Nanomaterials EH&S – A Perspective

Panel 5 “How environmental exposures occur and change under different environmental conditions”

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NNI Workshop on Nanomaterials and the Environment and Instrumentation
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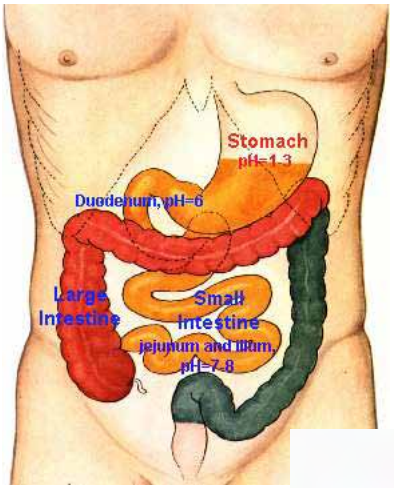
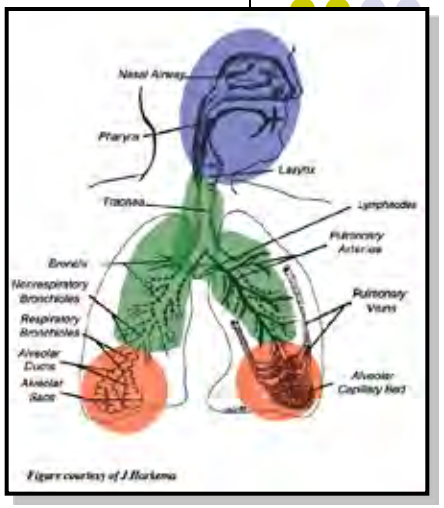
How environmental exposures occur?

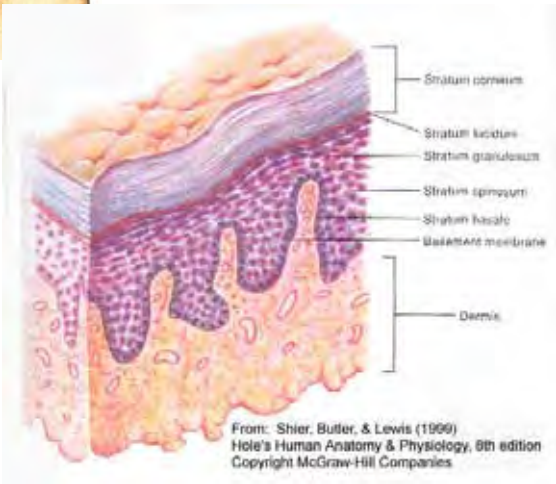
Points of Entry
- Gastrointestional track
- Respiratory track
- Skin

Unintended Exposure
- Research
- Manufacturing
- Consumer

Intended Exposure
- Drug delivery
- Imaging
- Cosmetics





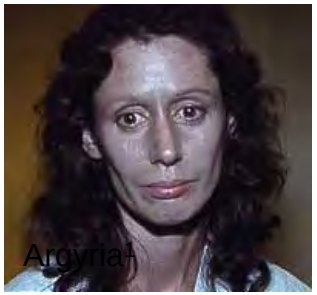


Exposures occur by direct application

NanoProducts CORPORATION

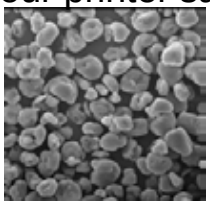
10,000 tons/yr

- Metal oxide NPs in sun screens, TiO_2, ZnO (<20 nm)
- Silver NP antimicrobial effects – surgical masks, refrigerators, wound care (Silvadene)
- Xerographic toner emissions
- Fe oxide contrast agent for MRI imaging

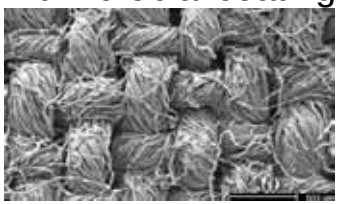


Argyria[1]

Is your printer safe?[2]



Antimicrobial coatings[3]



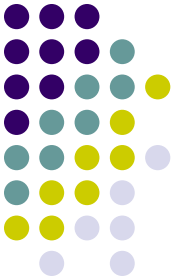
>9800 personal care products[3] chronic exposure

Helping Make Products Better™
BASF
The Chemical Company

~$65B 2010

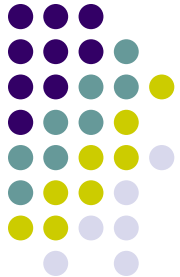
1. www.krank-durch-toner.de/English.htm
2. http://www.ewg.org/
3. Gupta et al. J.Cotten Science 2008 12, 280.

…change under different environmental conditions?



- Different skin types (light/dark)
- Follicular density
- Different skin conditions
  - Mechanical cuts
  - Chemical
  - Environmental (UV)
  - Disease

Prioritize Test Nanomaterials



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

- 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.

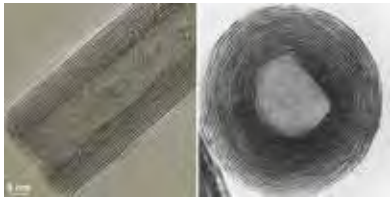
(Photo by J.S. Carras)

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.**

Science and Technology Barriers

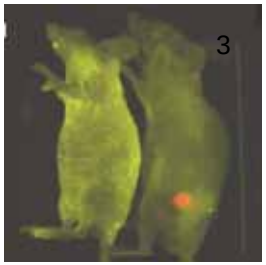
Exposure Assessment

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

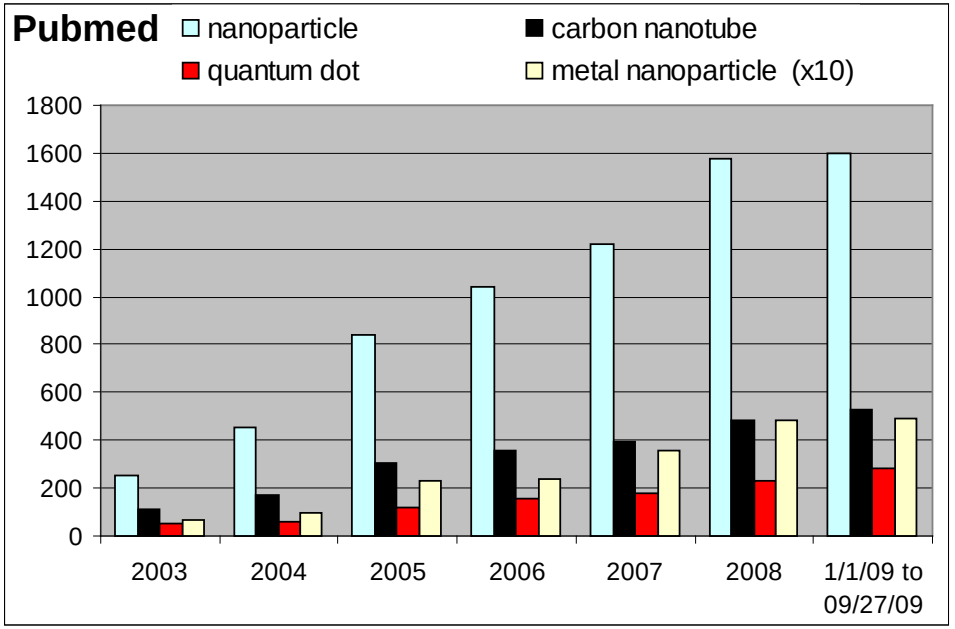


2-10 nm

CdSe



Can they penetrate through my gloves?



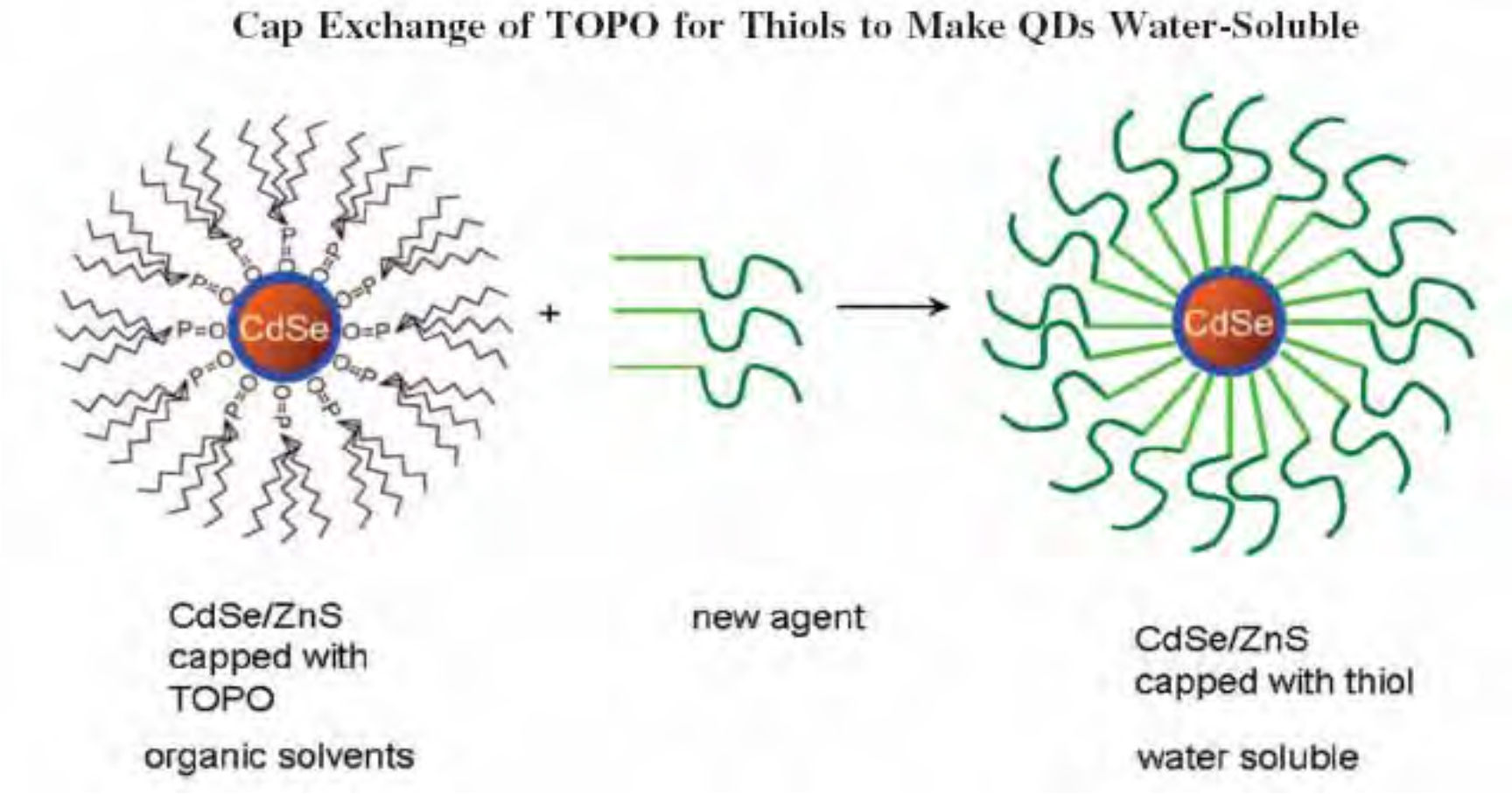
State of Science

Hypotheses:

