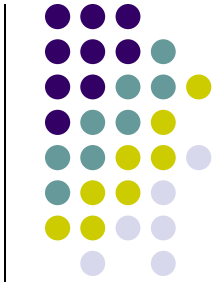
Nanomaterials EH&S – A Perspective



Panel 6 “Transformations in the organisms and in the environment: what do we measure and how do we develop testing strategies to measure impacts of particles that may be transformed over time in the environment”

Lisa DeLouise, PhD

University of Rochester Medical Center

Departments of Dermatology and Biomedical Engineering

NNI Workshop on Nanomaterials and the Environment and Instrumentation

October 6-7, 2009

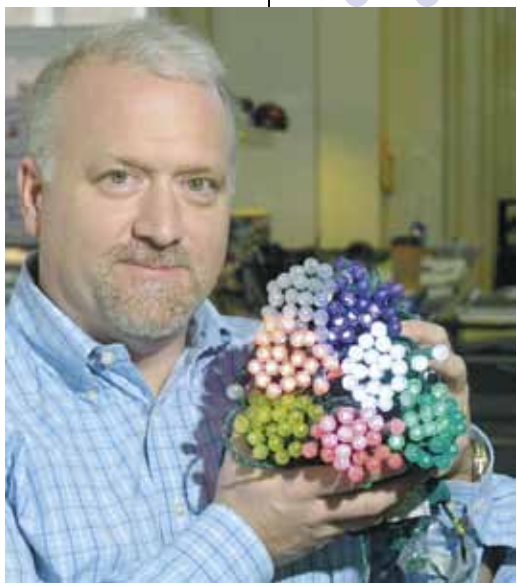
State of the Science

Exposure?

- Research should be guided by materials that are likely to be economically important

- Obviously has driven EPA top 7
  - single-walled carbon nanotubes,
  - multi-walled carbon nanotubes,
  - fullerenes,
  - cerium oxide,
  - silver,
  - titanium dioxide,
  - zero-valent iron.

The Problem is Much Bigger



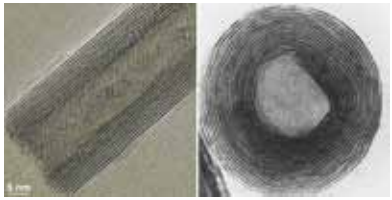
Clint Ballinger, CEO of Evident Technologies holds a selection of LED lights in this 2007 file photo.

(Photo by J.S. Carras)

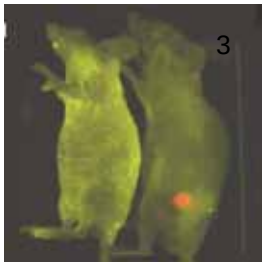
2008 We'd like to introduce you to one of Evident's newest products - dotstrandTM Energy Efficient LED Lights - **the world's first consumer product to utilize quantum dot technology.**

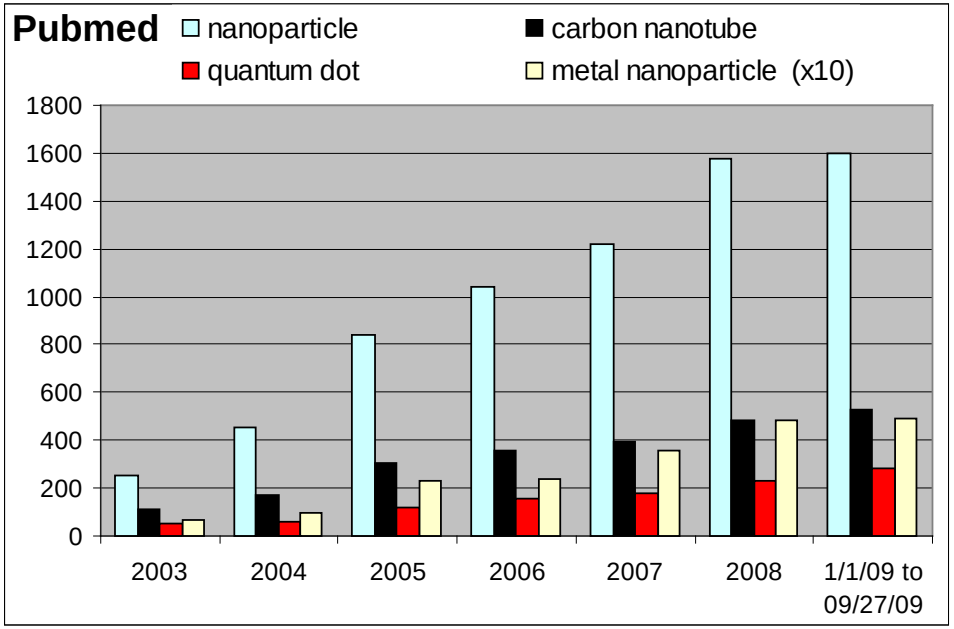
Nanomaterials in Research

- From published literature it is clear the NP research increasing CNT 2x QD
- How to quantify exposure in academics and industrial R&D?



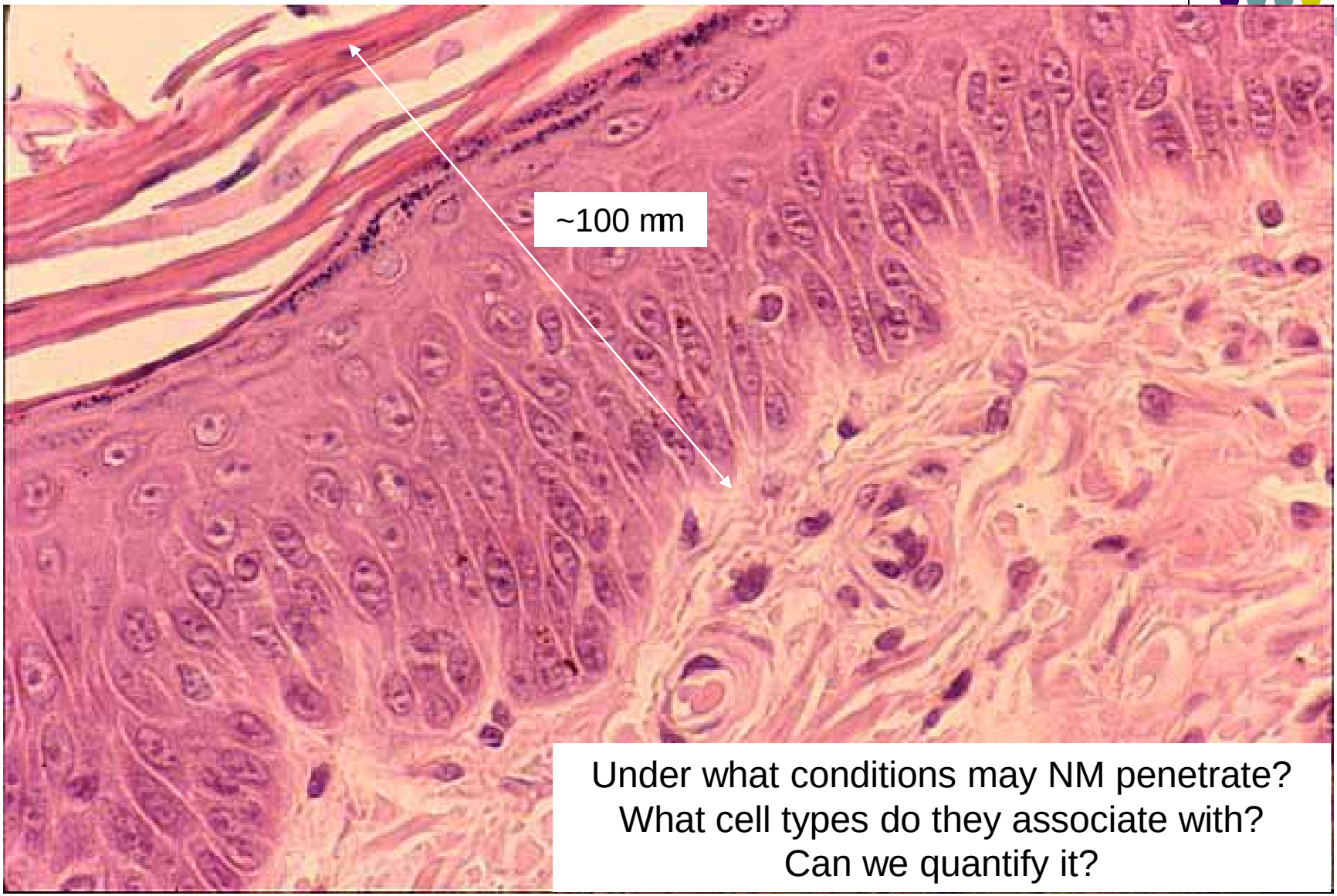
2-10 nm





Can they penetrate through my gloves?

Histology - Healthy Human Epidermis

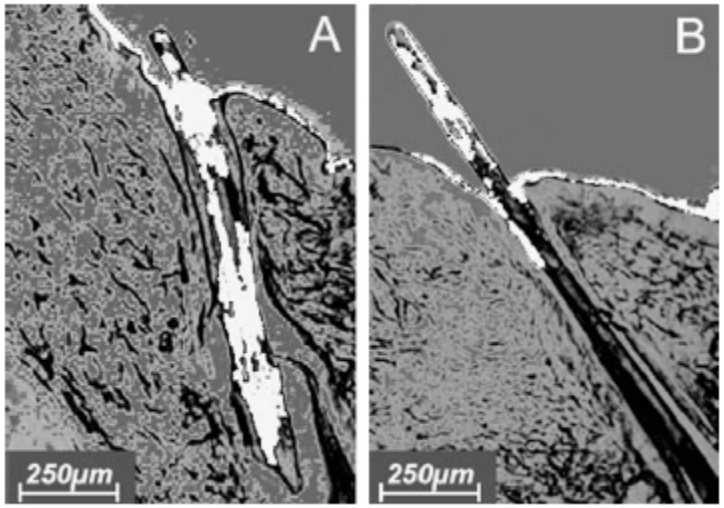


Under what conditions may NM penetrate?
What cell types do they associate with?
Can we quantify it?

