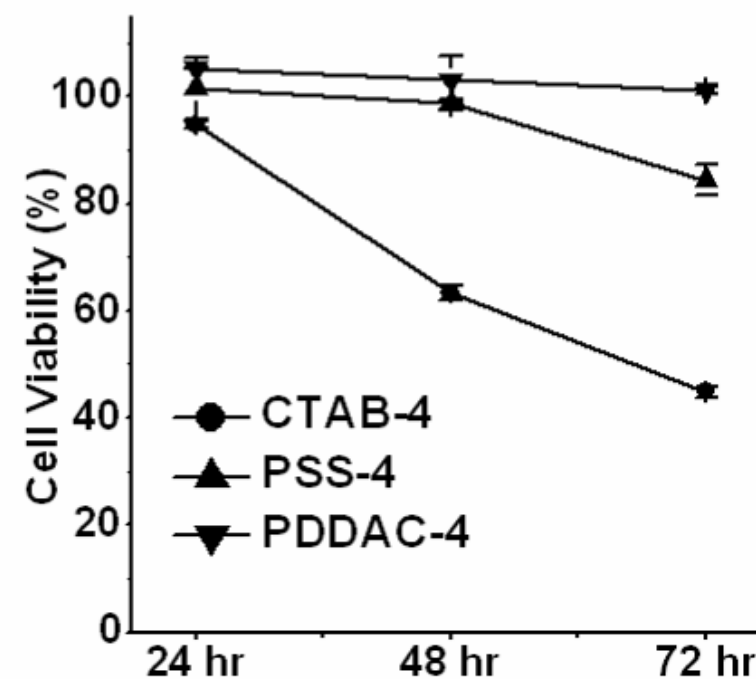
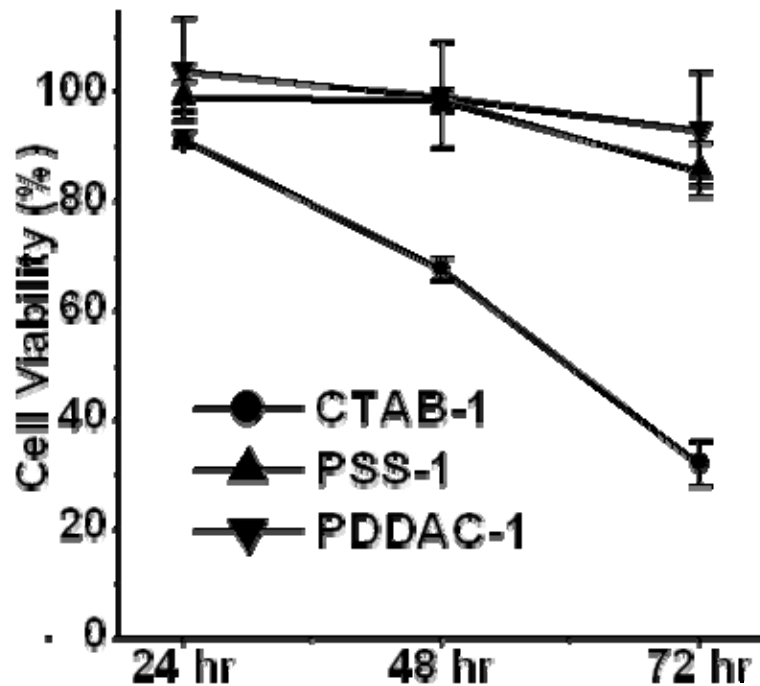
PDDAC-4