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

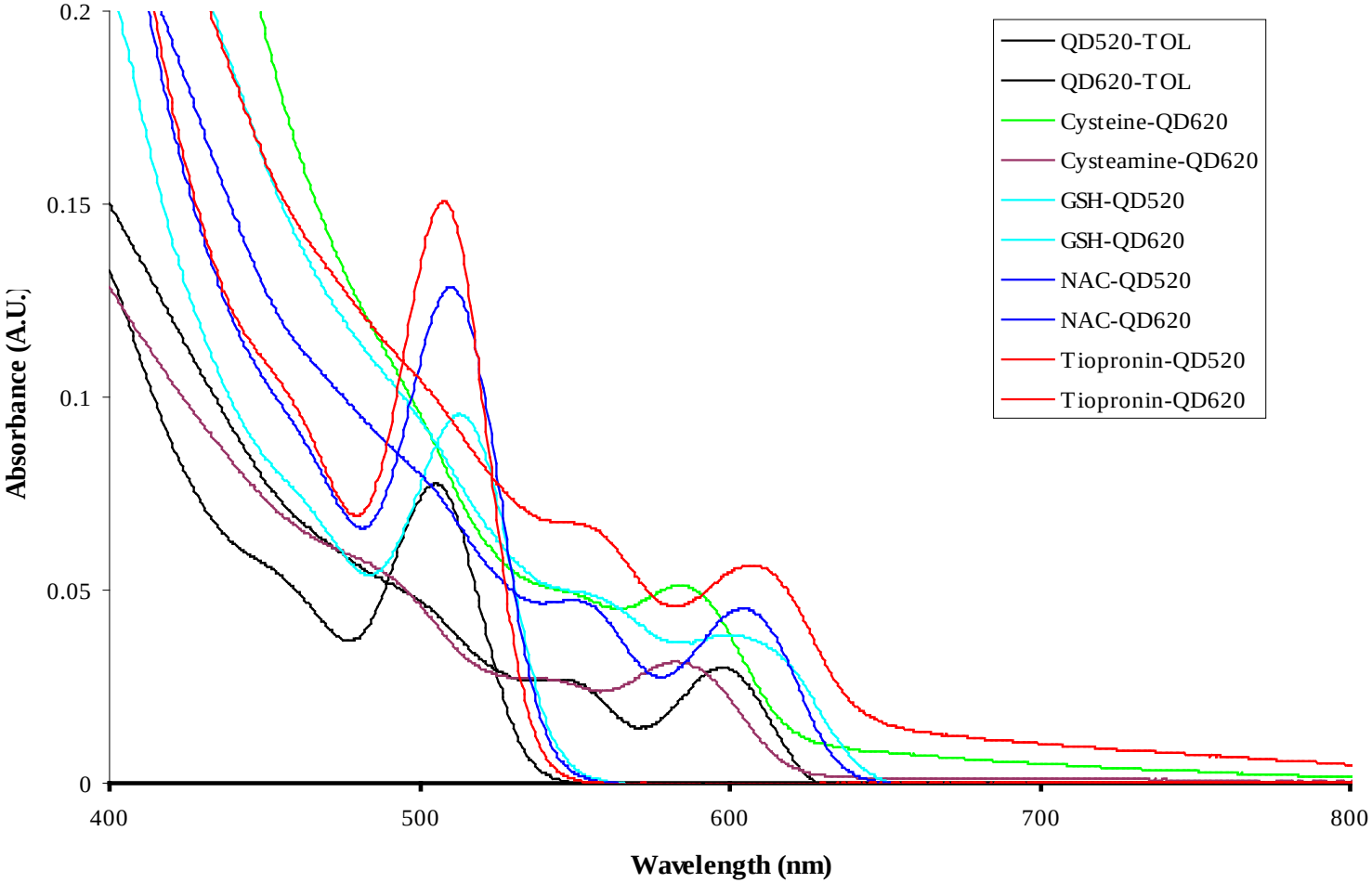
Are current methods/models/materials sufficient to prove this or produce useful data for EHS risk assessment? – No