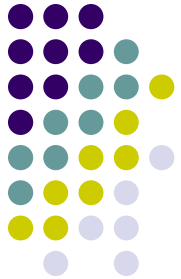
Skin Conditions Vary

- Different skin types (light/dark)
- Follicular density
- Different barrier defects
  - Mechanical cuts
  - Chemicals
  - Environmental (UV, microbes, allergens)
  - Disease

Lademann 2006



Hypotheses:



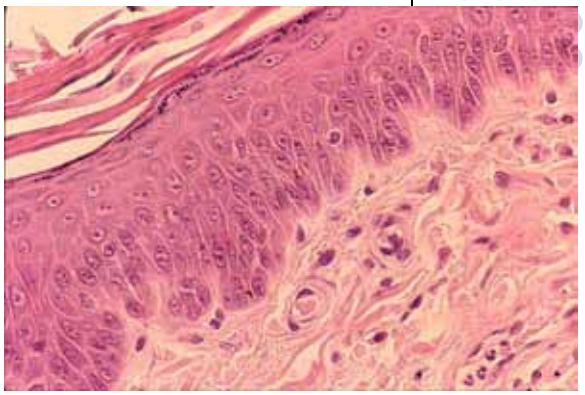
1. Size, shape, charge and surface energy are key NM properties that determine epithelial penetration upon first exposure
2. Composition, dissolution properties, and translocation determine toxicity secondarily

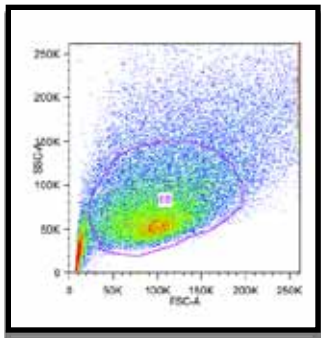
Are current methods & models sufficient to prove this? – No

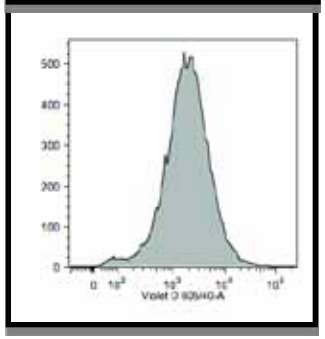
State of the Science

Skin Barrier Status and NM Detection Techniques

- Histology / Immunofluorescence
  - Slicing may introduce artifacts, slow
  - Background autofluorescence
- Transepidermal water loss (TEWL)
  - Only accepted method
  - Measures inside-out barrier only
- Franz/Ussing Diffusion Studies
- TEM
  - Limited tissue analysis, slow, expensive
  - Skin structures are also dark and small
  - Need amplification strategy
- Flow cytometry (FACS)
  - Quantitative with good statistics
  - Destructive
- NIR microscopy
  - Major innovation and future

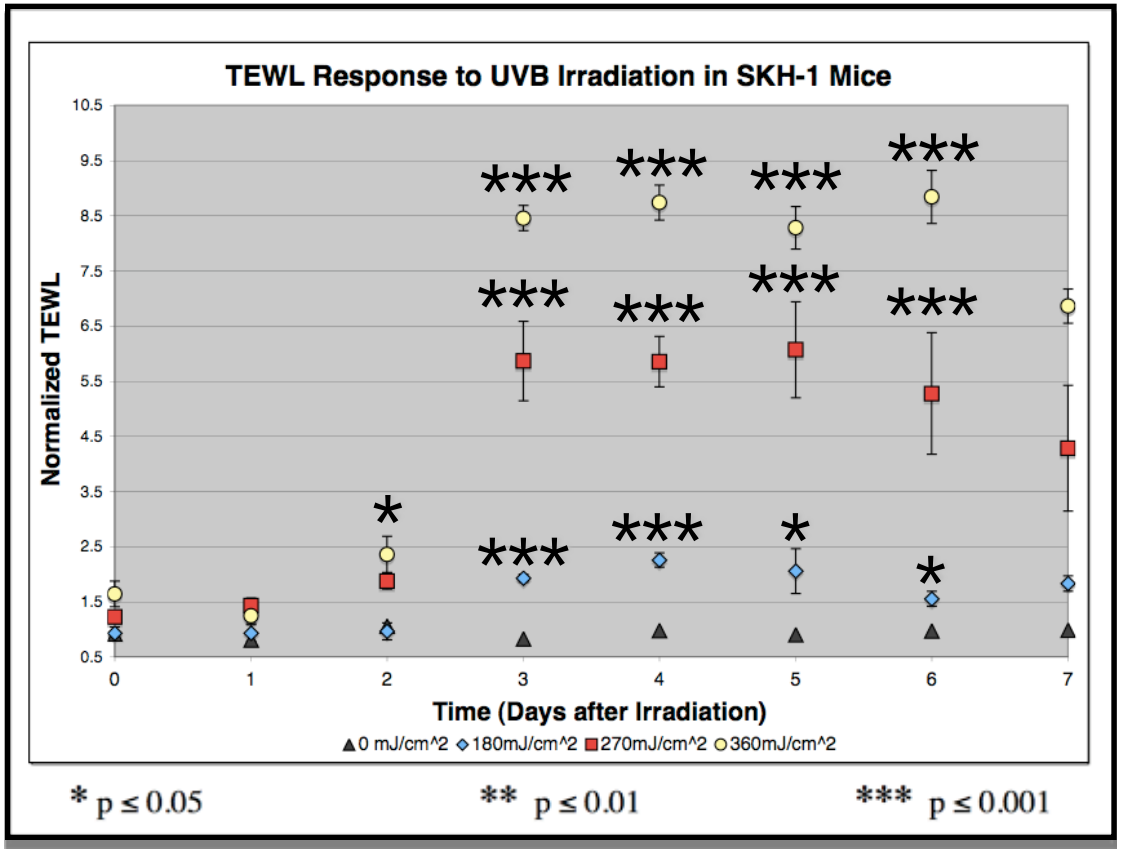






http://www.odakecza.com/bilder/reviscisondarm.jpg

TEWL - UVB Induced Barrier Damage

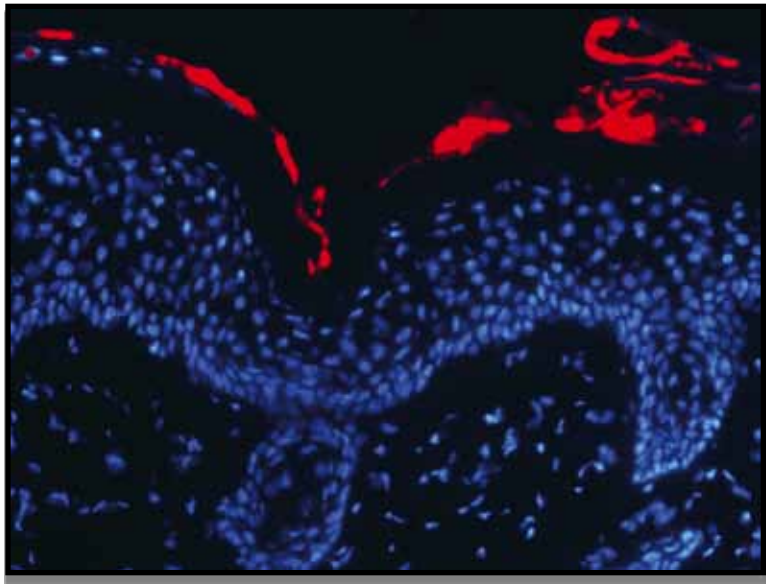


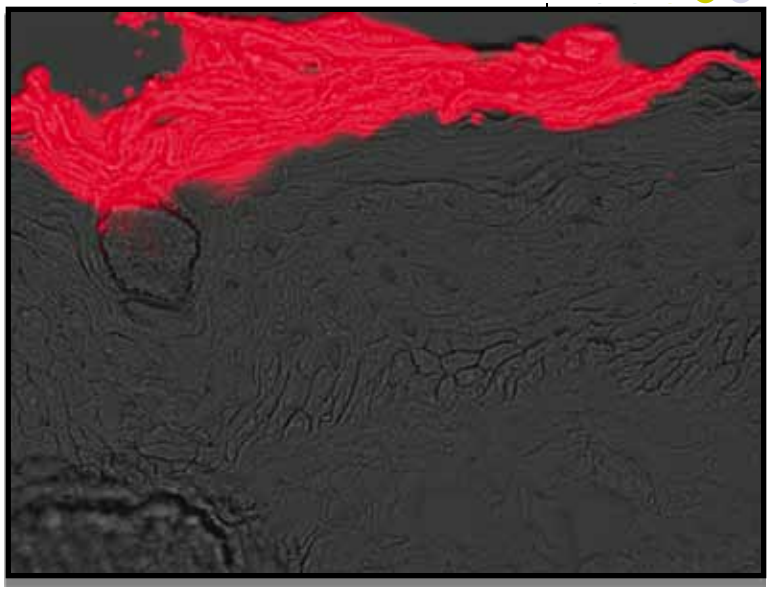
- UVB induced barrier impairment is quantifiable with TEWL quantified?
- Can this be correlated to QD penetration levels?