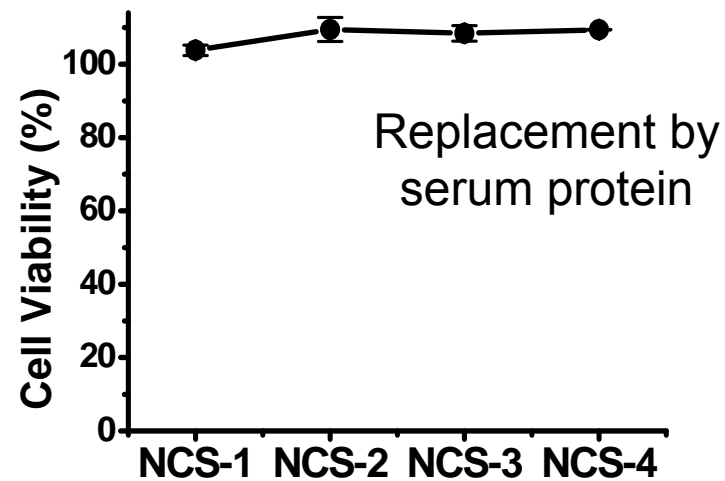
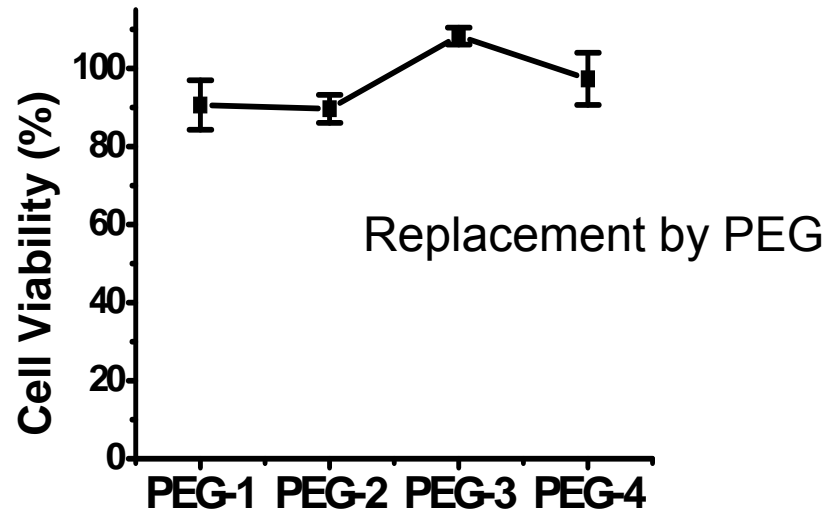
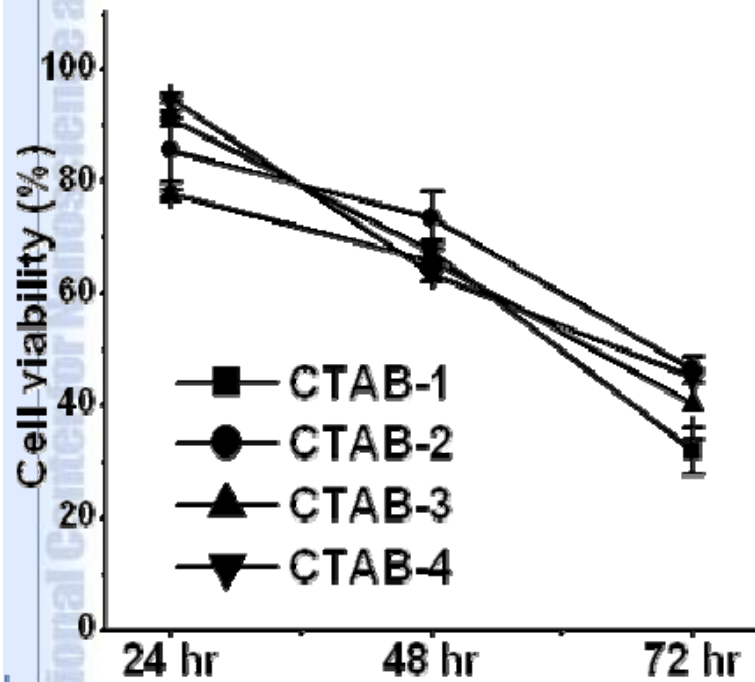
Surface coating dependent cytotoxicity