State of Science – NM reactivity

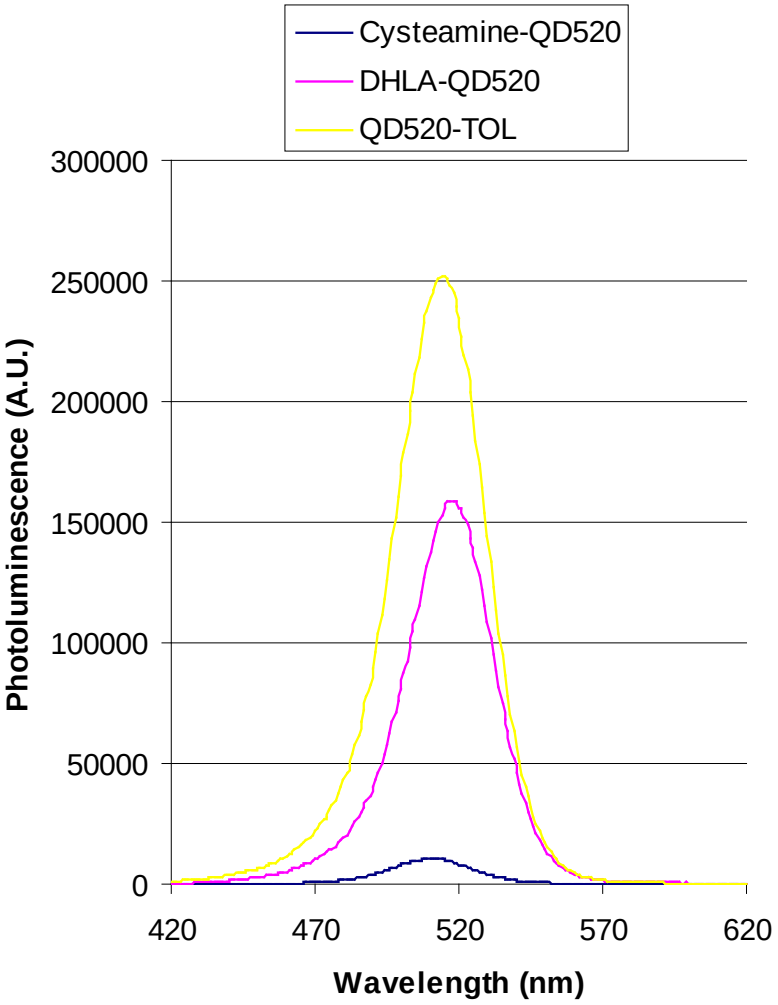


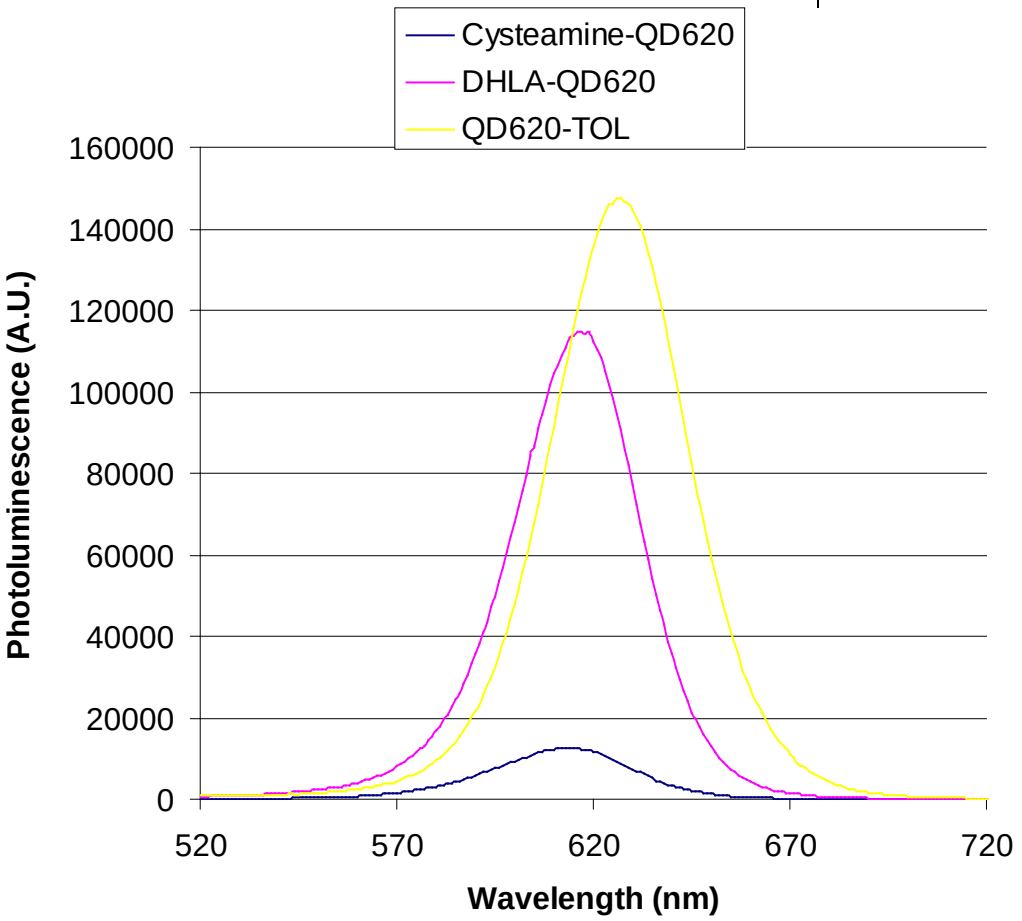
Langmuir **2008,** *24,* 9194-9197

Absorbance Company A

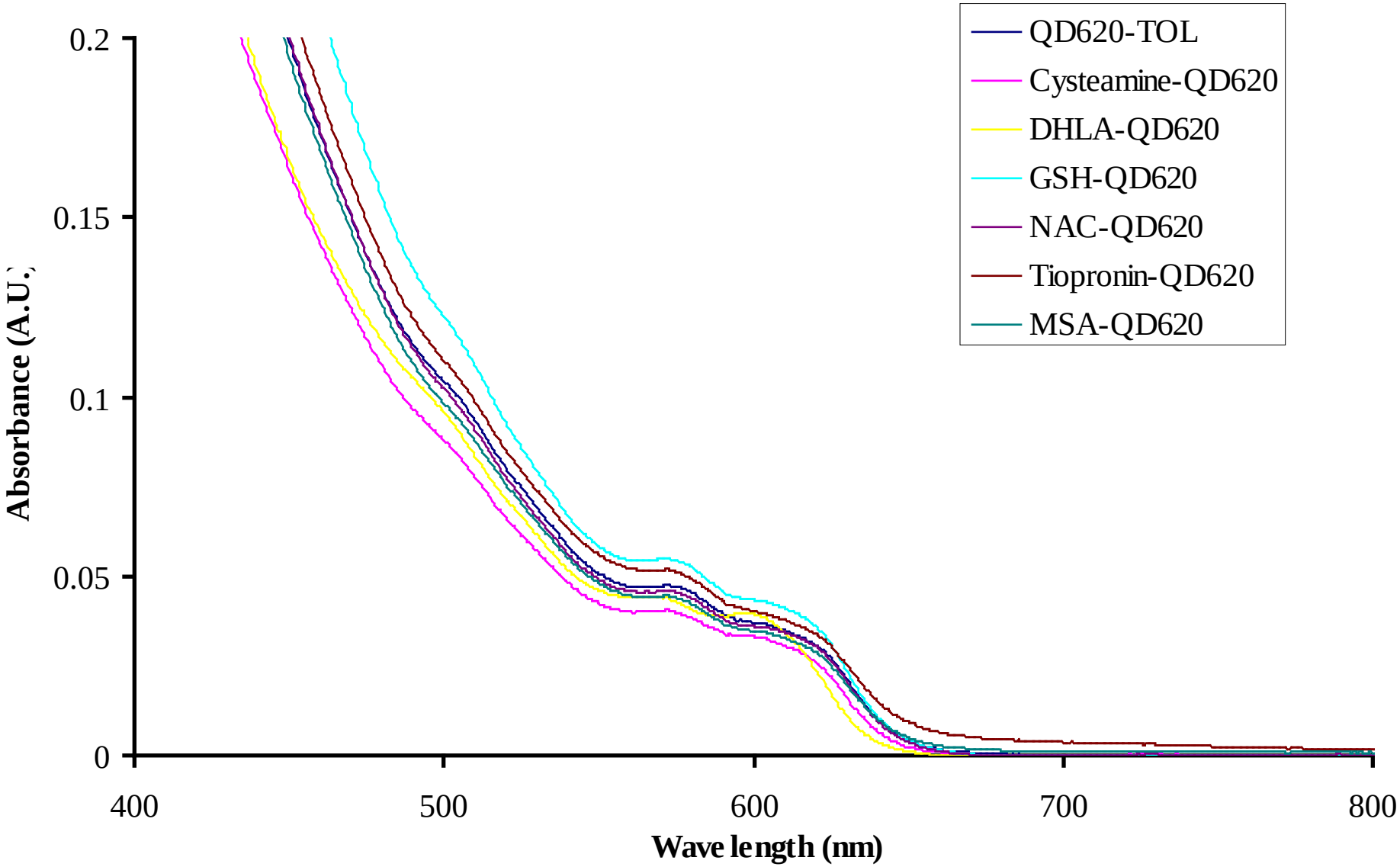


Fluorescence Company A

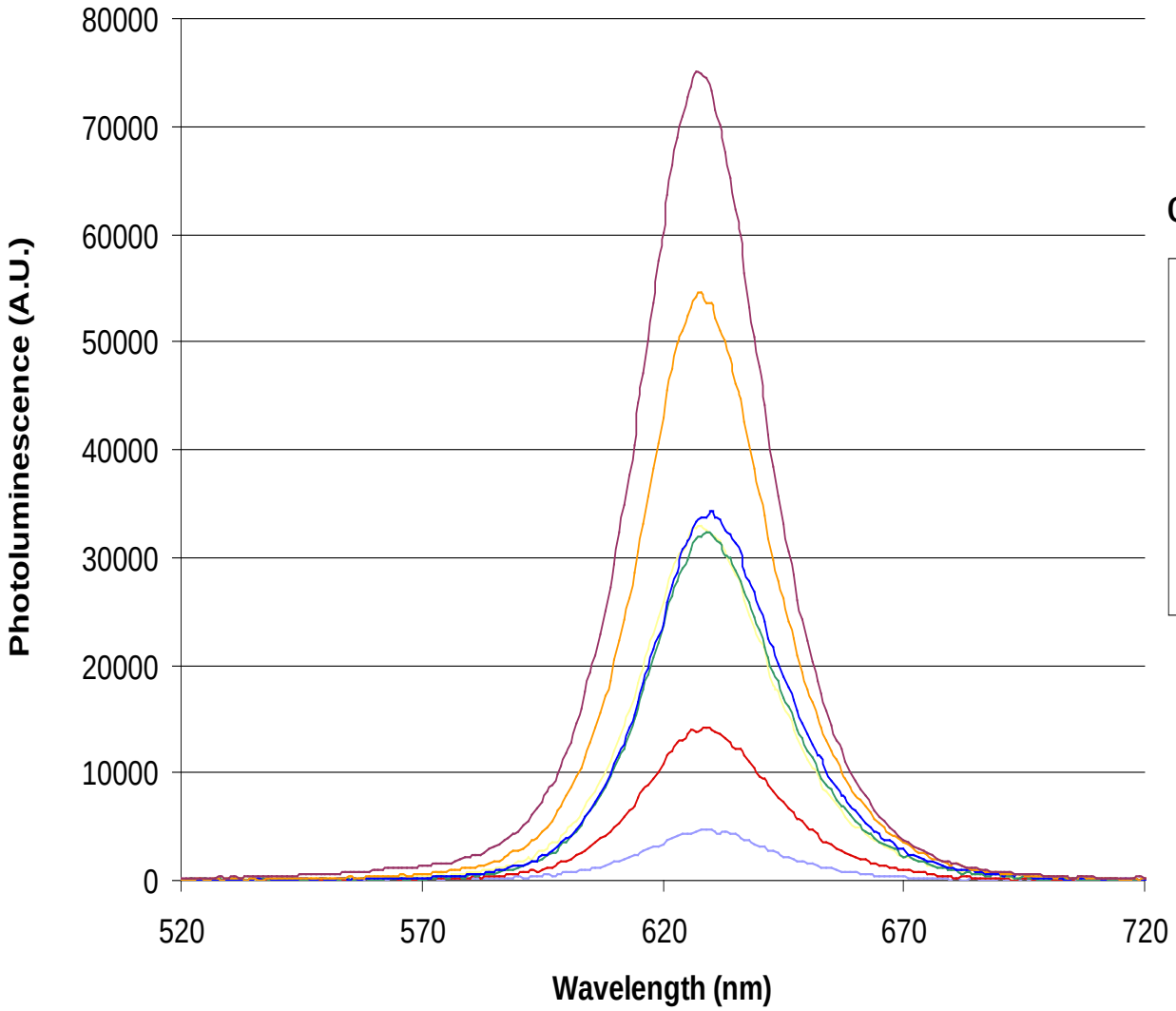




Absorbance Company B



Fluorescence Company B

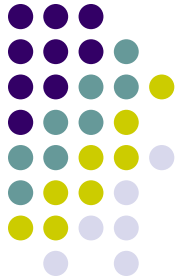


Equal concentrations

- CYS-QD620
- DHLA-QD620
- TP-QD620
- MSA-QD620
- NAC-QD620
- GSH-QD620
- QD620-TOL

- Stable core
- But QY varies

Science and Technology Barriers

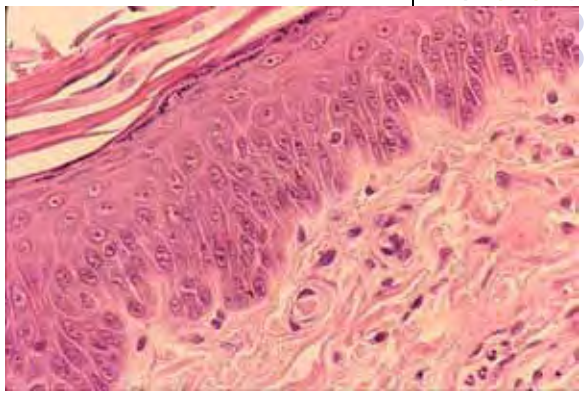


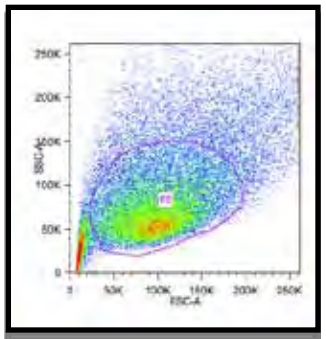
First Points

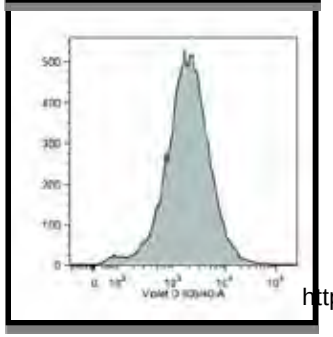
1. Standardized NM worthy goal
2. Vendors need to provide more quantitative data on their products
3. Researchers need to quantify NM properties

State of the Science – 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
  - Quantitative with good statistics
  - Destructive
- NIR microscopy
  - Major innovation and future





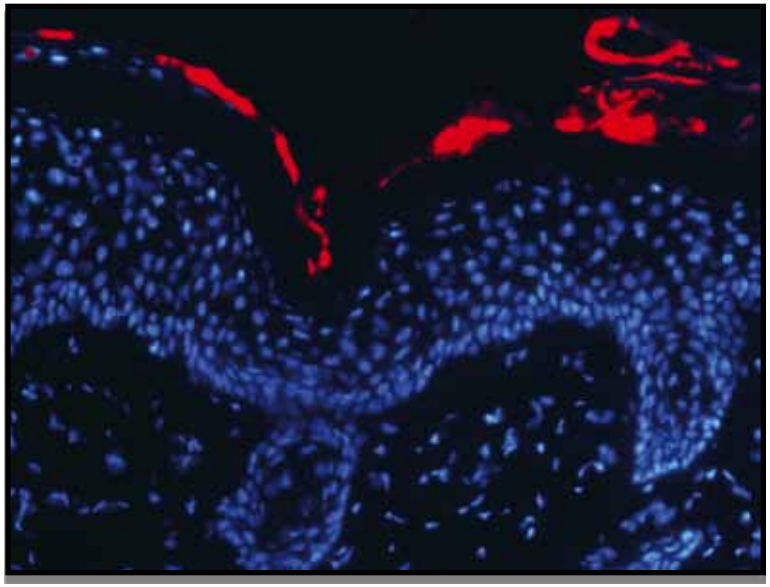


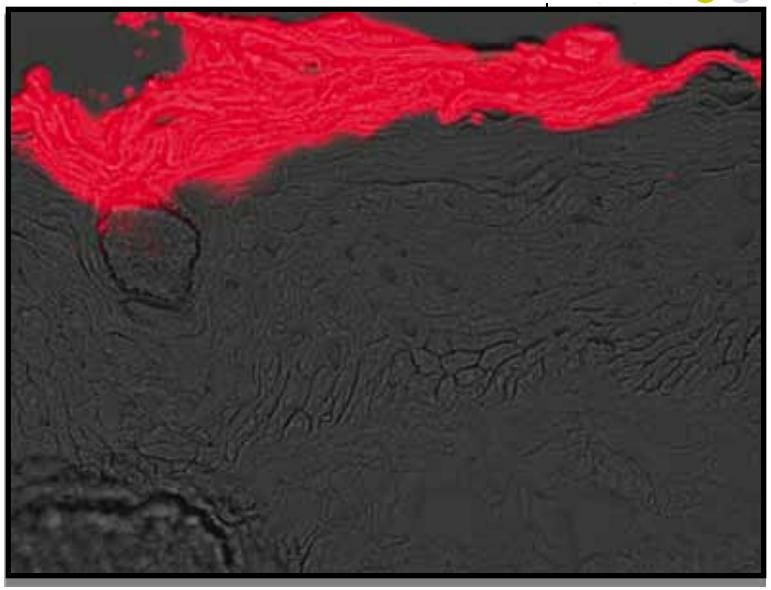
http://www.odakecza.com/bilder/reviscisondarm.jpg