DAPI/ QD Fluorescence

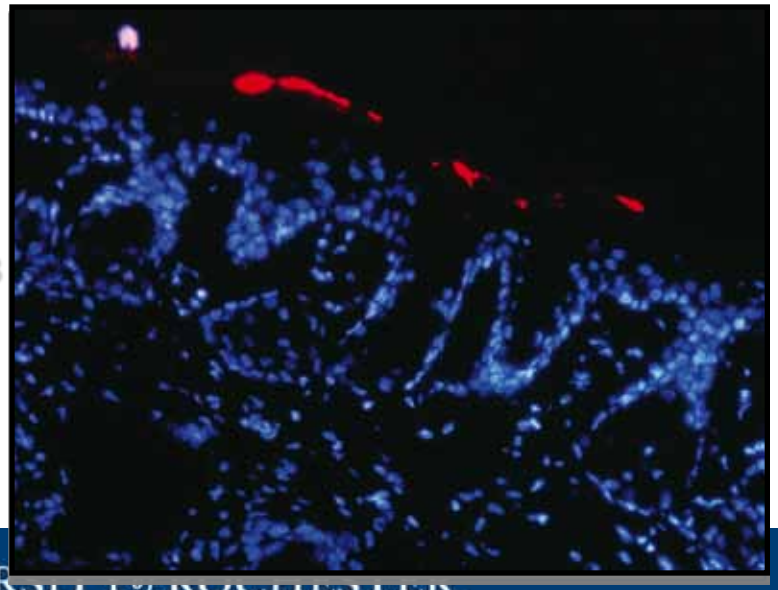
Brightfield/ QD Fluorescence

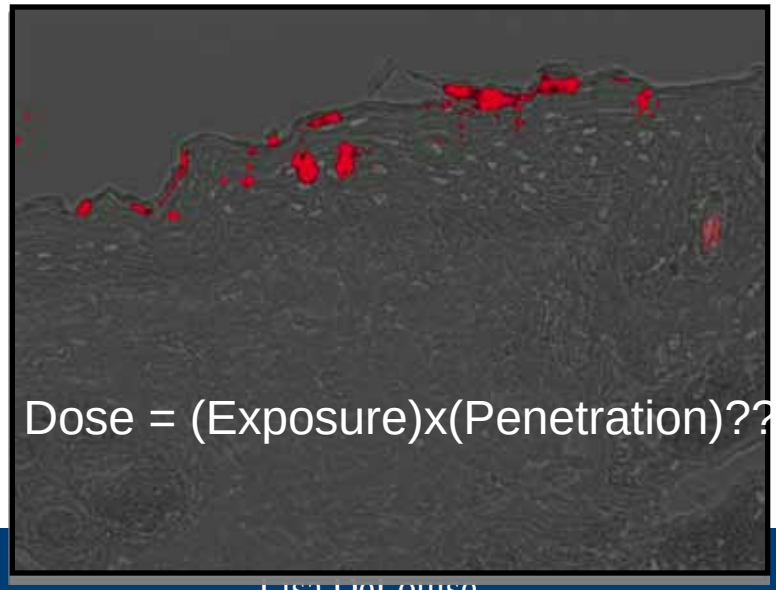
180mJ/cm^2 UVB
DHLA QD 620



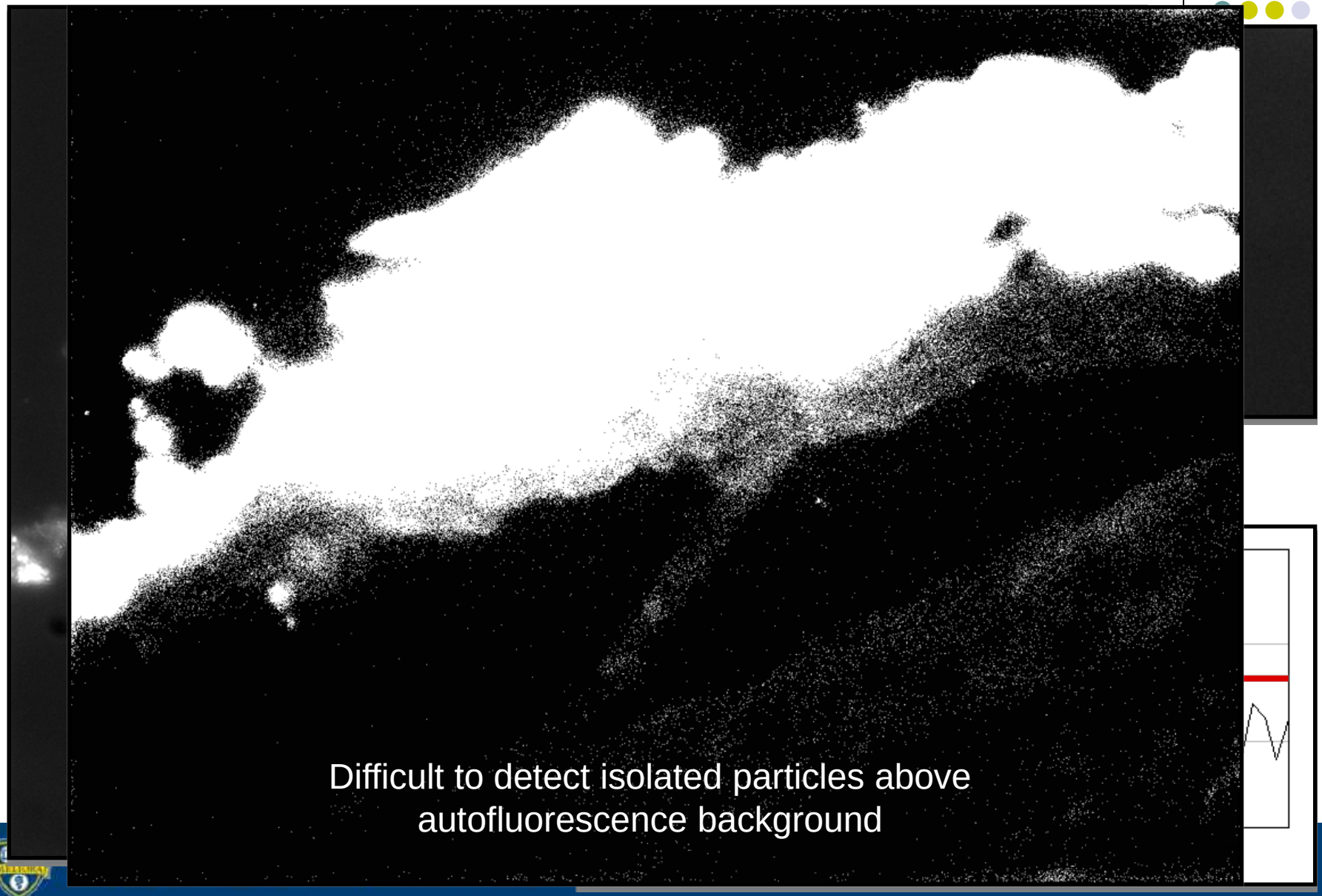


360mJ/cm^2 UVB
DHLA QD 620



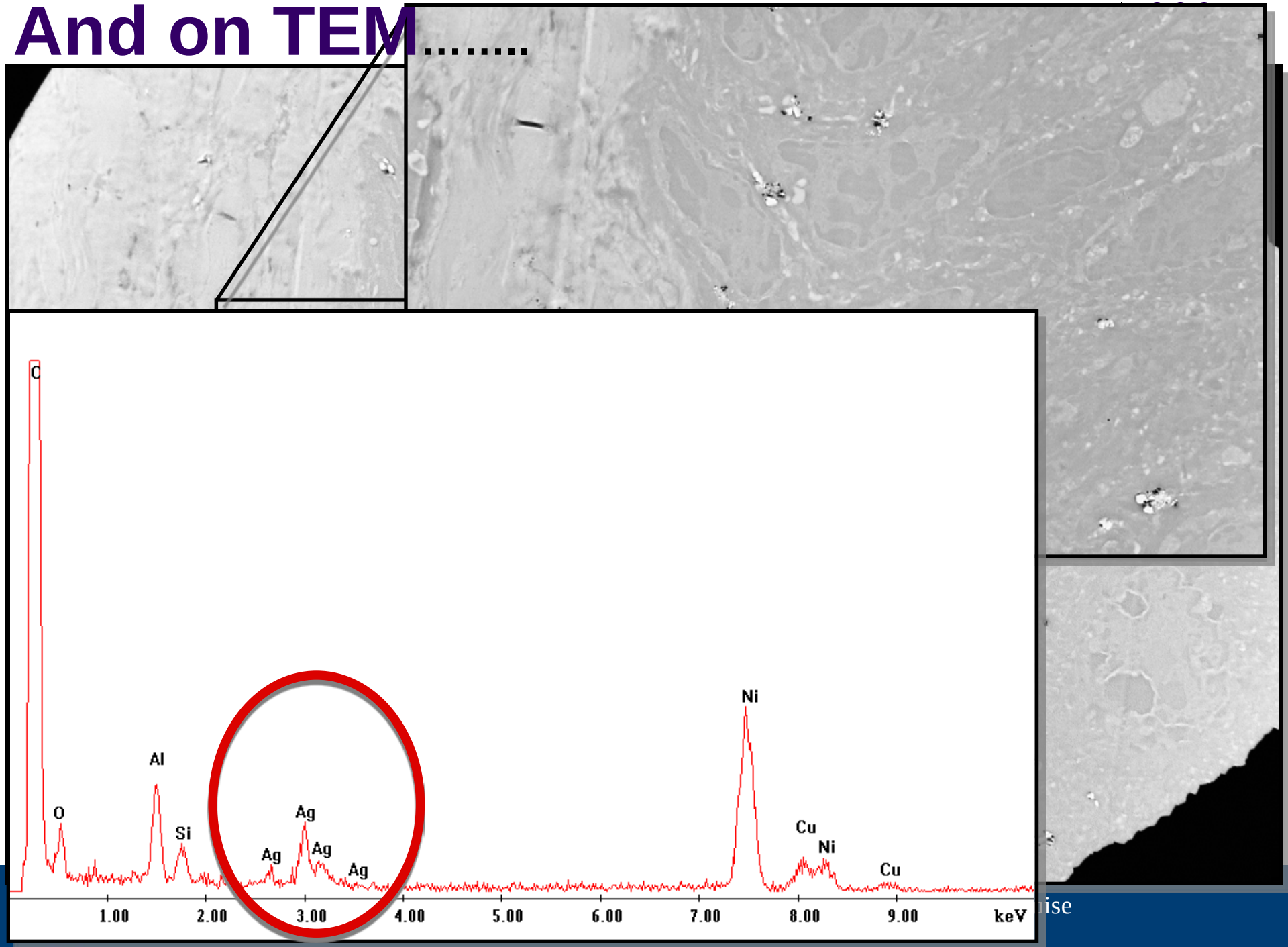


The Trouble Is...



Difficult to detect isolated particles above autofluorescence background

And on TEM……


C
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8.00
9.00
keV

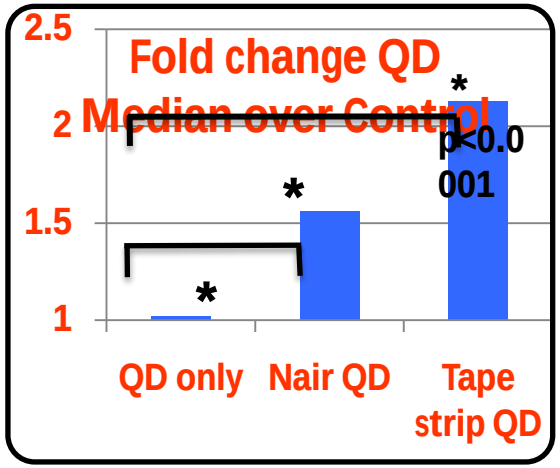
Important Points

- TEWL on UV-induced barrier (in vivo mouse) correlated with QD penetration
- Need to resolve TEM/Histology results
- Whole tissue imaging preferred
- Quantification of NM penetration via visible fluorescence above tissue background is problematic

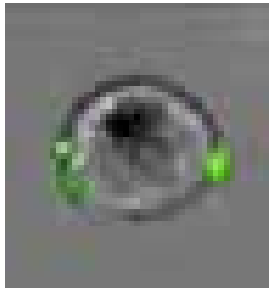
Fluorescence-activated cell sorting (FACS)

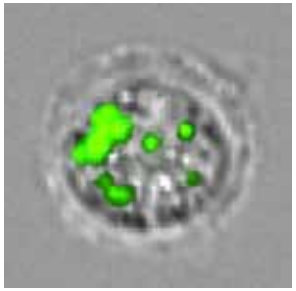
Fluorescence intensity used to quantify NM uptake

- Measure 20000 cells
- Quantify % of fluorescent cells or the relative fluorescence magnitude relative to control



QD applied to ex vivo skin 24 hr





But….uptake or associated?

State of the Science - Models

- Are tissue models appropriate (in vitro, ex vivo, in vivo)?
- Are exposure methods relevant?

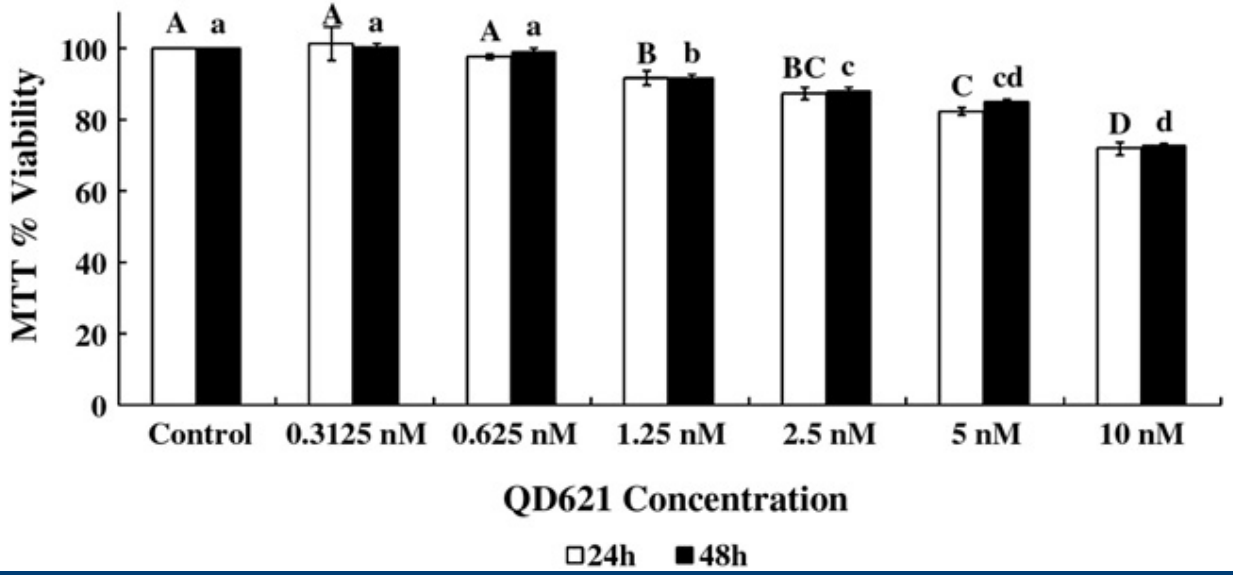
In vitro cell culture ubiquitously used to:
Screen NM cytotoxicty
Uptake mechanisms

State of the Science – Models

Problem #1 - Dose

- Cytotoxicity studies done at acute NP exposure levels far greater than is anticipated to occur.
  - Skin Cell QD toxicity >20 nM
  - 10^7 QD/cell

Is this realistic?