Surface coating can affect the cytotoxicity of Au NRs

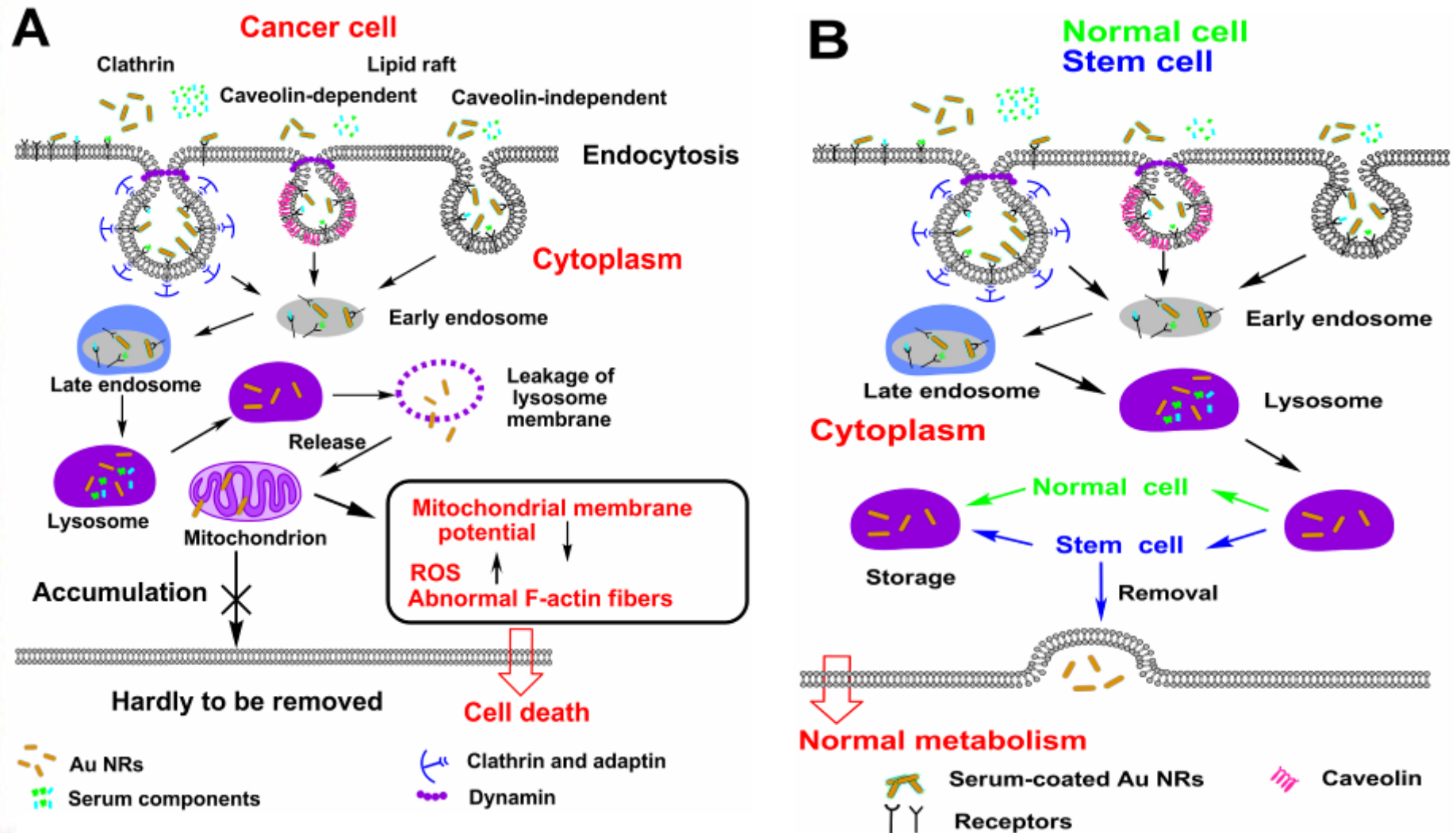


Shape independent cytotoxicity

Shape cause **no influence** upon the cytotoxicity of Au NRs with toxic or non-toxic coatings.



Selective Targeting of Gold Nanorods at the Mitochondria of Cancer Cells: Implications for Cancer Therapy





Full Assessment of Fate and Physiological Behavior of Nanomaterials in vivo

cell

Daphnia

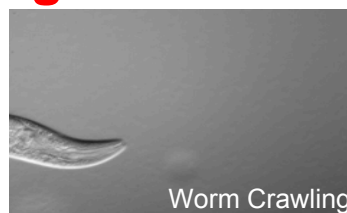
Drosophila

Zebra fish

Rat/mouse



C. Elegan



- ◆ Caenorhabditis elegans (C. elegans)
- ◆ Important model system
- ◆ About 1000 somatic cells.
- ◆ A life cycle of about 3 days
- ◆ The body length: 1 mm.

Metabolism

Uptake & accumulation
Distribution
Elimination

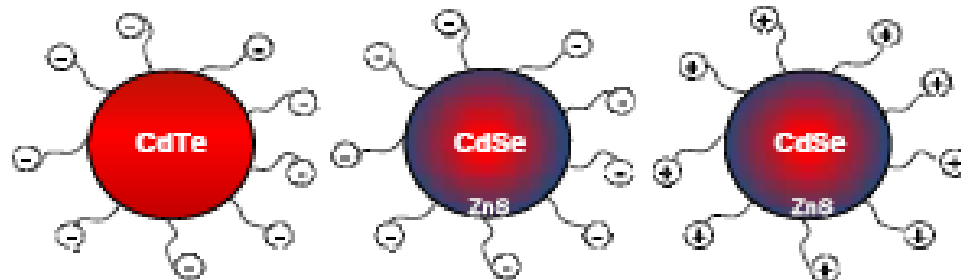
Toxicity

Lethality
Life span
Behavior

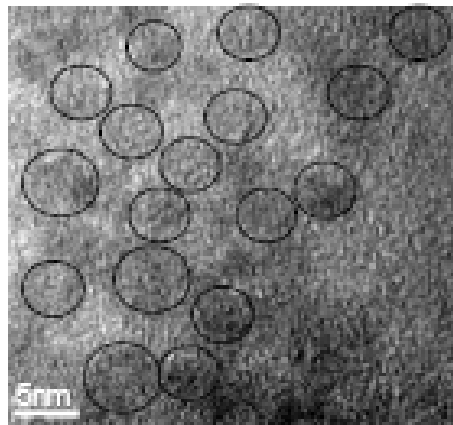


Comparison of toxicological effects of different types of QDs.