DAPI/
QD Fluorescence

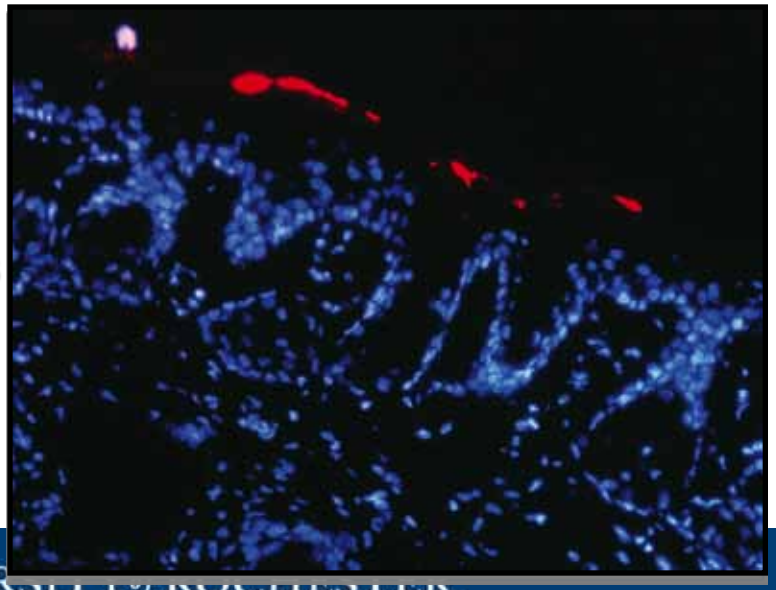
Brightfield/
QD Fluorescence

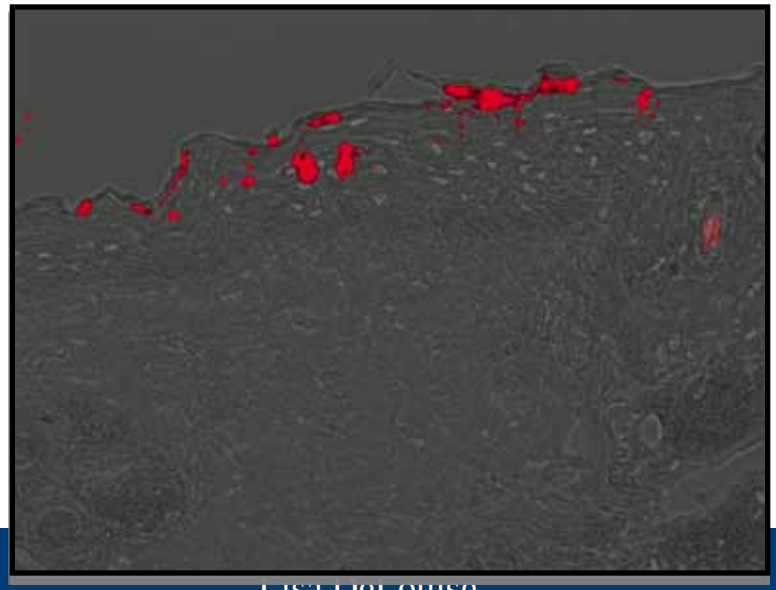
180mJ/cm^2 UVB
DHLA QD 620



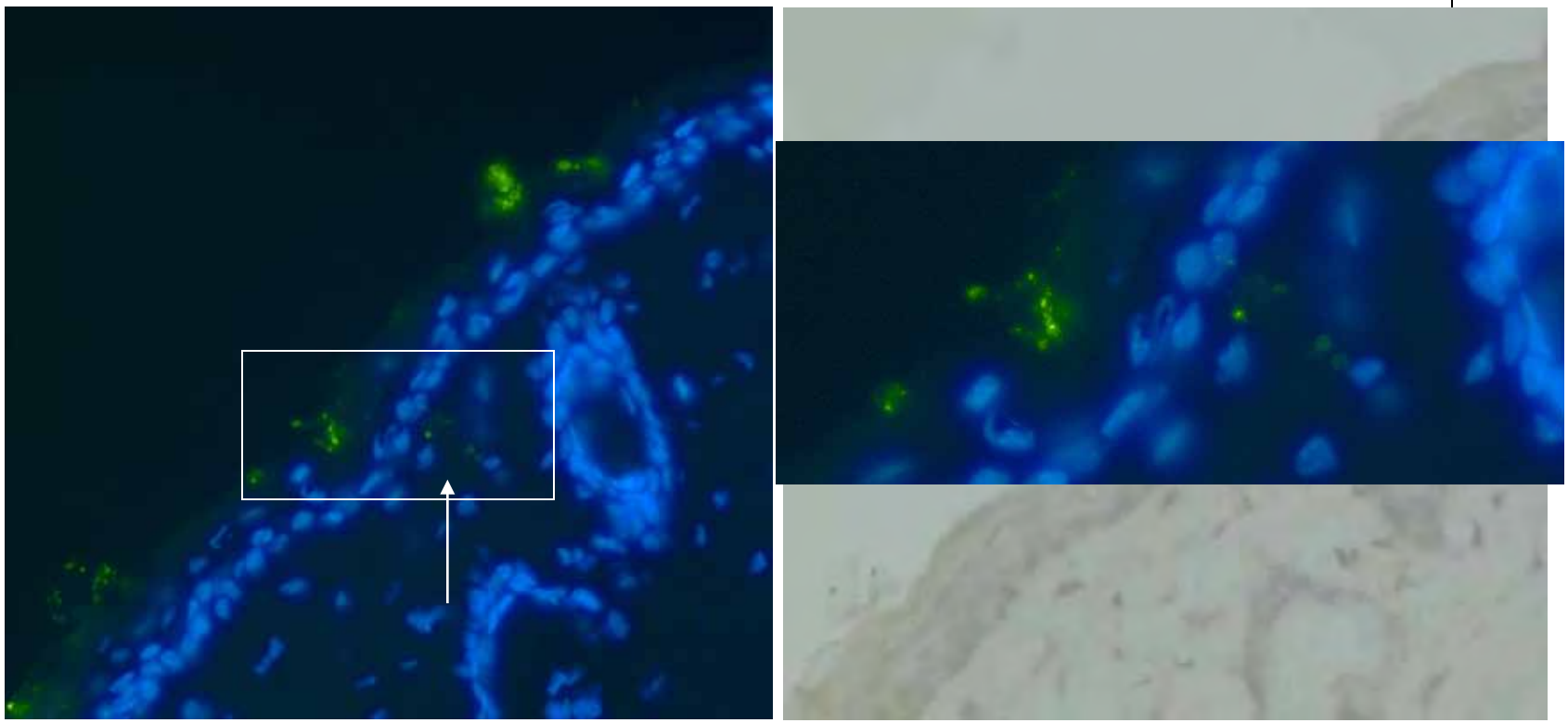


360mJ/cm^2 UVB
DHLA QD 620





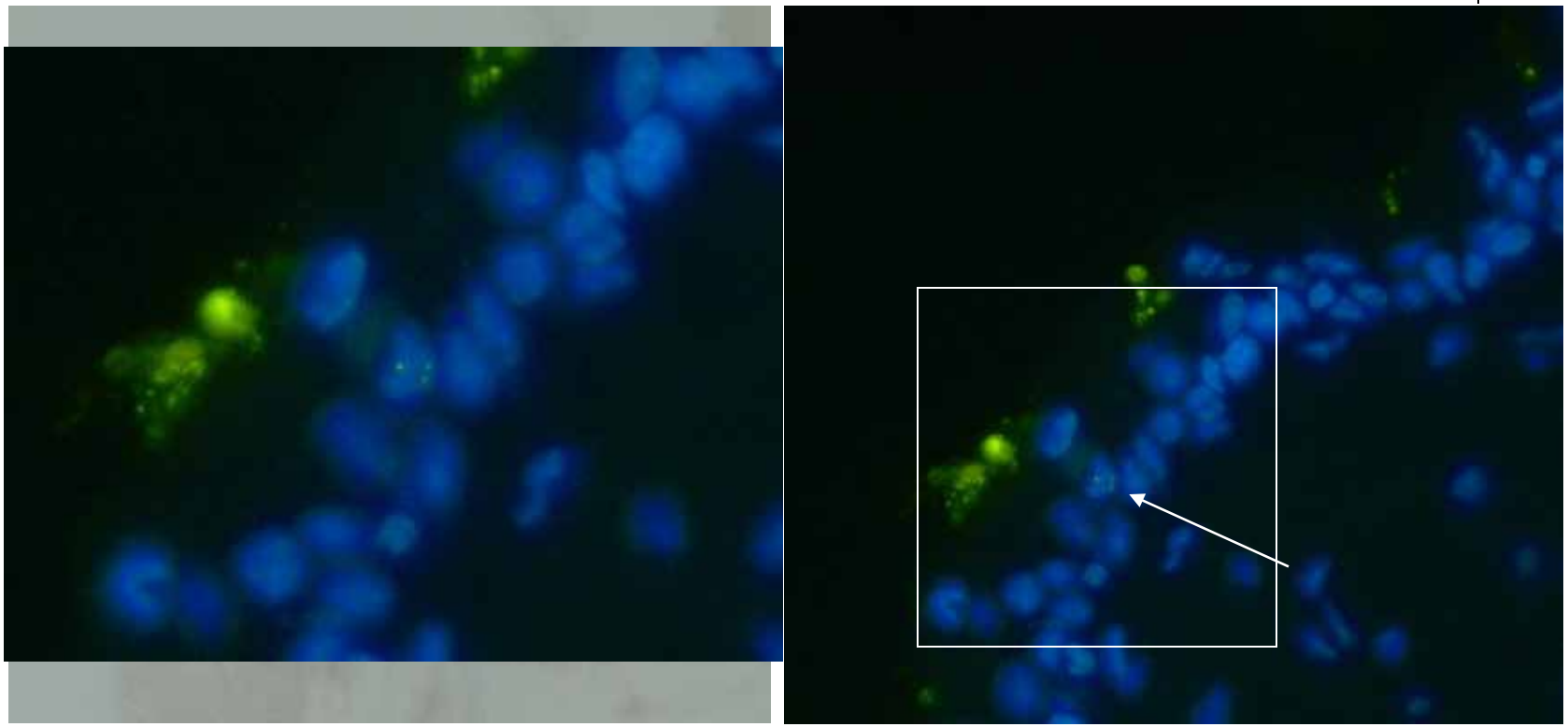
UV exposed, 24hr after COOH



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

40X

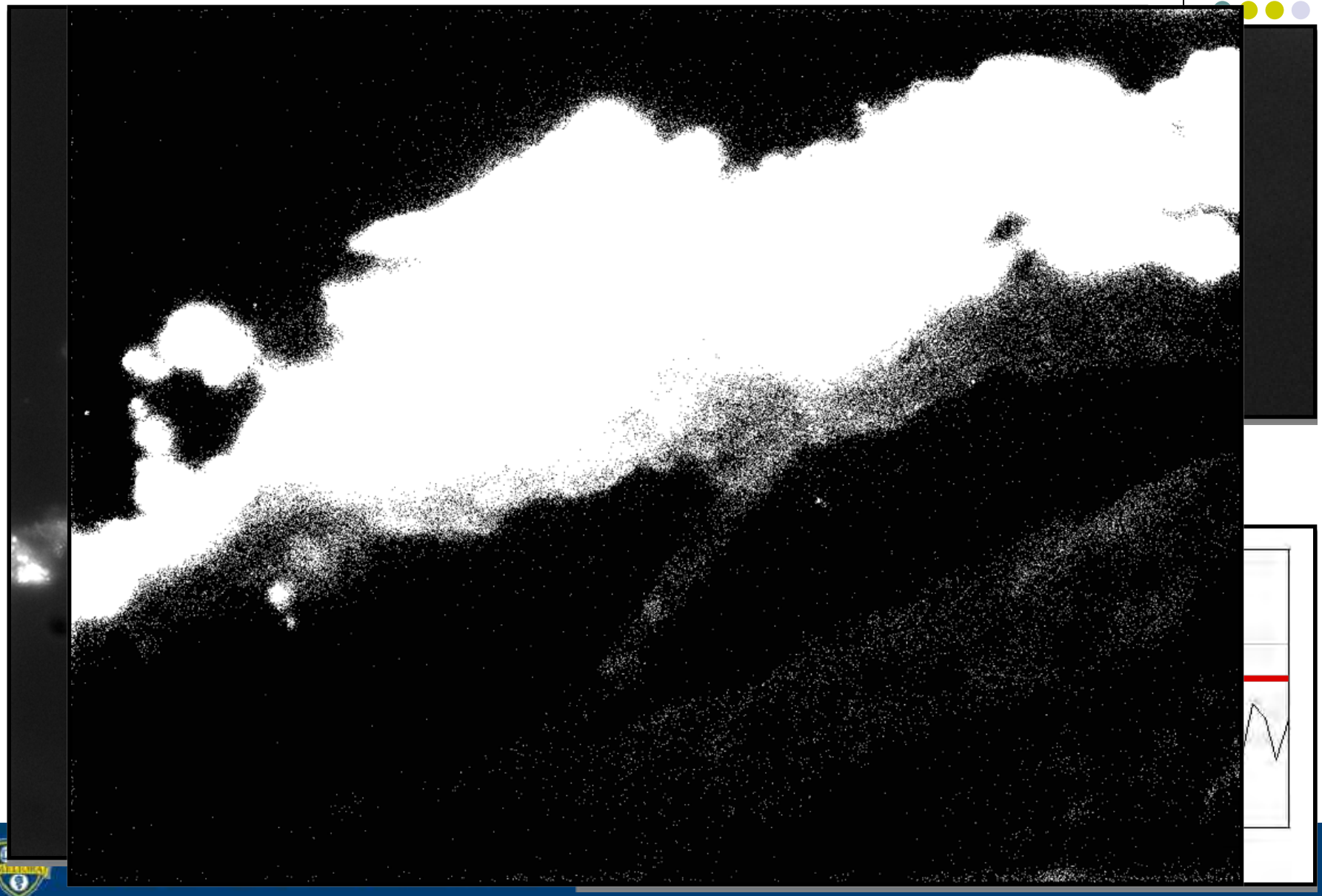
UV exposed, 24hr after QD-COOH Cryosection, DAPI