Zhang et al. Toxicology and Applied Pharmacology 228 (2008) 200–211

State of the Science – Models

Problem #2 Validity

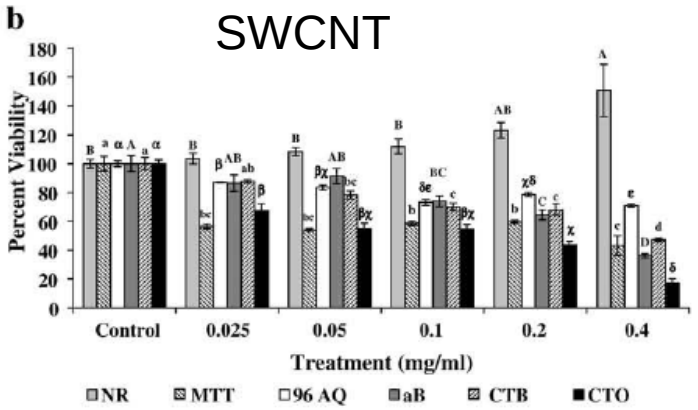
- NM can interfere with standard assays used to quantify cytotoxicity
- HEK line

Table 3
Percent difference of HEK viability relative to HEK controls at the highest NM concentration

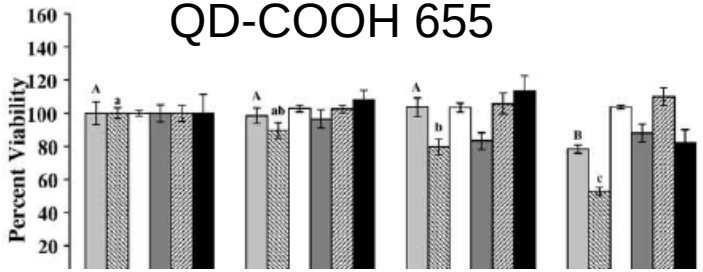
	CB (0.4 mg/ml)	SWCNT (0.4 mg/ml)	C_{60} (0.4 mg/ml)	nC_{60} (0.047 μg/ml)	QD-COOH (20 nM)
NR	+108.9*	+50.7*	+17.9	+31.6*	−21.6*
MTT	−19.2*	−56.7*	−14.6*	−21.4	−47.3*
96 AQ	−58.4*	−29.1*	+3.1	−7.6	+3.8
aB	−99.5*	−63.7*	−10.1	−0.2	−12.0
CTB	−93.0*	−52.7*	−29.4*	−5.0	+10.0
CTO	−81.5*	−82.5*	−32.7*	−17.4*	−17.9

* Significantly ($p<0.05$) different from paired control.

SWCNT



QD-COOH 655



N.A. Monteiro-Riviere et al. / Toxicology and Applied Pharmacology 234 (2009) 222–235

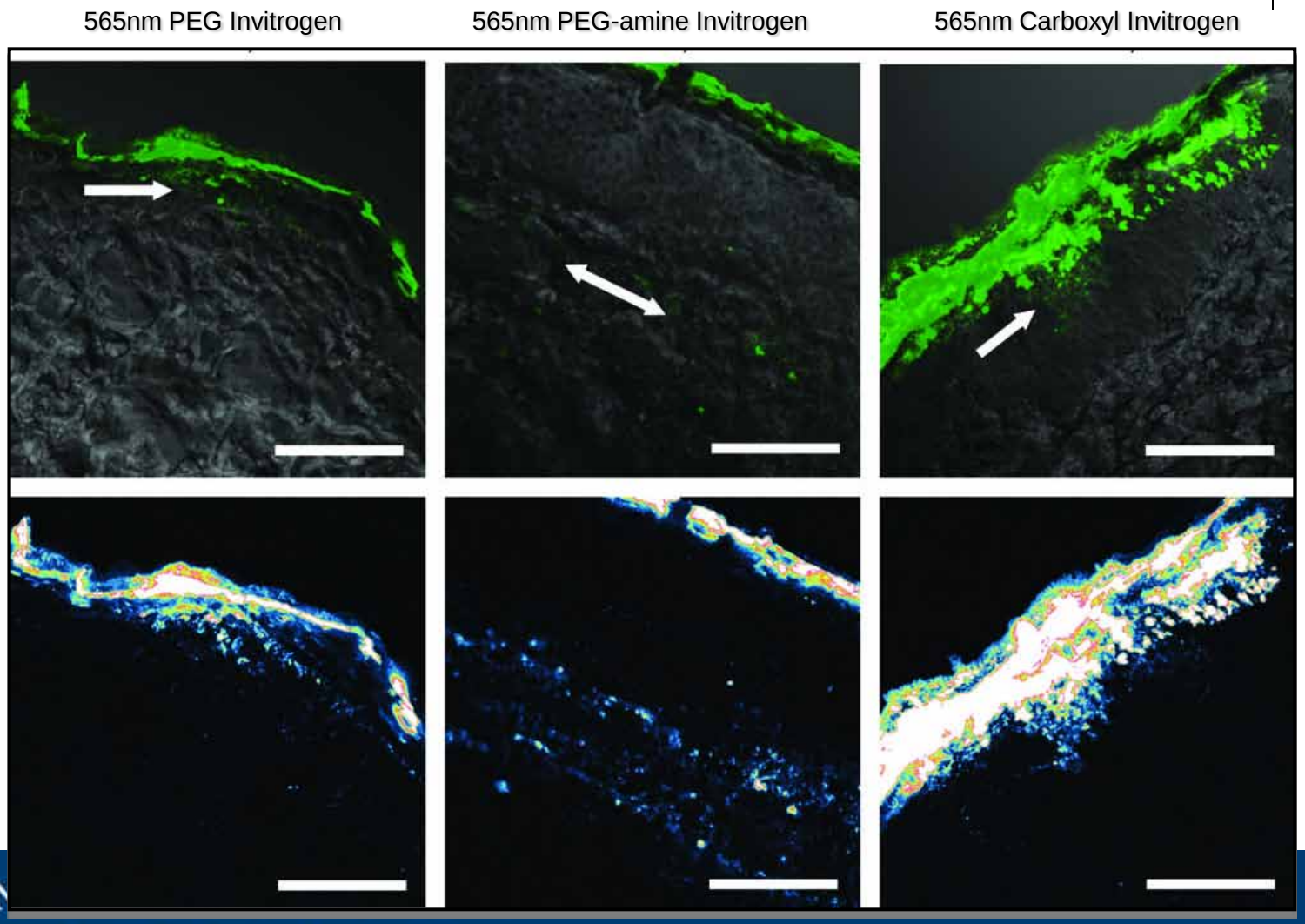
State of the Science – Models

Ex vivo Tissue Models

- Pig, human skin – they are different
- Storage – frozen skin is dead
- Application of materials – no standard vehicle

QD Ex vivo Pig Skin Penetration

- First studies (2006) demonstrated high permeability levels and surface chemistry dependent.

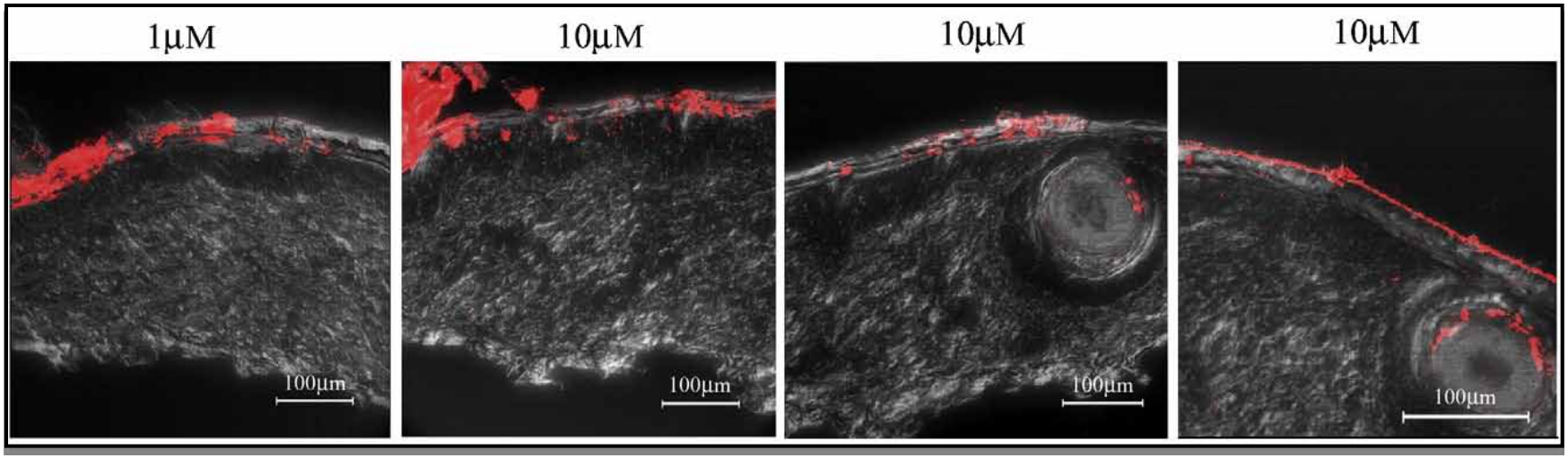


JP Ryman-Rasmussen, et al. Toxicol Sci **2006**, 91, 159.

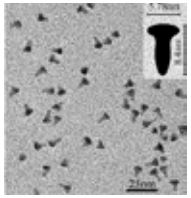
QD Skin Penetration

- More recent work (2008) *ex vivo* porcine skin suggests much lower levels of permeability

621nm PMAO-PEG, 24 hours



- Is it NM shape?
- Tissue processing?
- Tissue Type?