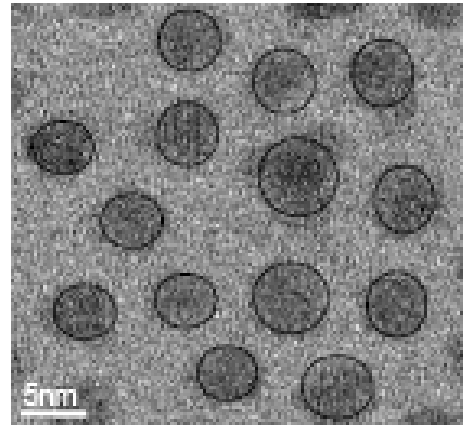
A MPA-CdTe MPA-CdSe@ZnS MEA-CdSe@ZnS



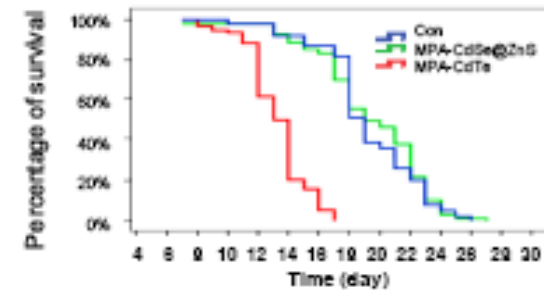
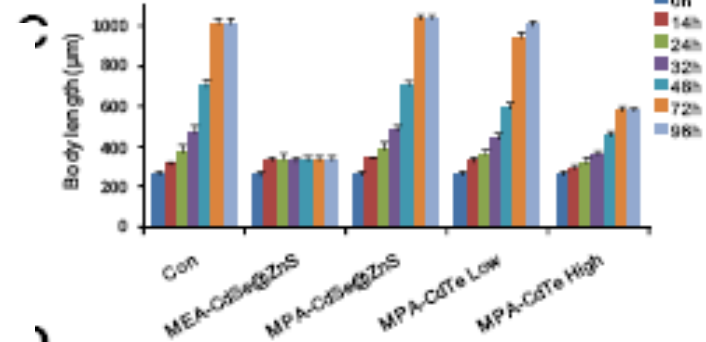
B MPA-CdTe