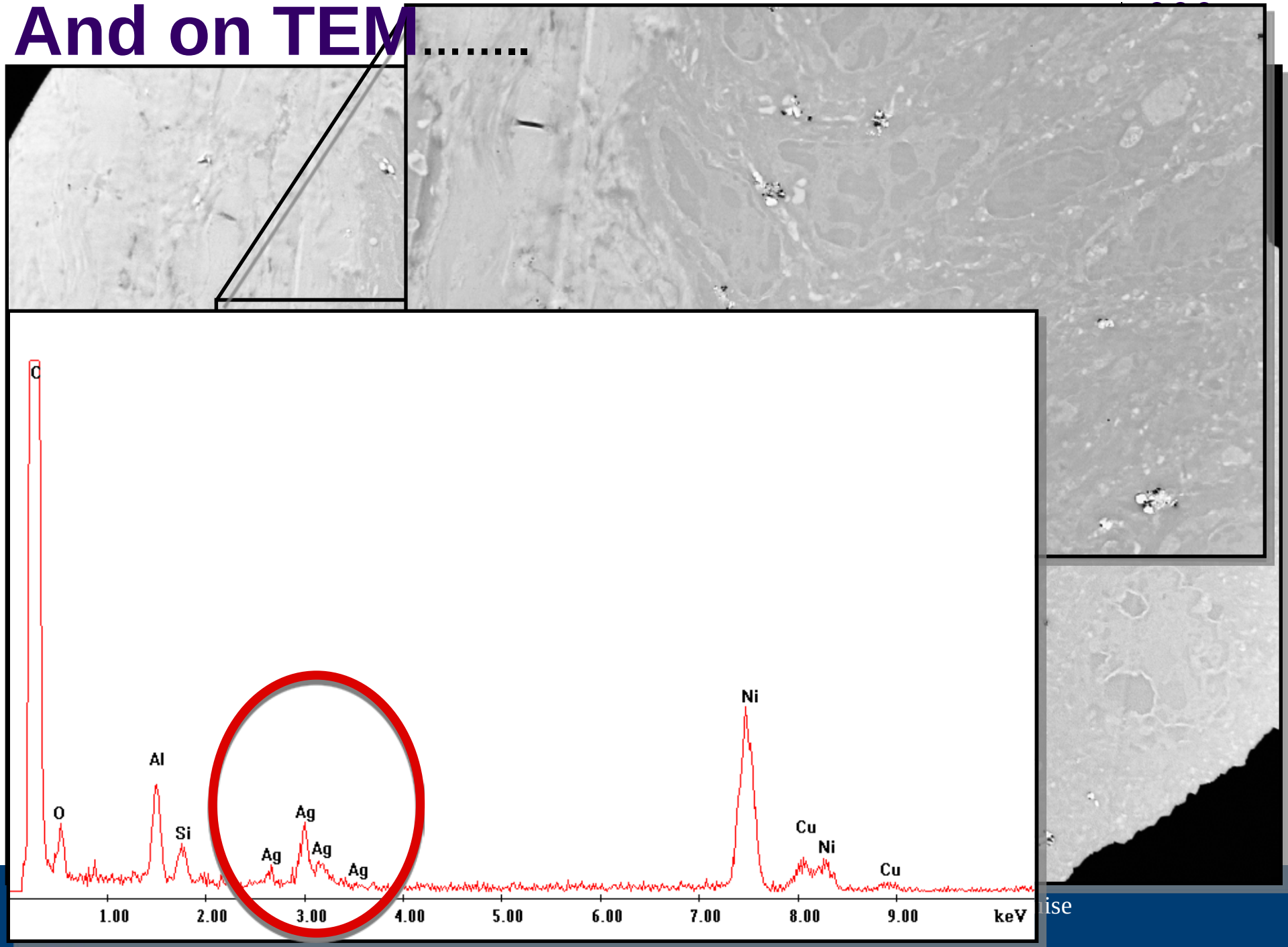
- QD fluorescence associated with cell nucleus
- Are these aggregates?

40X

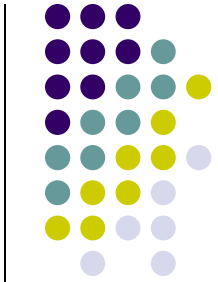
The Trouble Is...



And on TEM.....


C
O
Al
Si
Ag
Ag
Ag
Ag
Ni
Cu
Ni
Cu
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
keV

Science and Technology Barriers



Further Points

- Instrument calibration – NM limit of detection
- NM amplification strategies
- Whole tissue imaging
- Innovative NIR technologies
- Instrumentation needs to be smaller, more portable, and less expensive than currently state.

State of the Science - Models

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

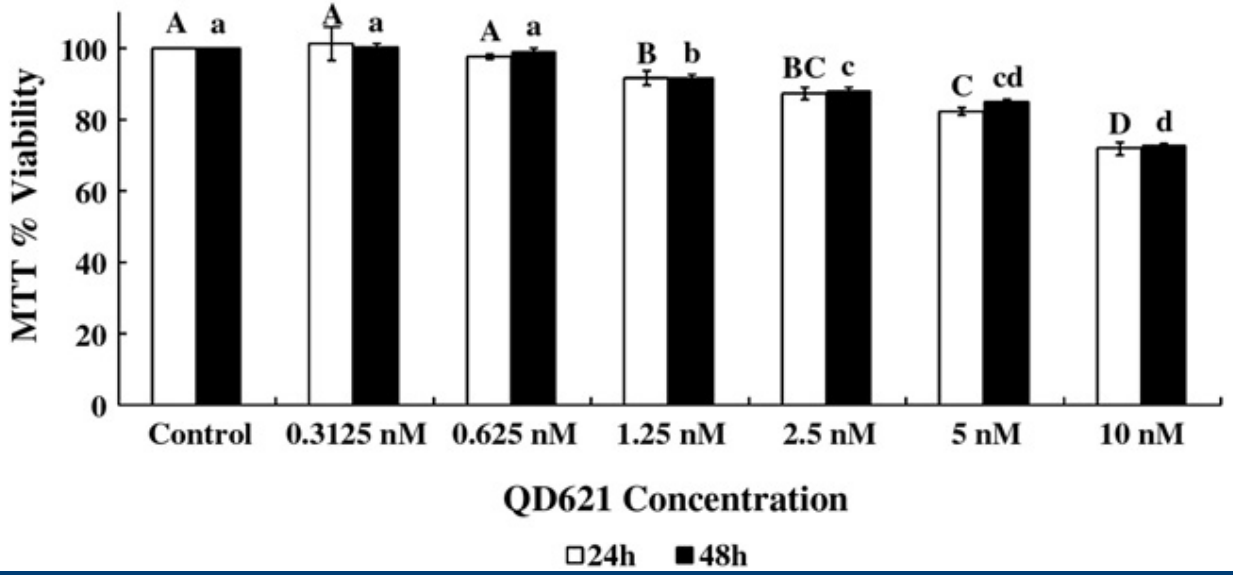
In vitro cell culture ubiquitously used to:
Screen NM cytotoxicty
Uptake mechanisms

State of the Science – Models

Problem #1 - Dose

- Cytotoxicity studies done at acute NM 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

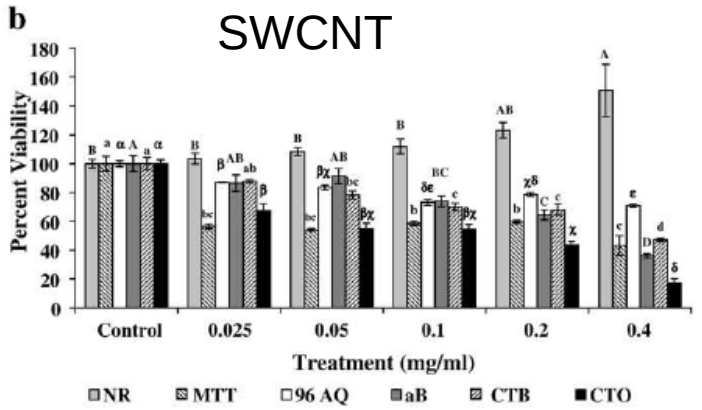
Problem #2 Assay Validity

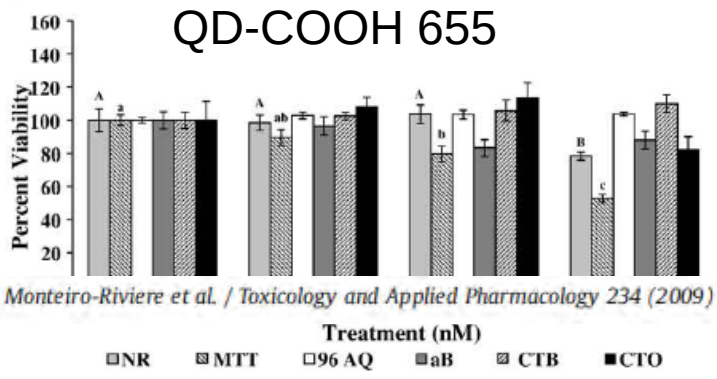
- NM can interfere with standard assays used to quantify cytotoxicity
- HEK line

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.





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

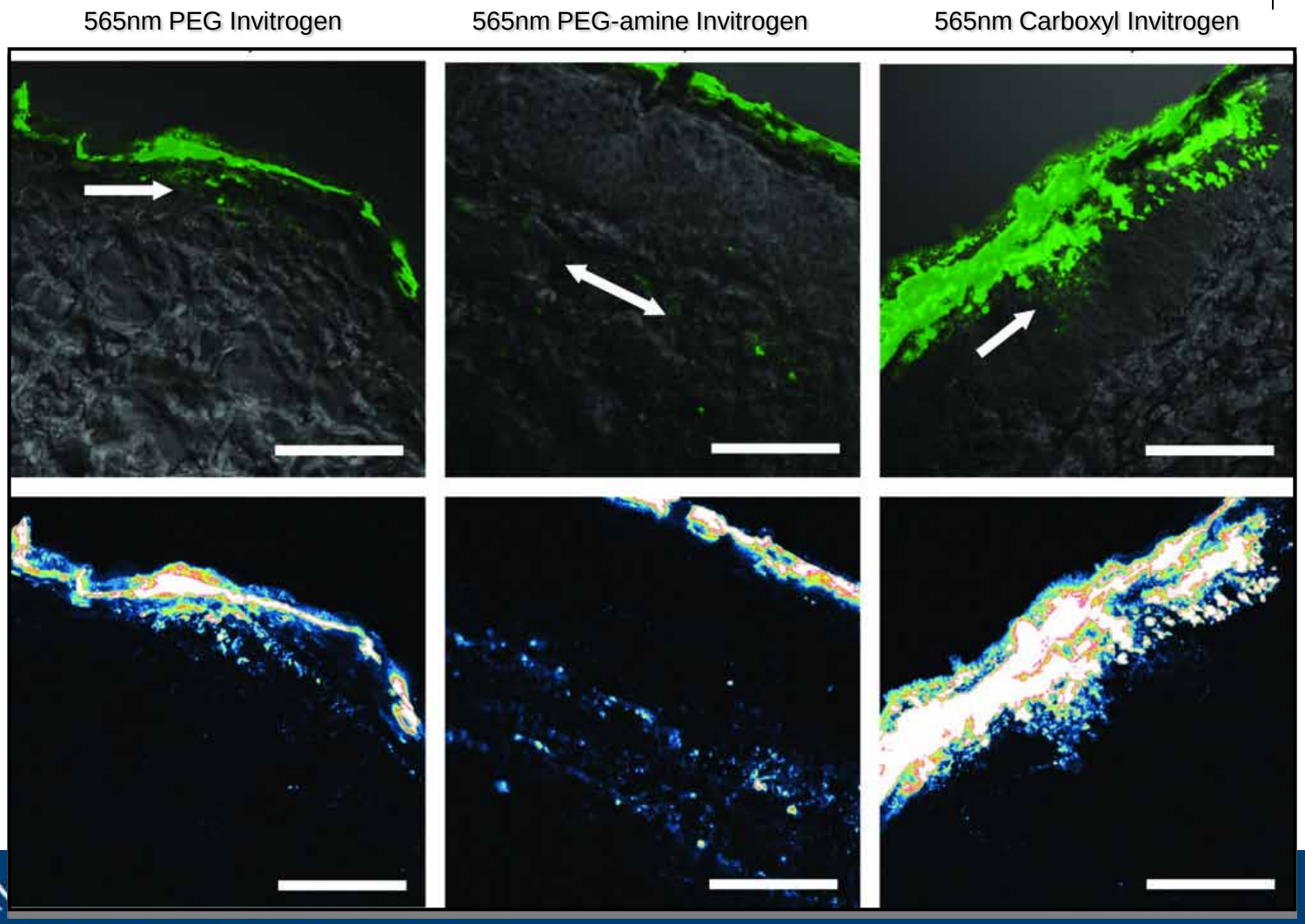
State of the Science – Models

Ex vivo Tissue Models

- Pig, human skin – they have different follicular density, lipids, thickness etc.
- Storage – frozen skin is dead
- Application of materials – no standard vehicle
- Sample Processing – can introduce artifacts
- Few models of barrier impaired skin (physical, chemical or diseased)