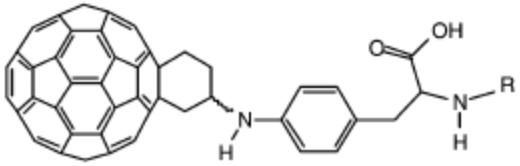
LW Zhang et al. Toxicol Appl Pharmacol **2008**, 228:200211

NANO LETTERS

2007
Vol. 7, No. 1
155−160

Effects of Mechanical Flexion on the Penetration of Fullerene Amino Acid-Derivatized Peptide Nanoparticles through Skin

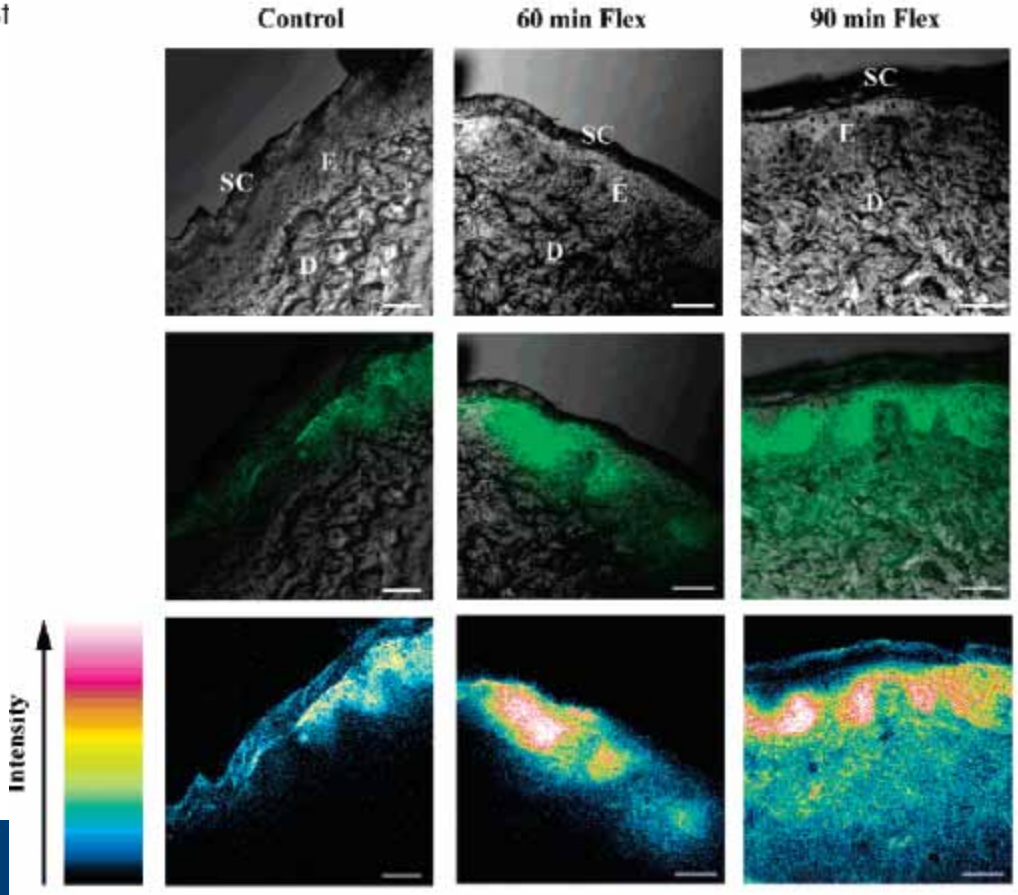
Jillian G. Rouse,[†,‡] Jianzhong Yang,[§] Jessica P. Ryman-Rasmussen,[†]
Andrew R. Barron,[§] and Nancy A. Monteiro-Riviere*[,†]



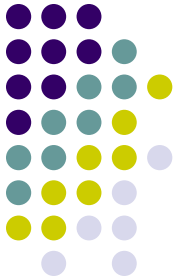
R = H (Baa, **1a**); C(O)O^{t}Bu (N-Boc-Baa, **1b**)

FITC
conjugated- NLS
(PKKKRKV)

Confocal Images



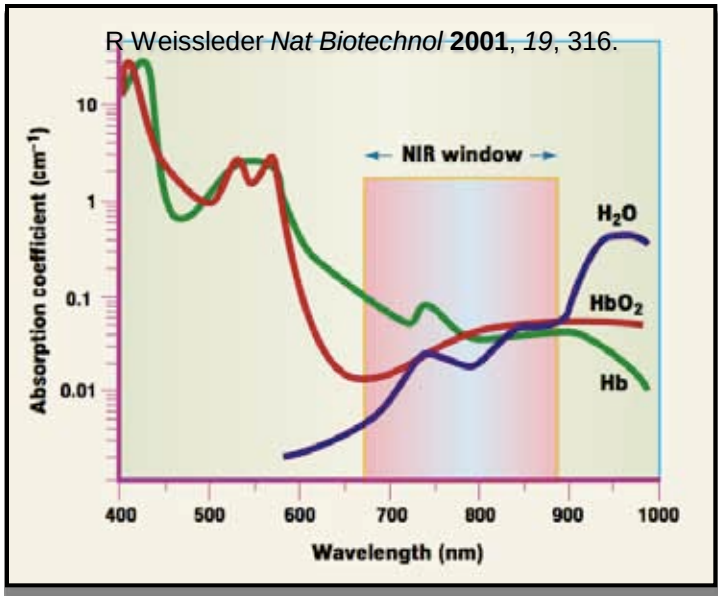
Science and Technology Barriers



- Standardized NM and viability assays
- Need to quantify tissue penetration at realistic exposure/dose levels
  - short-term/high dose vs. long-term/low dose
- Need accurate models of barrier impaired skin (physical, chemical or diseased)
- Need methods to quantify tissue penetration
  - Fluorescent techniques need to be calibrated- what is the detection limit?
  - How does quantum yield of NM effect detection?
  - Need novel NM amplification strategies.
  - Need NIR-based whole tissue imaging (no slicing, larger sample)
  - Can we take advantage of NIR two-photon imaging?

State of the Science

- NIR Tissue Window
- Some NM exhibit strong absorbance in NIR
- Two-photon excitation microscopy



State of the Science

Wild and Jones Environ. Sci. Technol. **2009,** *43,* 5290–5294 (Lancaster UK)

TPEM used to image TiO_2, CeO_2, and MWCNTs in living wheat roots

- Pump at 720 nm
- Image MWCNT ex 710 nm, em 300-390 nm
- Image TiO_2 ex 720 nm, em 410-600 nm
- Wheat root ex 710 nm, em 500-530 nm

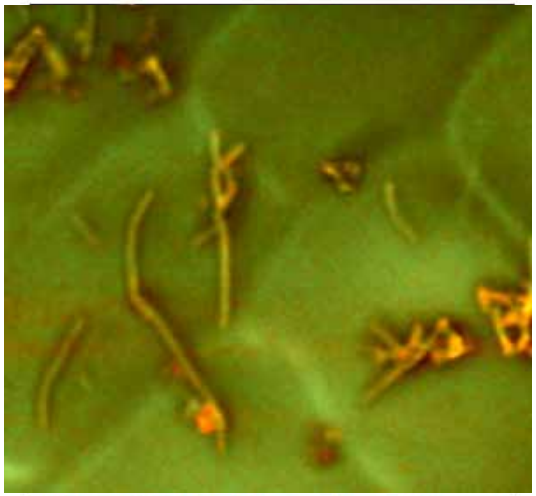


FIGURE 1. Unstained MWCNTs at the surface of a living root. Individual and aggregated MWCNTs are shown in orange, and the root surface is shown in green. The MWCNTs were detected and imaged using two-photon excitation microscopy

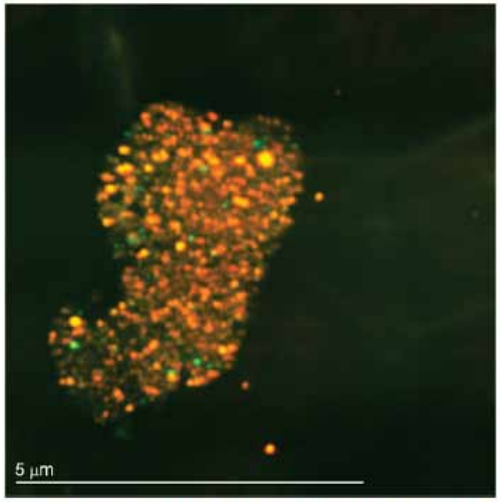
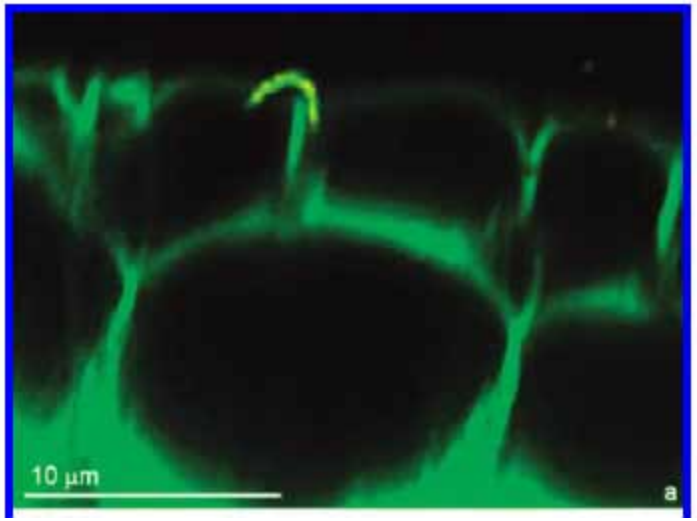


FIGURE 2. Unstained aggregated TiO_2 nanoparticles detected and visualized using two-photon excitation microscopy and autofluorescence.



Discovered MWCNT pierces cell membrane and is a portal for transport of PAH

QD Limit of Detection

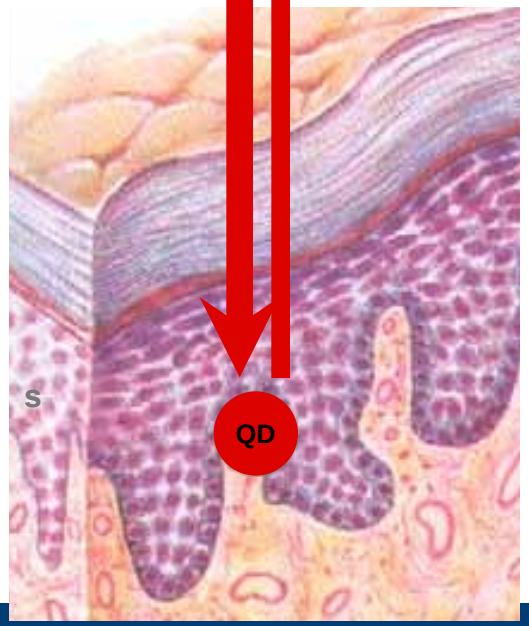
$$F_{out} = 8.89 \times 10^{-4} mW$$

Near-IR Wavelength

$$F_0 = 500 mW$$

Detector

Laser



Tissue Scattering

$$\frac{1}{e^{-2\mu_s' z}}$$

μ_s'= Scattering Coefficient

z=Tissue Depth

Beam Waist

$$\frac{r_b^2}{r_{qd}^2}$$

r_b= radius of the laser beam

r_{qd}= radius of the QD being illuminated

Lens Collection

$$\frac{4\pi}{W}$$

$$W = 2\pi\left(1 - \cos\left(\sin^{-1}\left(\tfrac{3}{4} NA\right)\right)\right)$$