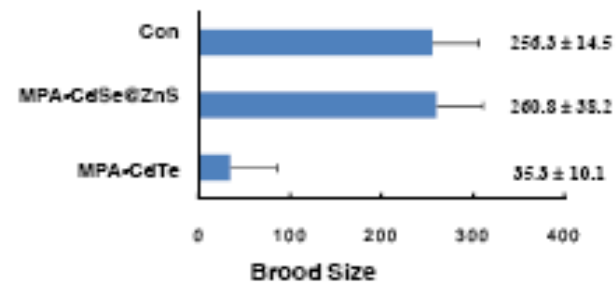
CdSe@ZnS



Larval development



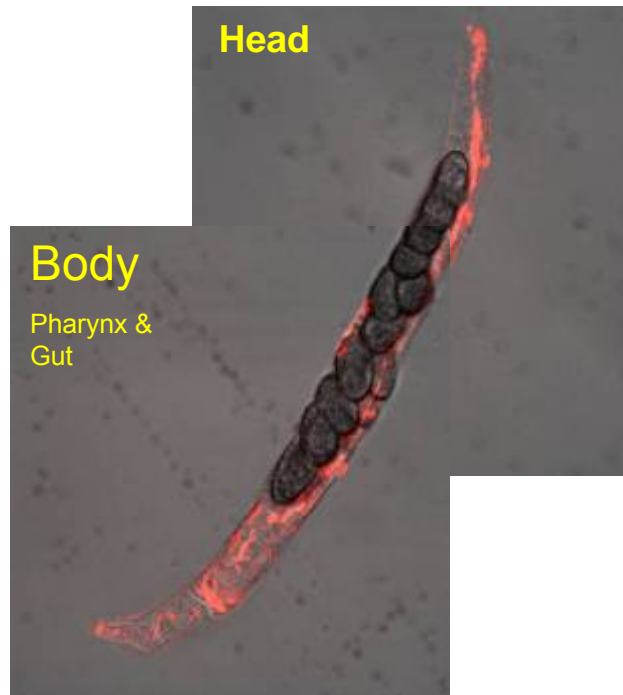
life span



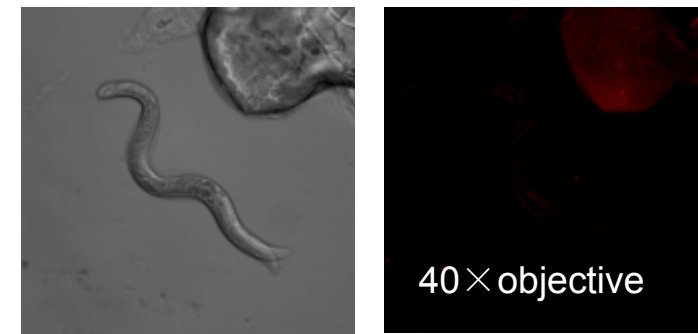
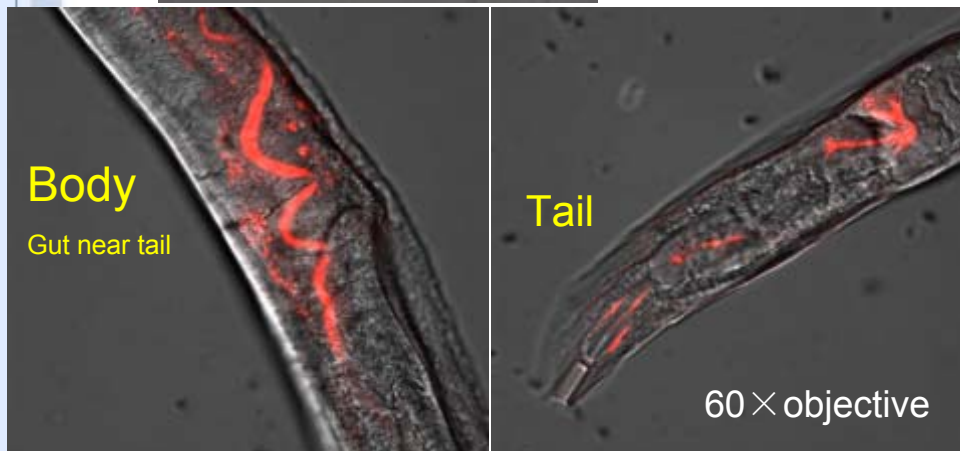
brood size

Body distribution of QDs in C. Elegans

Material: 620 nm QDs (CdTe MPA)



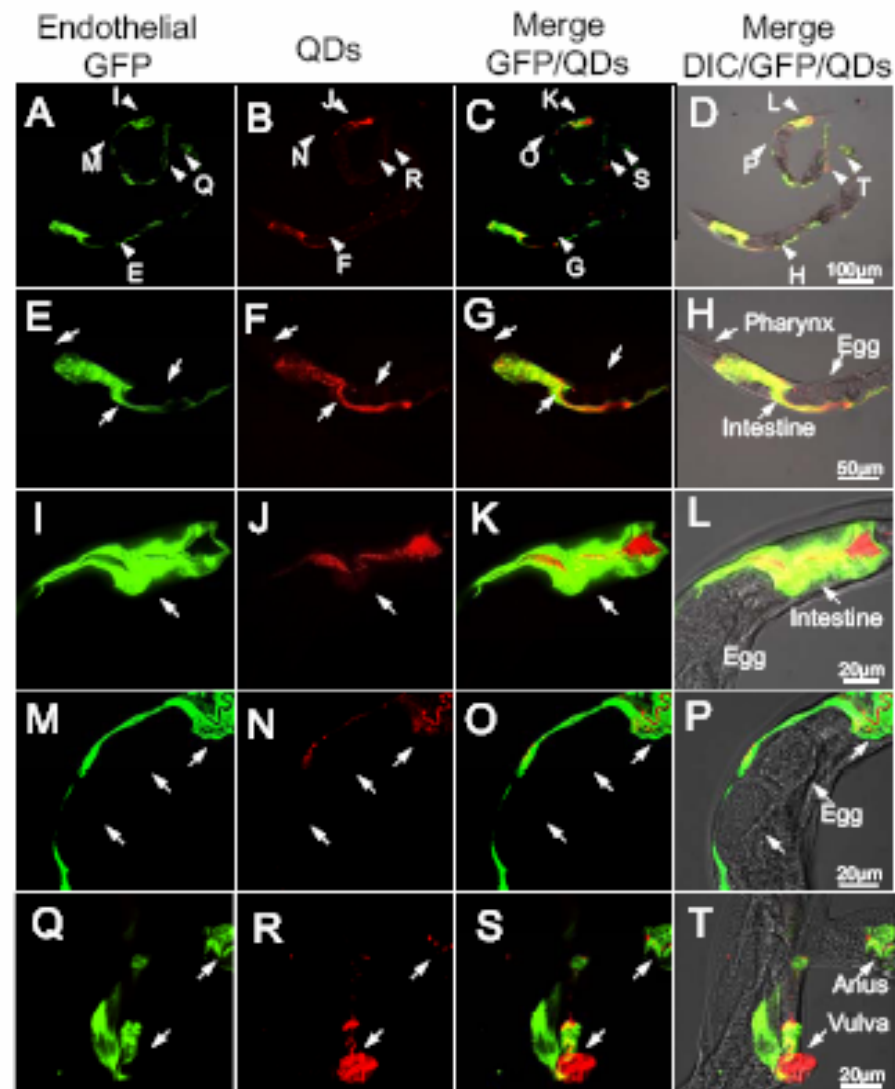
Treatment: Adult feed with live OP50;
 exposed 36h, wash and move to clean plate;
 4day (96h) wash and move to clean plate;
 4.5 day(108h) confocal image;