QD Ex vivo Pig Skin Penetration

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

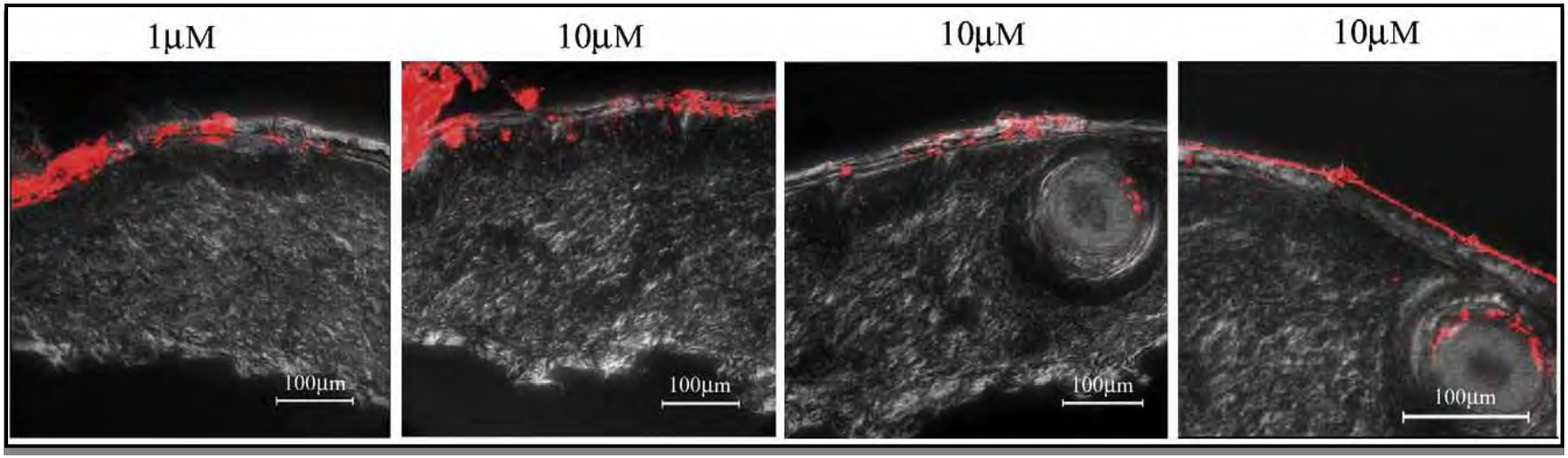


JP Ryman-Rasmussen, et al. Toxicol Sci **2006**, 91, 159.

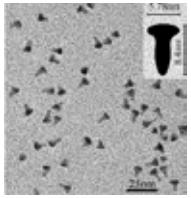
QD Skin Penetration

- 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
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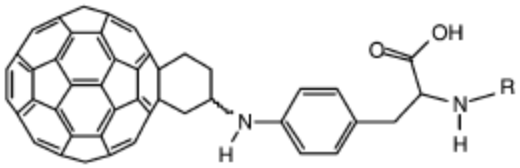
155−160

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

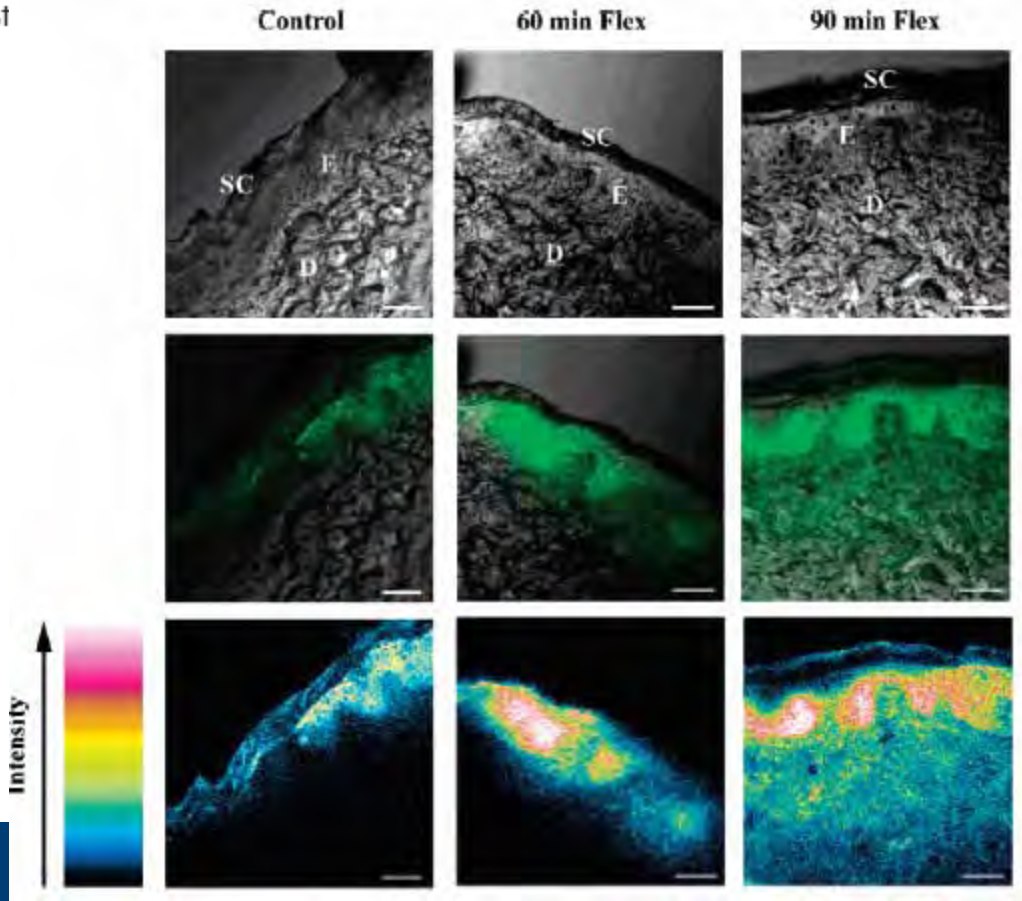
Jillian G. Rouse,[†,‡] Jianzhong Yang,[§] Jessica P. Ryman-Rasmussen,[†] Andrew R. Barron,[§] and Nancy A. Monteiro-Riviere*[,†]



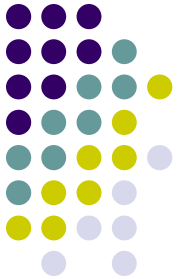
R = H (Baa, **1a**); C(O)O^{t}Bu (N-Boc-Baa, **1b**)

FITC
conjugated- NLS
(PKKKRKV)

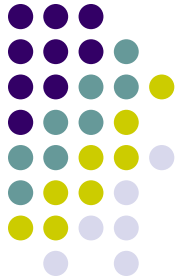
Confocal Images



Science and Technology Barriers



- Standardized viability assays
- Need to quantify tissue penetration at realistic exposure/dose levels
  - short-term/high dose vs. long-term/low dose
- Need models to quantify NM epithelial penetraion (skin, respiratory and GI) in diseased state or barrier comprised state
- For skin – no studies to date on lipid NM interaction, photostability of NM
- How does conjugation to NM effect stability and penetration?



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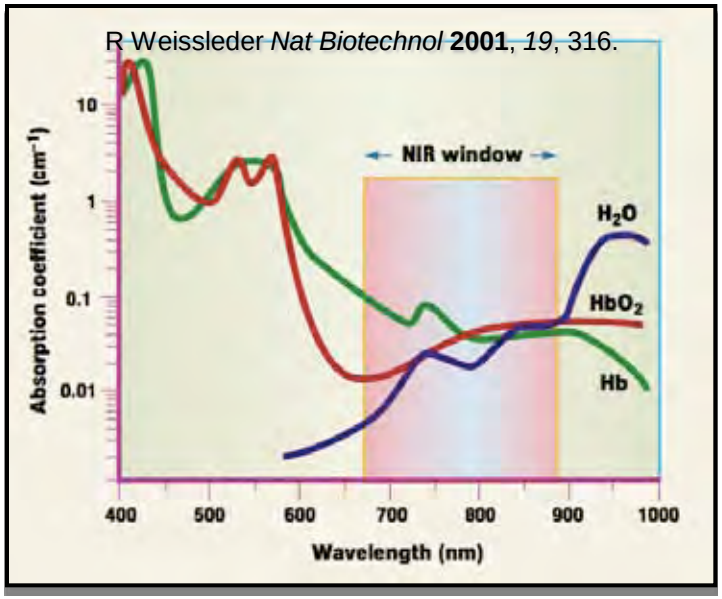
State of the Science

- 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 TiO2 ex 720 nm, em 410-600 nm
- Wheat root ex 710 nm, em 500-530 nm

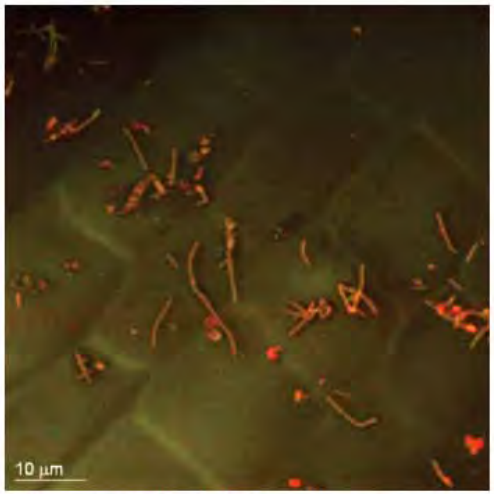


FIGURE 1. Unstained MWCNTs at the surface of a living root. Individual and aggregated MWCNTs are shown in orange, and the root surface is shown in green. The MWCNTs were detected and imaged using two-photon excitation microscopy combined with root and MWCNT autofluorescence.

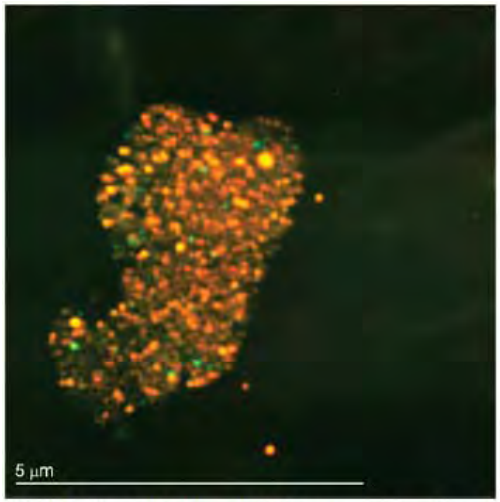


FIGURE 2. Unstained aggregated TiO_2 nanoparticles detected and visualized using two-photon excitation microscopy and autofluorescence.

Visible emission fluorescence still prone to high background, but one can take advantage of excited state lifetime differences to gate NM detection.

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

Moving Forward – What is needed?