NA=numerical aperture

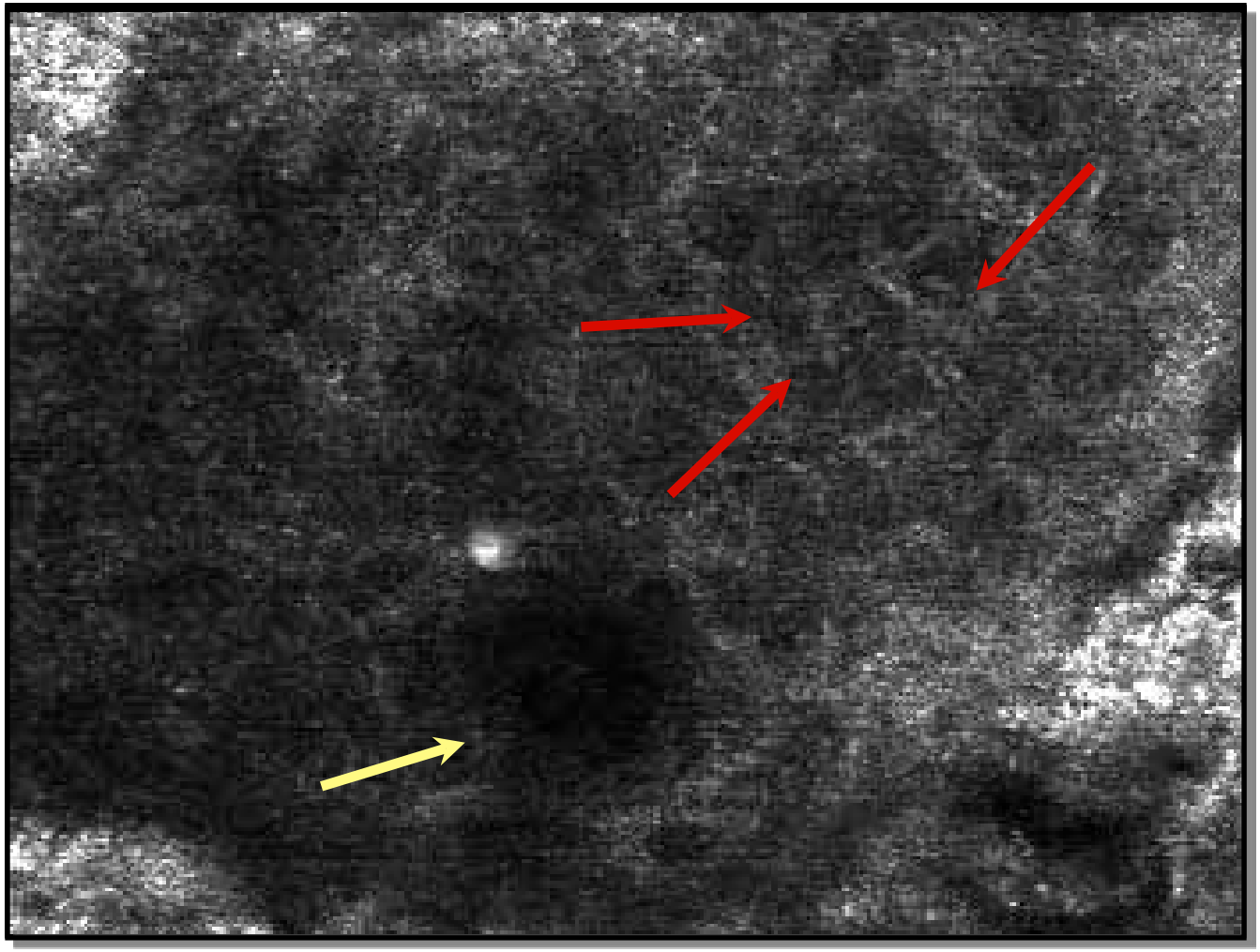
Q= Quantum Yield

Near-IR Wavelength

$$N = \frac{4\pi r_b^2 F_{out}}{W Q r_{qd}^2 \exp(-2\mu_s' z) F_0}$$

RA Wilson, J Zavislan Submitted to *Optics Lett* **2008**

Preliminary Data: Reflectance



Stratum Basaltum

In Collaboration with the Zavislan Lab

yellow arrow reference, red arrow areas of interest

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Hong Zheng, PhD

Gunter Oberdorster, PhD
Alison Elder, PhD
Jim Zavislin, PhD

EHSC Pilot
Project Funding

NSF

NYSTAR®

CEIS

National Institutes of Health
The Nation's Medical Research Agency

NIH / NIAID 5K25AI060884

EPA Recent Awards

http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.rfa/rfa_id/461

R833856	Development of an In Vitro Test and a Prototype Model to Predict Cellular Penetration of Nanoparticles	Grant	July 1, 2008 through June 30, 2011
R833857	"Effects of Surface Oxides on the Behavior of Carbon Nanotubes and their influence on the Mobility of Contaminants in Aquatic Environments"	Grant	July 1, 2008 through June 30, 2011
R833858	Quantum Dot Weathering and its Effects on Microbial Communities	Grant	July 1, 2008 through June 30, 2011
R833859	Analysis and Fate of Single-Walled Carbon Nanotubes and Their Manufacturing Byproducts in Estuarine Sediments and Benthic Organisms	Grant	July 1, 2008 through June 30, 2011
R833860	Functionalized Metal Oxide Nanoparticles: Environmental Transformations and Ecotoxicity	Grant	July 1, 2008 through June 30, 2011
R833861	Environmental Transport, Biodegradation, and Bioaccumulation of Quantum Dots and Oxide Nanoparticles	Grant	July 1, 2008 through June 30, 2011
R833862	Bioavailability, Environmental Transformation, and Detoxification of Core/Shell Nanomaterials	Grant	July 1, 2008 through June 30, 2011
R833891	Transformation and Fate of Manufactured Metal Oxide and Metal Nanoparticles in Aqueous Environments	Grant	January 15, 2009 through January 14, 2012
R833892	Platinum-Containing Nanomaterials: Sources, Speciation, and Toxicity in the Environment	Grant	March 1, 2009 through February 29, 2012
R833893	Bioavailability of Metallic Nanoparticles and Heavy Metals in Landfills	Grant	April 1, 2009 through March 31, 2012
R834091	Nanocavity sensor array for the isolation, detection and quantitation of engineered nanoparticles	Grant	December 1, 2008 through November 30, 2011
R834092	Influence of Water Quality on the Bioavailability and Food Chain Transport of Carbon Nanoparticles	Grant	October 1, 2008 through September 30, 2011
R834093	Interactions of Natural Organic Matter with C60 Fullerene and their Impact on C60 Transport, Bioavailability and Toxicity	Grant	January 1, 2009 through December 31, 2011
R834094	Environmental Behaviors of Solubilized Carbon Nanotubes in Aquatic Systems: Transformation, Sorption, and Toxicity Exposure	Grant	September 1, 2008 through August 31, 2011

State of the Science – Models

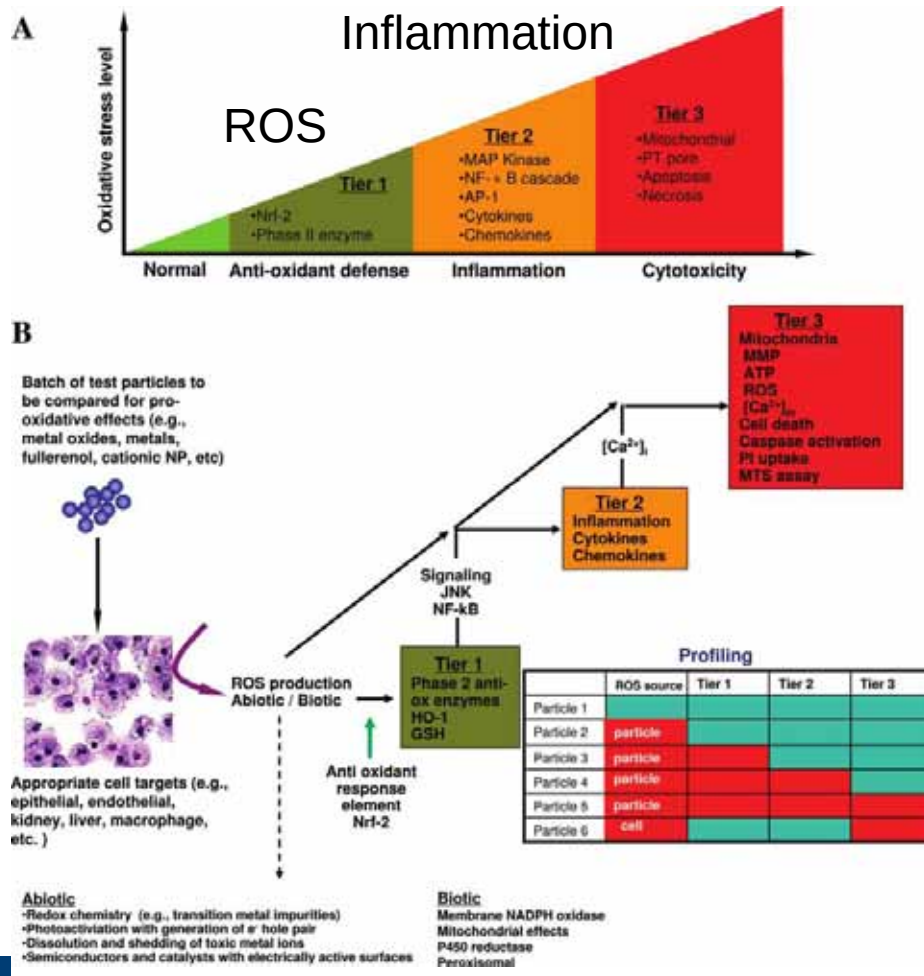
In vitro cell culture ubiquitously used to:

- Screen NM cytotoxicty
- Uptake mechanisms

How useful are these results?

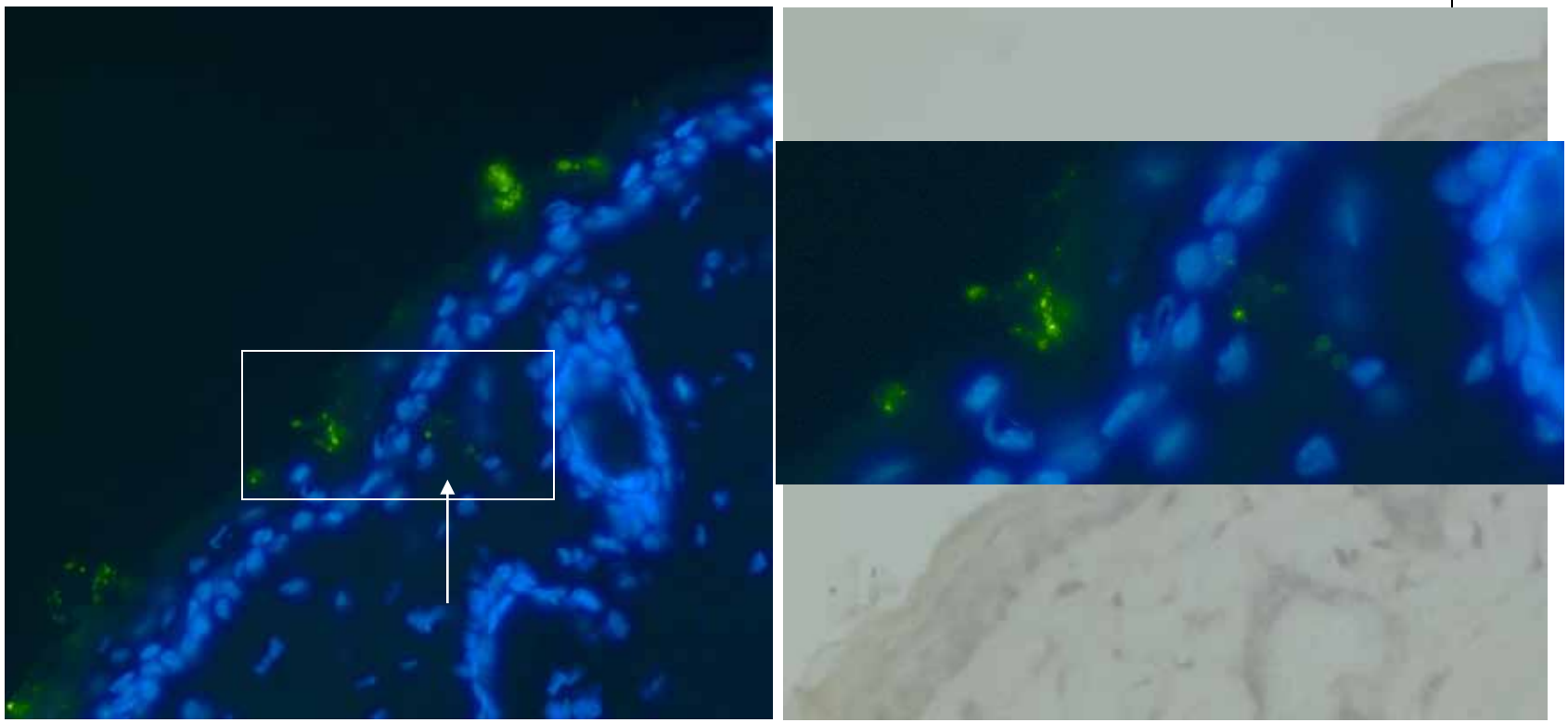
Nel's "Hierarchical Oxidative Stress Paradigm"

If a link exists between an in vivo disease (eg. allergic airway inflammation) and a mechanistic pathway at the cellular level (eg. oxidative stress) one can use a cell line (bronchial epithelial cells, macrophage) for NM high-throughput screening.