QDs cannot enter eggs and neonatal lava



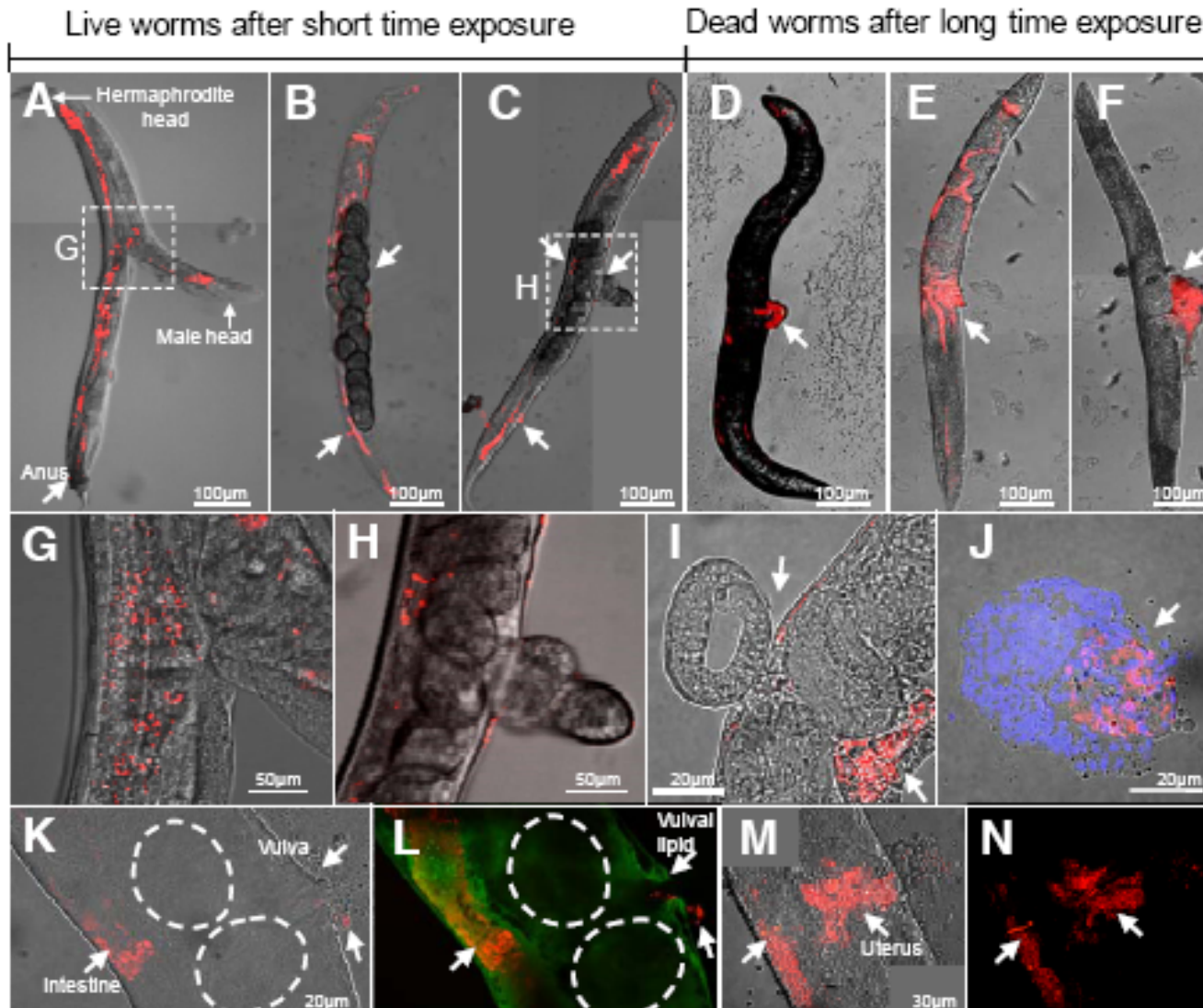
QD distribution in intestinal GFP-labeled *C. elegans*.