Nanomaterials Synthesis and Characterization

- Explosive growth of various NM with varied properties
- Can not test all materials
- Need objective descriptors (composition, synthesis method, size, shape etc.) to classify and group NM to predict biological activity/stability/risk

State-of-Science

- Characterization of NM Physical Properties
  - Size – light scattering, TEM, AFM
  - Surface Area – BET gas adsorption (hardly ever reported)
  - Charge - Zeta potential
  - Quantum Yield (rarely measured/reported)

These properties will strongly effect surface reactivity

State of the Science

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

Pulmonary toxicity

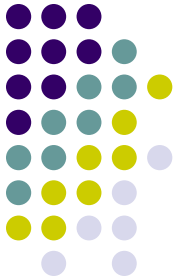
- Oberdörster et. al. (2005) - particle surface area
- Wittmaack et. al. (2007) -particle number
- Warheit et. al. (2009) - chemical reactivity

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.

Science and Technology Barriers



Nanomaterials Synthesis and Characterization

- *Greater emphasis for researchers to quantify NM properties as vendor spec are often inaccurate or unavailable.*
- Methods to measure adhesion force between particles.
- Methods to determine tendency to agglomerate/aggregation to sizes larger than NP.
- Methods to measure agglomerate/aggregation on inorganic/biological surfaces under environmental/physiologic conditions (eg. skin pH, sweat etc) .
- How to control agglomerate/aggregation?
- Methods to quantify redox surface chemistry.
- Are they soluble?
- How does fluorophore conjugation effect properties ?
- What role do contaminants have in determining toxicity?
- How does shape and size effect accumulation in soil, in polymers (clothes, carpets etc)., hair follicles etc.
- How does drag/mechanical force (foot traffic on carpet, massaging skin) effect accumulation, adhesion, agglomerate/aggregation

QD Limit of Detection

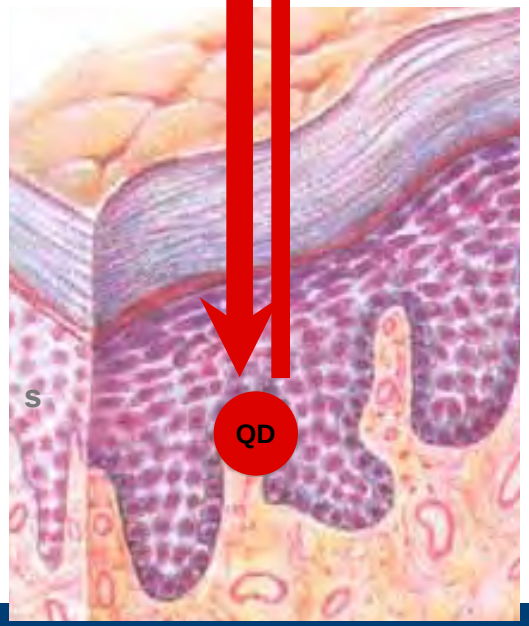
$$F_{out} = 8.89 \times 10^{-4} mW$$

Near-IR Wavelength

$$F_0 = 500 mW$$

Detector

Laser



Tissue Scattering

$$\frac{1}{e^{-2\mu_s' z}} \qquad \mu_s' = \text{Scattering Coefficient}$$

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

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

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**

Moving Forward – What is needed?

Nanomaterials and the Environment

- Q: Are there good models for long term bioaccumulation / chronic exposures?
- Q: What do we know about the life cycle in the environment?
- Under what conditions do air borne NP deposit onto surfaces (leaves, tissue etc)
- Novel methods to image NM in plant tissues needed.
- How to identify and quantify NP in complex media (soil, tissue, water, air)?

EPA Recent Awards

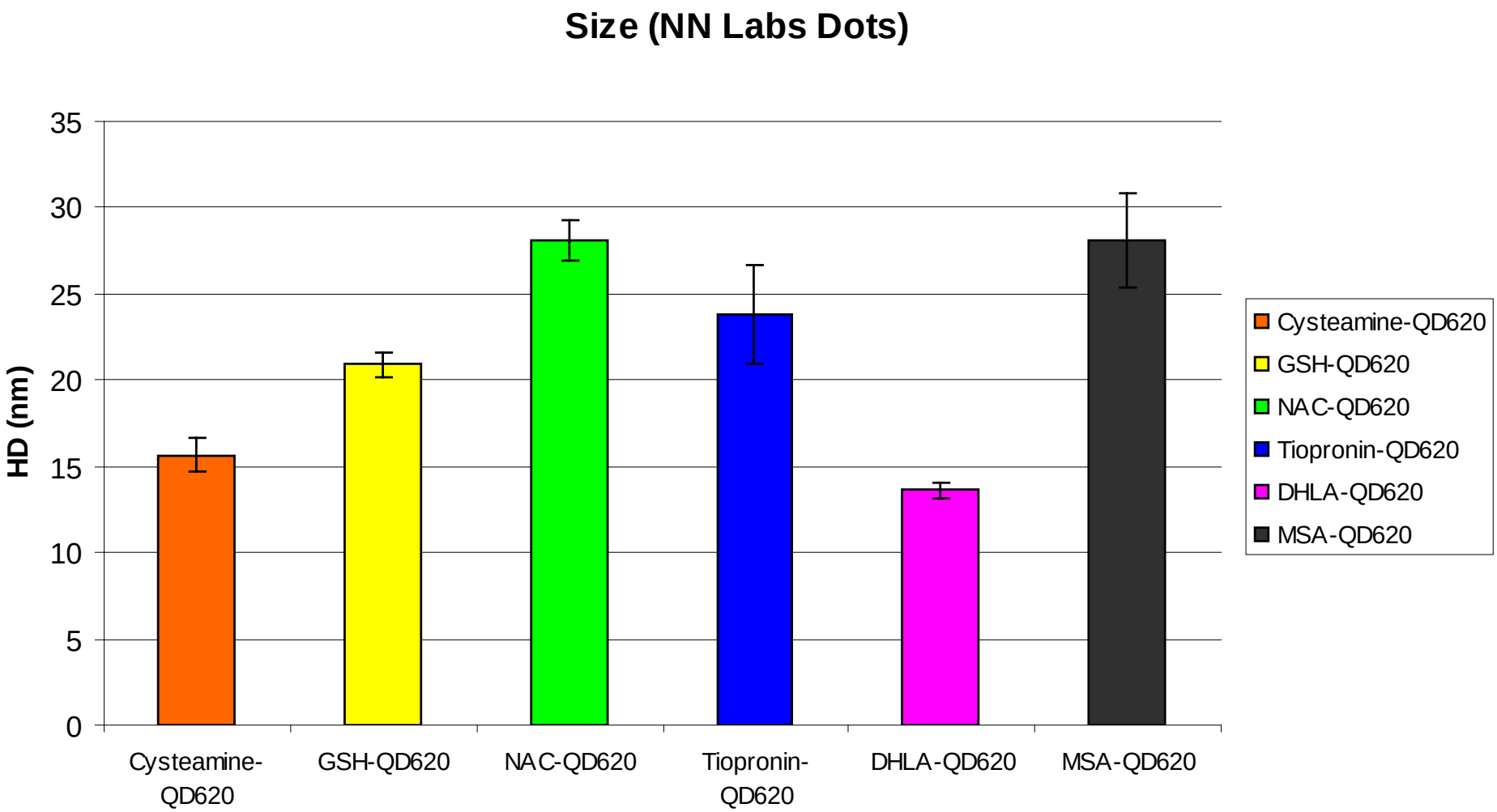
http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.rfa/rfa_id/461

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

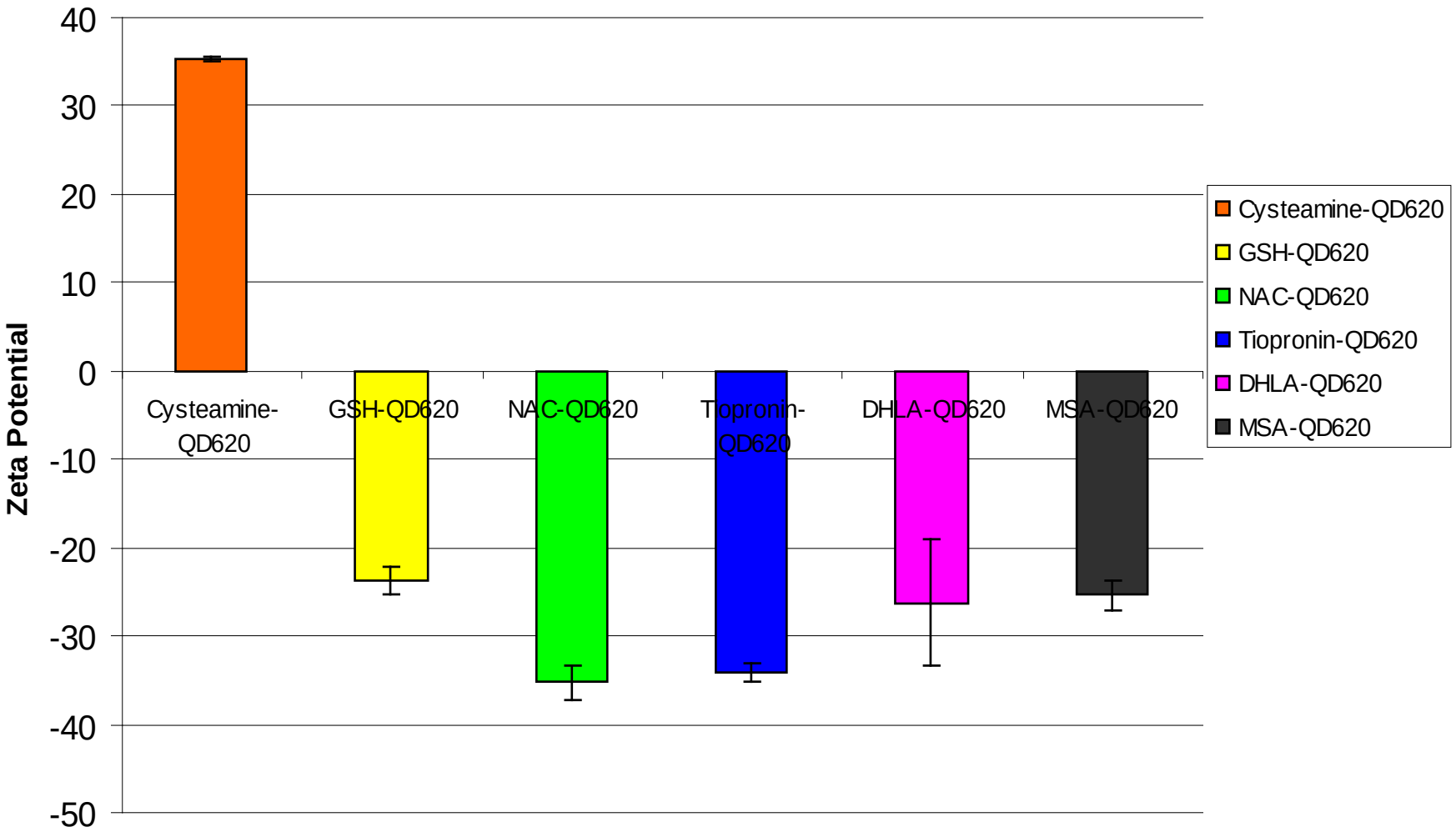
Critique of the NNI Strategy Document

Risk Management Methods

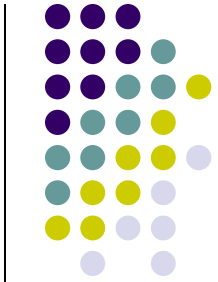
- Operate with maximum caution
- Employers undertake risk assessment take into acct 10 factors (Giacobbe et al. Hum exp toxicol 2009; 28; 401)
  - Physiochemical NP properties
    - dimension and concentration of NP, NP behavior (aggregation, agglomeration), toxicological characteristic of substance, risk of fire/explosion,
  - Work place
    - Number of exposed workers, frequency of exposure, freq. of direct handling, effectiveness of personal protection devices, work organization/procedures,, suitability of workplace.