Nel and coworkers ACS Nano **2009** 3(7) 1620.

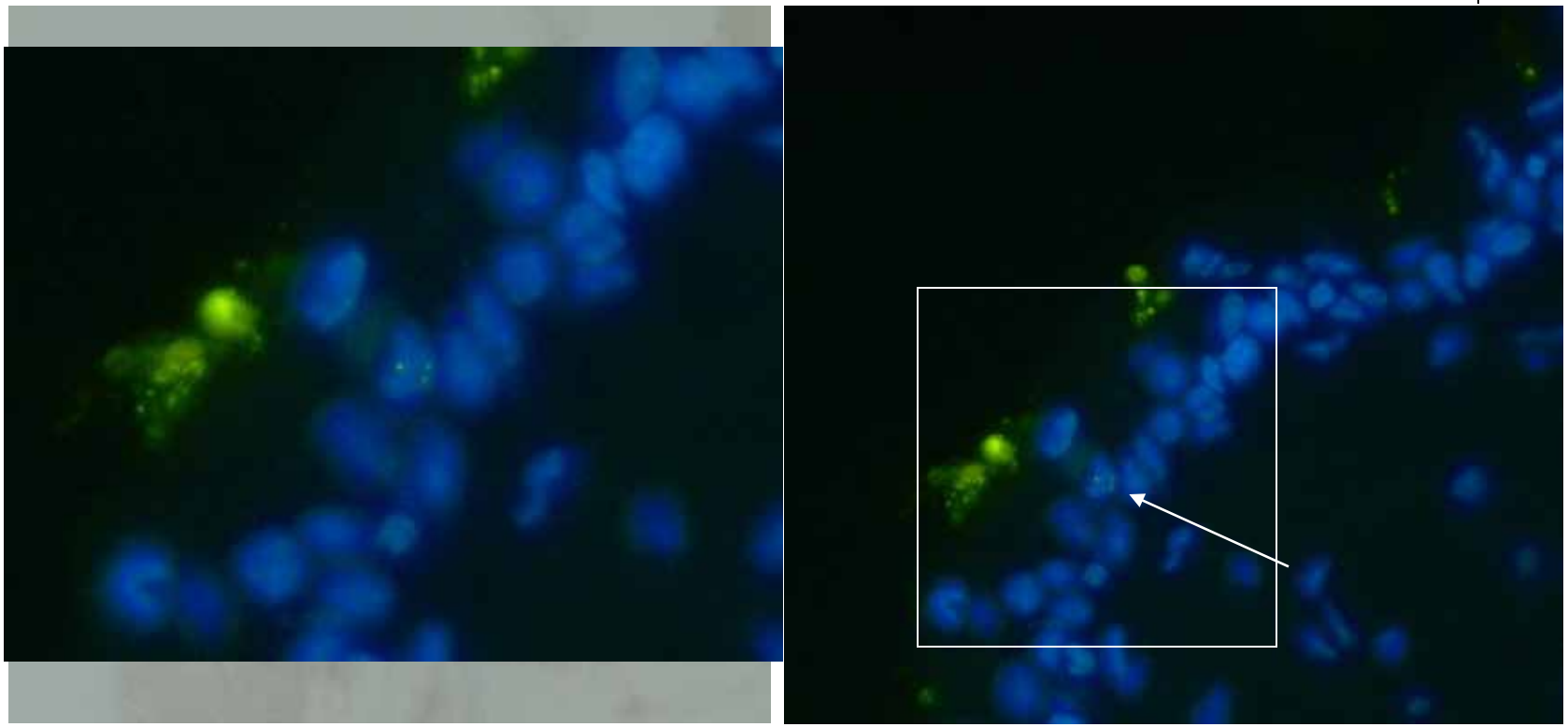
UV exposed, 24hr after COOH, cryosection



•QD fluorescent evident in viable epidermis
•What cell types do QD interact with?

40X

UV exposed, 24hr after QD-COOH Cryosection, DAPI



- QD fluorescence associated with cell nucleus
- Are these aggregates?

40X

State of the Science

What is the appropriate dose metric - mass, surface area, particle number, composition?

Pulmonary toxicity

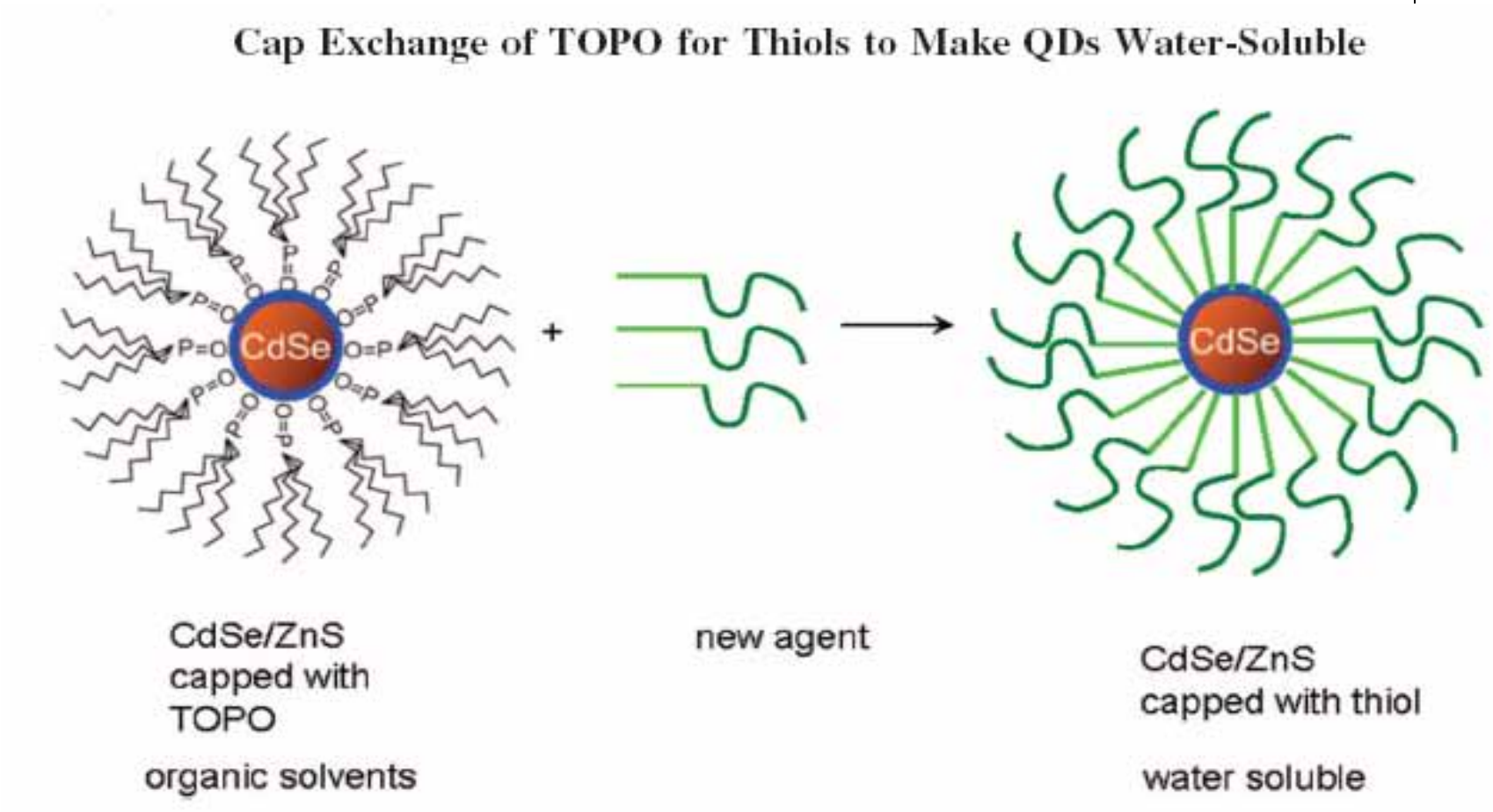
- Oberdörster et. al. (2005) - particle surface area
- Wittmaack et. al. (2007) -particle number
- Warheit et. al. (2009) - chemical reactivity

Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect. 2005;113:823–839.

Wittmaack K. 2007. In search of the most relevant parameter for quantifying lung inflammatory response to nanoparticle exposure: particle number, surface area, or what? Environ Health Perspect 115187–194

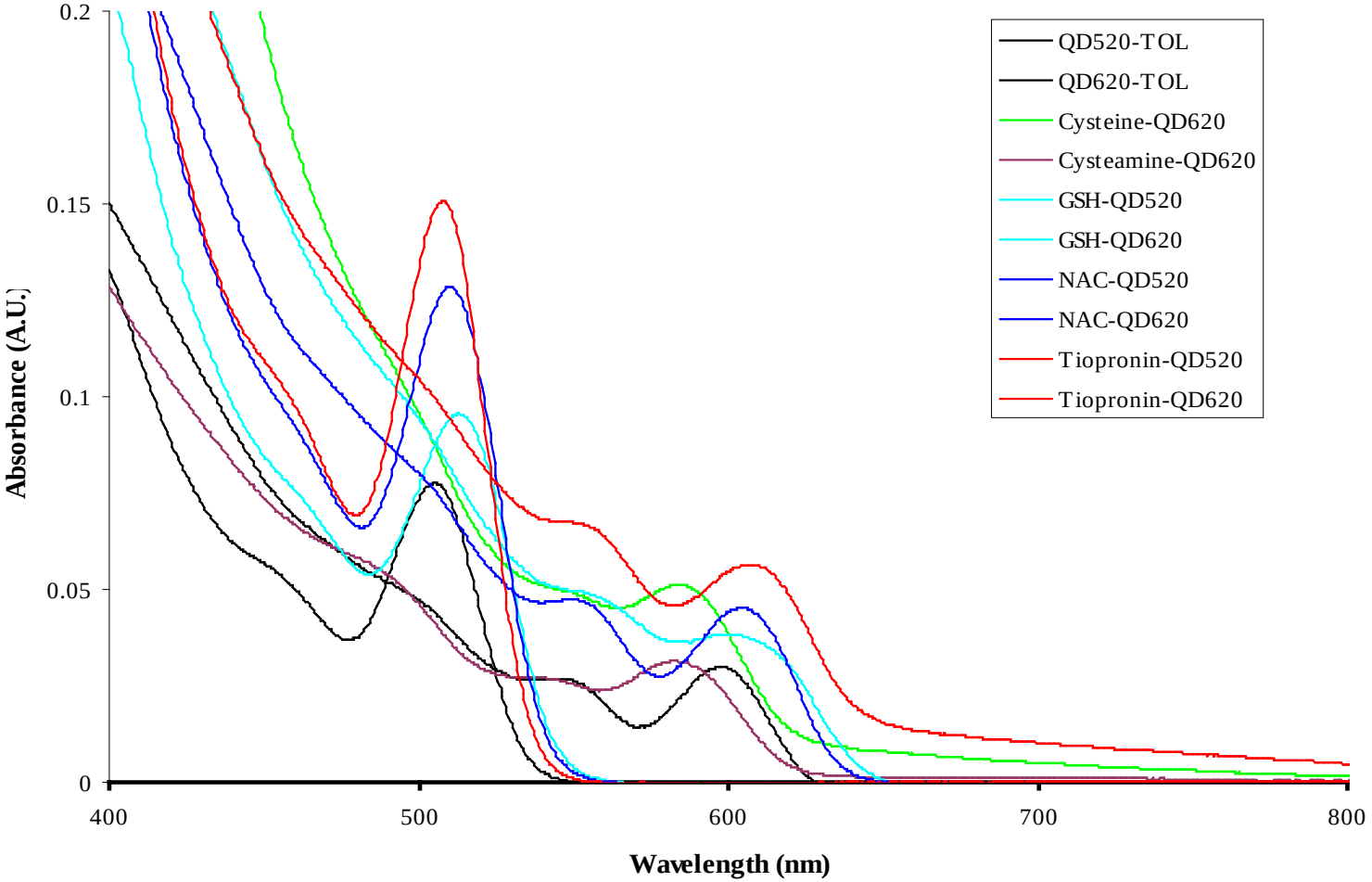
Warheit DB, Reed KL, Sayes CM. A role for nanoparticle surface reactivity in facilitating pulmonary toxicity and development of a base set of hazard assays as a component of nanoparticle risk management. Inhal Toxicol. 2009 Jul;21(S1):61-67.