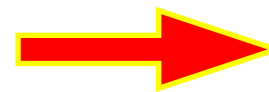
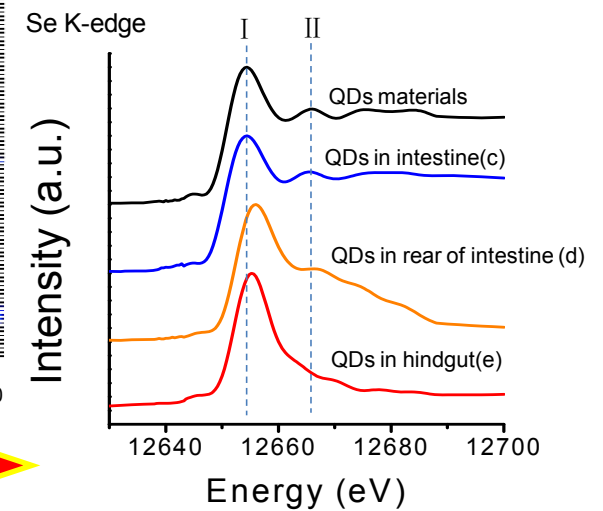
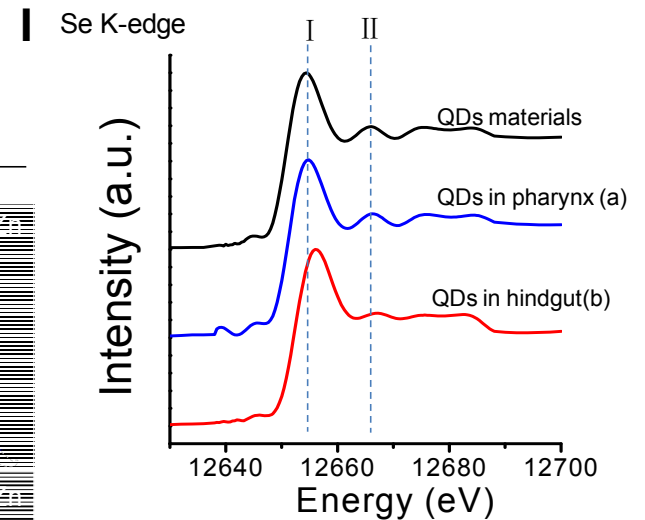
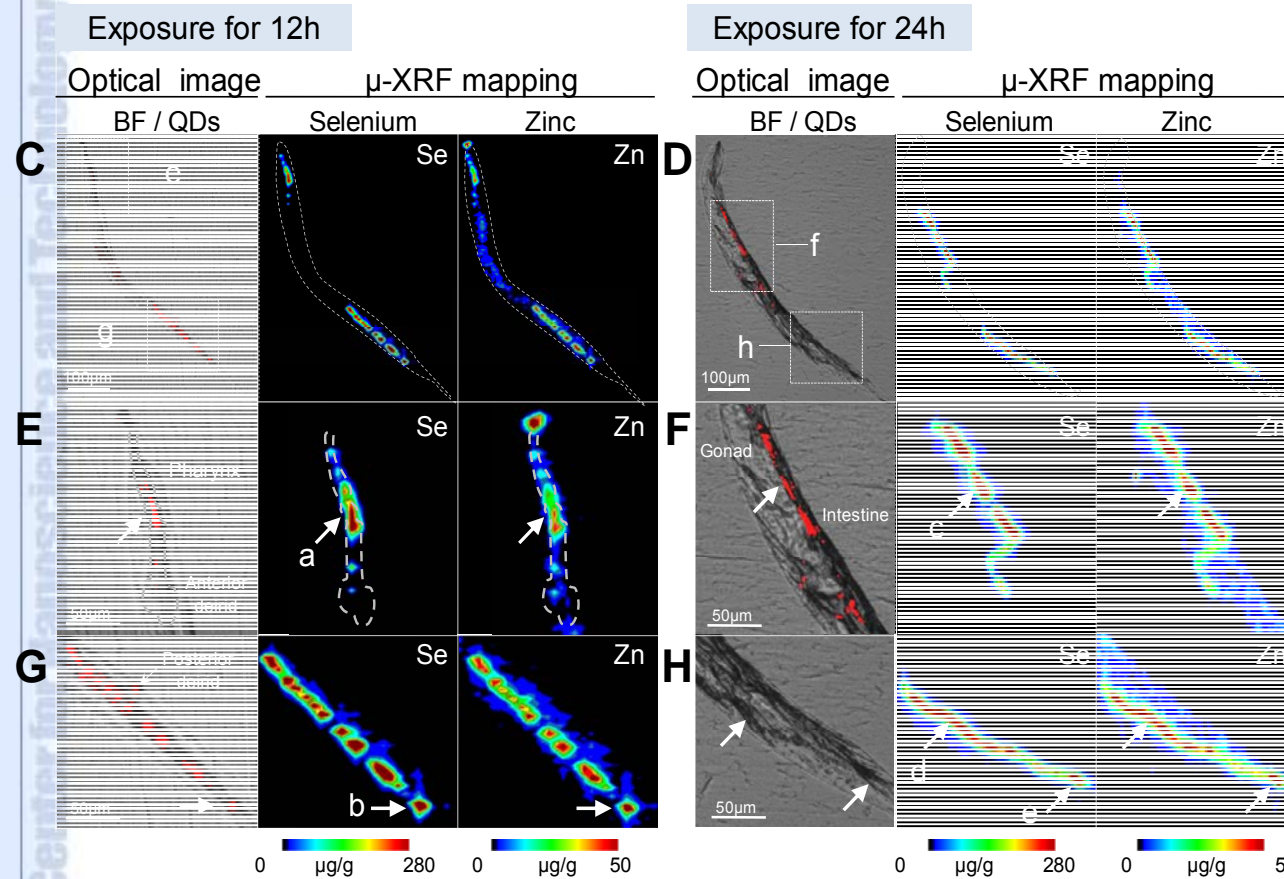
Qu, Tang, Chen, et al. Nano Lett, 2011