State of the Science –reactivity

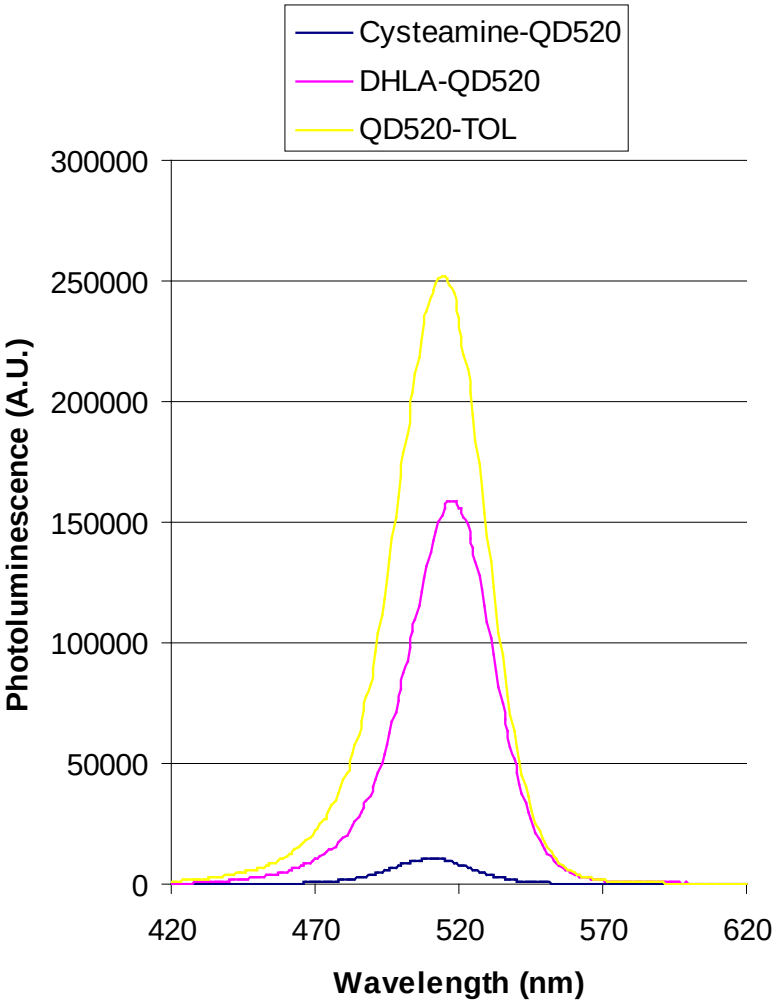


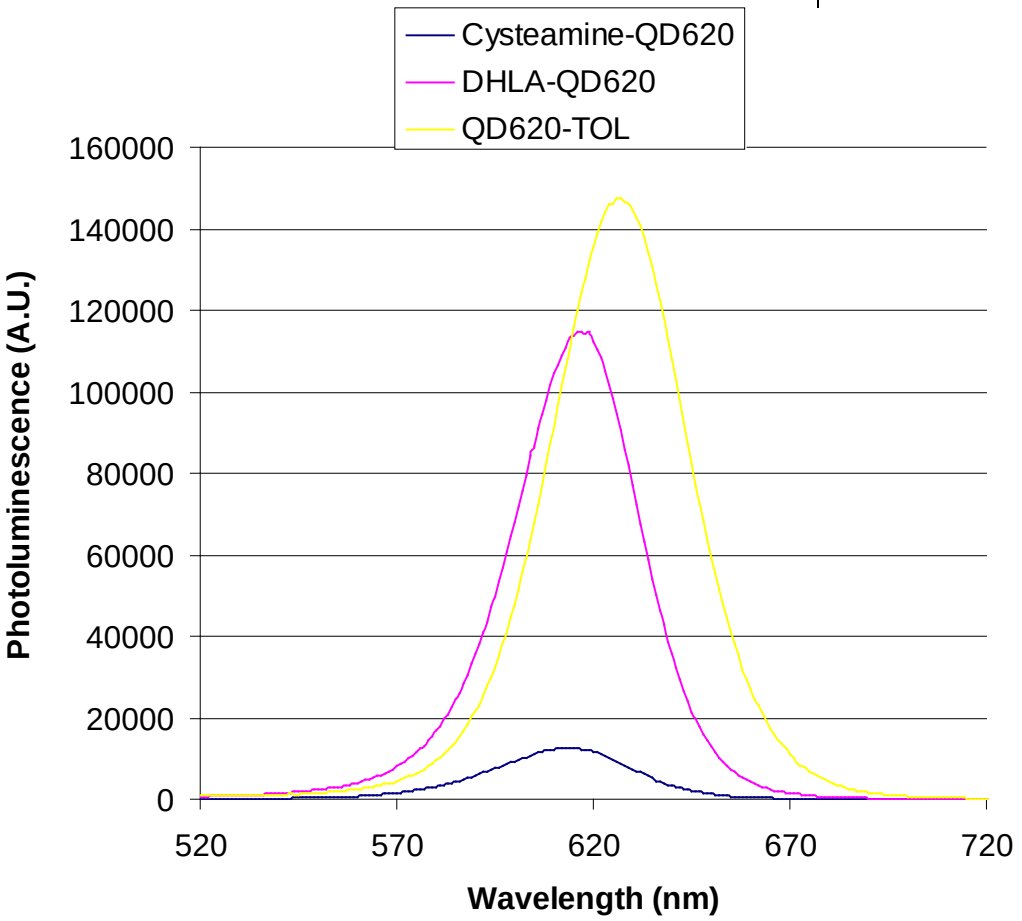
Langmuir **2008,** *24,* 9194-9197

Absorbance Company A

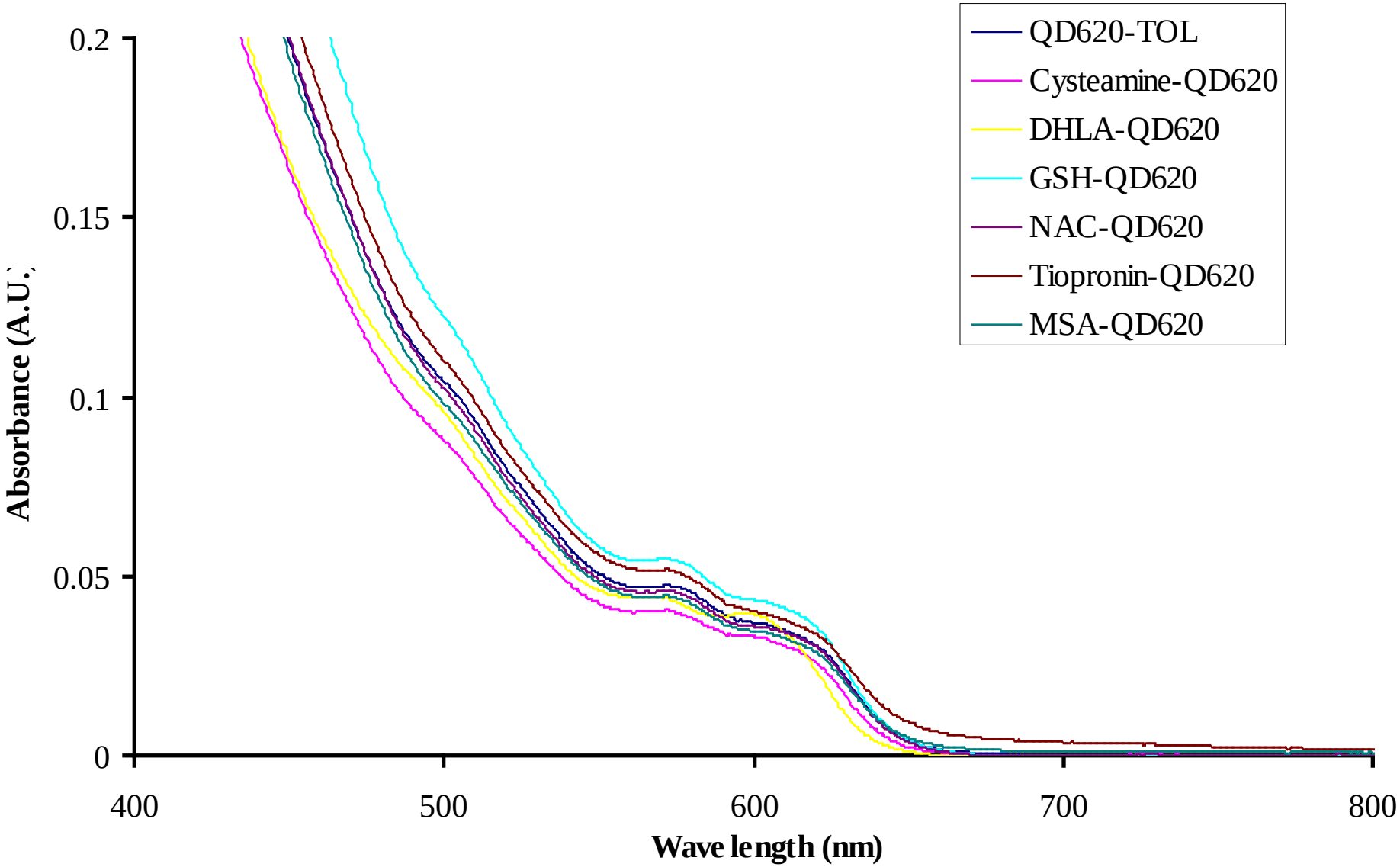


Fluorescence Company A





Absorbance Company B



Fluorescence Company B