Reproductive behavior and egg-laying difficulty after long time exposure.



In situ elemental analysis and degradation of QDs





Understanding Interactions of Nanoscale Materials with Biological Systems

- **Nanotoxicology is a new and highlighted field, which opens a great opportunity and challenge to chemist, biologist, and toxicologist.**
- **The rules are different for living matters when materials become nanoscale. Some concepts of traditional toxicology need to be modified in nanotoxicology.**
- **The ability of NPs for biological barriers**
- **Key factors influence the nano-bio interactions.**
 - **All studies are a function of particle size, size distribution, shape, surface coating, pH, reactivity, vehicles, agglomeration / aggregation.....**
- **More issues will be taken into consideration**



Models for Risk Evaluation of Nanoparticle Exposure

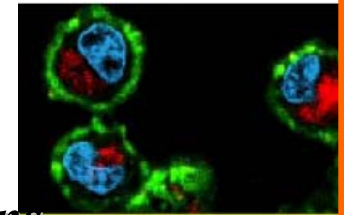
In vivo testing

- Bronchia Instillation
- Bronchia Injection (without surgery)
- Nasal Instillation
- Inhalation (ambient air)
- Blood vein injection
- Oral gavage



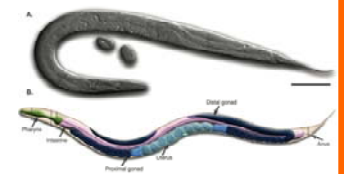
High-throughput screening

Primary and cell lines



The nematode *C. elegans*

about 1000 somatic cells.
a life cycle of about 3 d
1 mm



Drosophila



Daphnia magna



Zebra fish





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- **National Nature and Science foundation of China (NSFC)**
- **Chinese Academy of Sciences (CAS)**
- **Ministry of Science and Technology of China (MOST)**





中国科学院
CHINESE ACADEMY OF SCIENCES

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Fang LAO, PhD
Ying LIU, Dr
Yang QIU

.....

Institute of High Energy Physics, CAS

Yuliang ZHAO, Prof.
Zhifang CHAI, Prof.
Wei LI, PhD
Jiangxue WANG, PhD
Yuxi CAO, Dr.
.....
..... PhD
..... nician



Nanotoxicology 2012

Sept 4-7, Beijing, China

Nanotoxicology 2012



History

2012, the 6th NT Conf., Beijing, China

2010, the 5th NT Conf., UK

2008, the 4th NT Conf., Switzerland

2007, the 3rd NT Conf., Italy

2006, the 2nd NT Conf., USA

2005, the 1st NT Conf., USA

Contact

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Email: nanotoxicology@nanoctr.cn

Tel: +86-10-82545526/

Fax: +86-10-62656765

National Center for Nanoscience and Technology, China
No.11, Beiyitiao Zhongguancun 100190 Beijing

Steering Committee Chair: Prof. Yuanfang Liu

Conference Chair: Prof. Yuliang Zhao

Secretary General: Prof. Chunying Chen

Organizers: National Center for Nanoscience and Technology, China

Supported by: MOST 973 program,
National Natural Science Foundation of China,
Chinese Academy of Sciences,
Chinese Society of Toxicology

Programme: The conference will be divided into sessions that focus on specific topics of all sciences for nano-bio interfaces. The paper presented at the conference will be published at a peer-reviewed SCI journal of nano-field.

Theme Covered: it includes but not limits to:
Nanotoxicology, Nanobiotechnology, Nanomedicine,
Bio-nanomaterials, Nanoecology, Nanochemistry,
Nano standardization, etc.



Technology, China

Thank you!



<http://www.nanoctr.cn>
<http://nanosafety.ihep.ac.cn>

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