Size (NN Labs Dots)
HD (nm)
35
30
25
20
15
10
5
0
Cysteamine-QD620
GSH-QD620
NAC-QD620
Tiopronin-QD620
DHLA-QD620
MSA-QD620
Cysteamine-QD620
GSH-QD620
NAC-QD620
Tiopronin-QD620
DHLA-QD620
MSA-QD620

Charge (NN Labs Dots)



Critique of the NNI Strategy Document



5 Research Needs (Priority my opinion)

- Exposure Assessment (1)
- Instrumentation, Metrology, and Analytical Methods (2)
- Nanomaterials and Human Health (3)
- Nanomaterials and the Environment (3)
- Risk Management Methods (4)

Should there be a 6th?

- Nanomaterial Synthesis and Characterization

This would be a cross cutting category supporting research in other categories

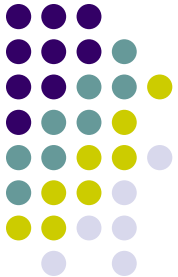
Critique of the NNI Strategy Document

- Many projects (>50%) funded in 2006 seem to vaguely fit the 5 Research Needs, eg. cancer therapeutics and BioMEMs neural probes are not directly relevant. Therefore gap analysis over estimates currently funded EHS activities.

- What does portfolio look like subsequent to 2007 EPA/DOD/NSF EPA-NSF-DOE Research Solicitation: "Nanotechnology Research Grants Investigating Fate, Transport, Transformation, and Exposure of Engineered Nanomaterials": $6M, ~ 30 awards
  - EPA-G2007-STAR-R1 Environmental and biological fate, transport, and transformation
  - EPA-G2007-STAR-R2 Human exposure/bioavailability

- Focus should be on what research should be done, not what are we doing and how does it fit in which our objective. The end goal is NM Risk assessment and management therefore critical input data needed for analysis.
- Support Industry/Academic/International collaborations? Minimize duplication of effort.

Science and Technology Barriers



Exposure Assessment

- Research funding should be guided by materials that are likely to are economically important and those that are actively investigated academics and industrial R&D and in manufacturing. (feel this is short term need)

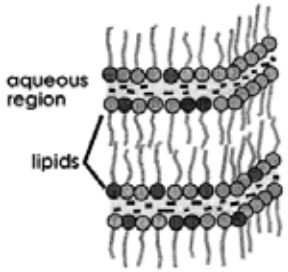
- How to account for NM exposures in academic setting?
  - A: from published literature (Pubmed search) it is clear the NM research rising CNT>QD

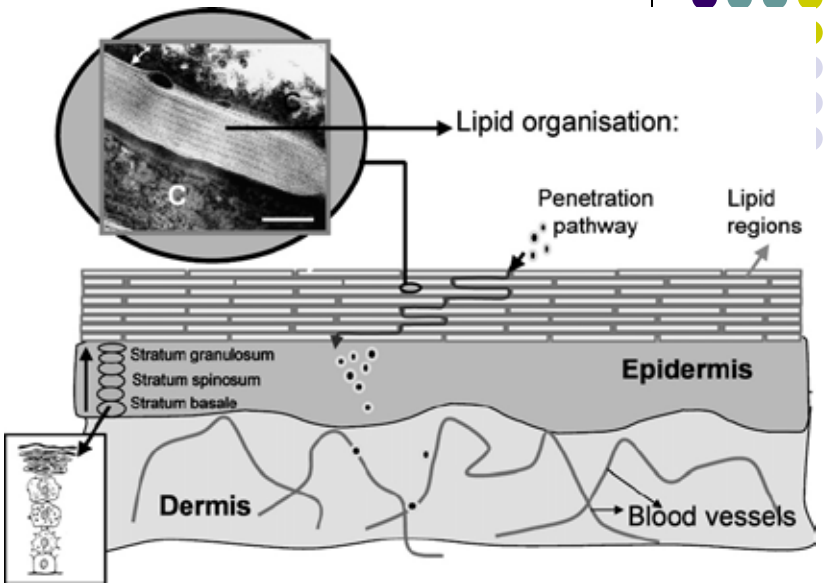
- Need: Research on consumer products
  - Are NP shed from products?
  - Do shed NP exhibit similar properties to raw material?

Premise

- Analytical methods for identifying and measuring the critical parameters related to NM in biological systems, the environment, and the workplace are not well developed or readily available.
- As a result, important metrics are infrequently or inaccurately reported.
- Further development of these methods is critical to all nanoEHS research.

Skin Barrier Function





SC

Gap junctions (<2 nm)

Lipid lamellar structure

Skin appendages

Hair follicle privileged site for accumulating NP

Scarred skin less transdermal drug delivery

External force - Mechanical Flexing and Massage

Skin Condition

- **hydration**
- **pH**
- **salt**
- **inflammation (UV, disease)**

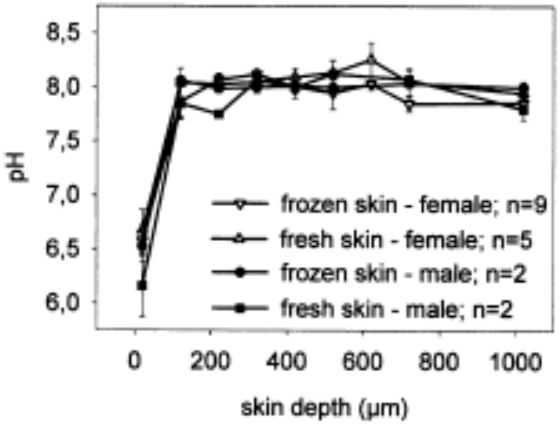
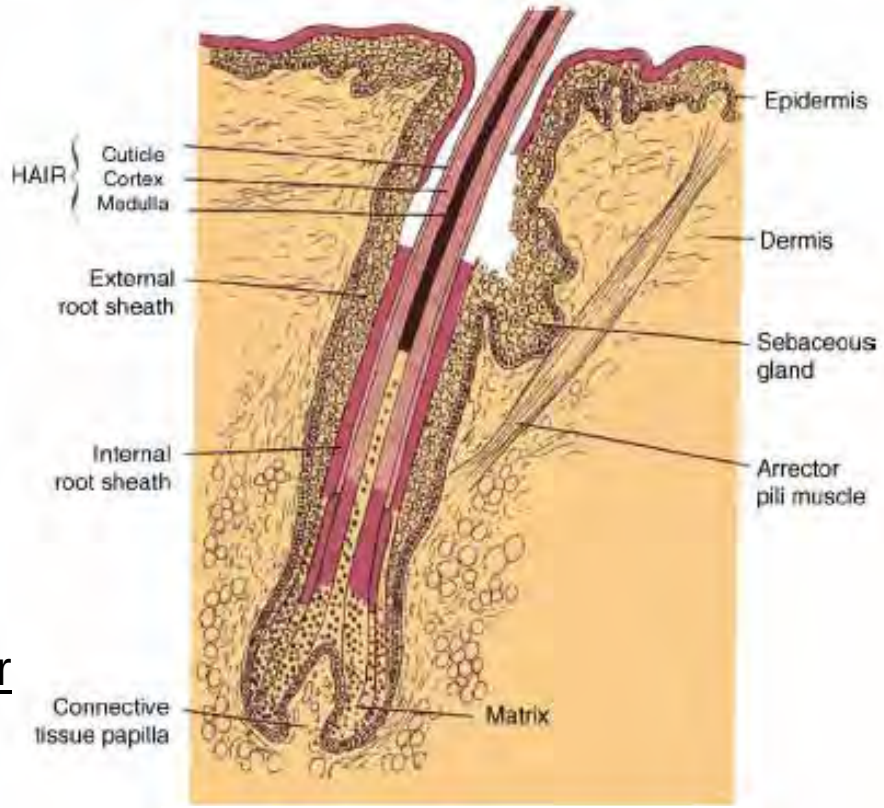


Fig. 2. pH profiles across human deeper skin layers in vitro (pH value ± SE versus skin depth (µm); 1–4 skin flaps with 2–3 replicates each).

Follicular Accumulation/Penetration

0.1 – 1.5 % skin surface
Racial and body site differences

- Efficient reservoirs for accumulation of substances - notably solid particulates.
- Accumulation and penetration depth are size dependent.
- Accumulation and penetration is enhanced by mechanical flexing.
- Substances applied in particulate form accumulate in the hair follicle and last longer than substances applied in pure form.



V.M. Meidan et al. / International Journal of Pharmaceutics 306 (2005) 1–14

Nanoparticles – An efficient carrier for drug delivery into the hair follicles

Juergen Lademann [a,*], Heike Richter [a], Alexa Teichmann [a], Nina Otberg [a], Ulrike Blume-Peytavi [a], Javiana Luengo [b,c], Barbara Weiß [b], Ulrich F. Schaefer [b], Claus-Michael Lehr [b], Roger Wepf [d], Wolfram Sterry [a]

[a] *Charité-Universitätsmedizin Berlin, Department of Dermatology, Berlin, Germany*
[b] *Saarland University, Department of Biopharmaceutics and Pharmaceutical Technology, Saarbruecken, Germany*
[c] *Universidad de Concepción, Facultad de Farmacia, Concepción, Chile*
[d] *Beiersdorf AG, Hamburg, Germany*

Received 14 June 2006; accepted in revised form 20 October 2006

Ex vivo Pig Ears

Massage

320 nm NP FITC

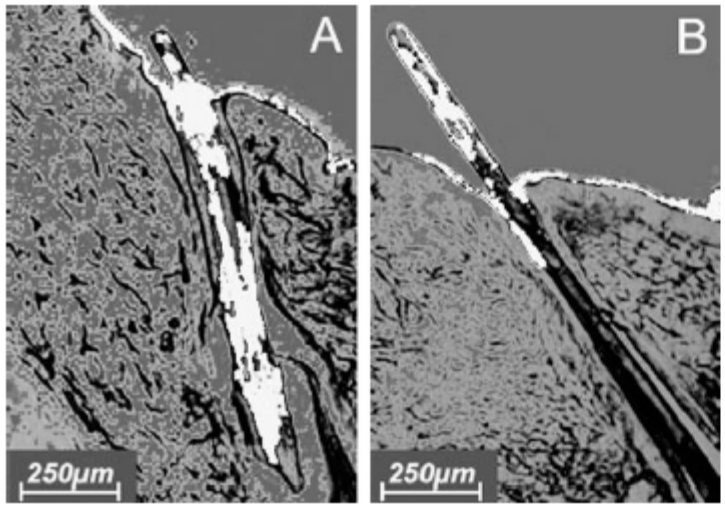


Fig. 1. Superposition of a transmission and fluorescent image, demonstrating the in vitro penetration of the dye-containing formulation into the hair follicles of porcine skin after application of a massage. (A) Dye in particle form. (B) Dye in non-particle form.

No Massage

320 nm NP FITC

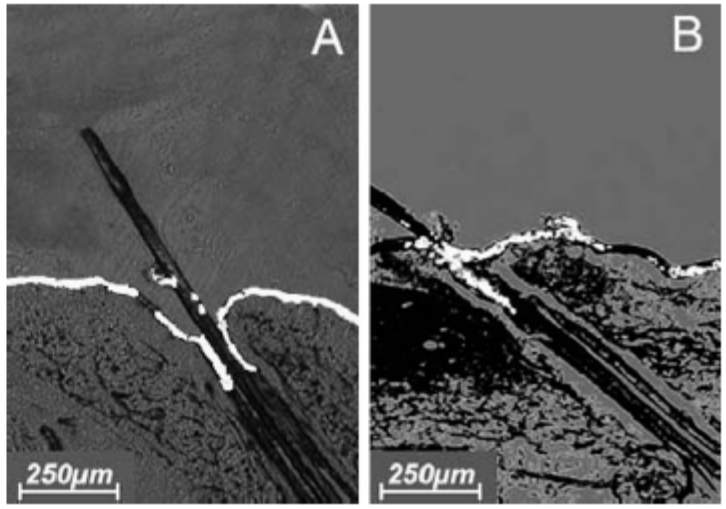
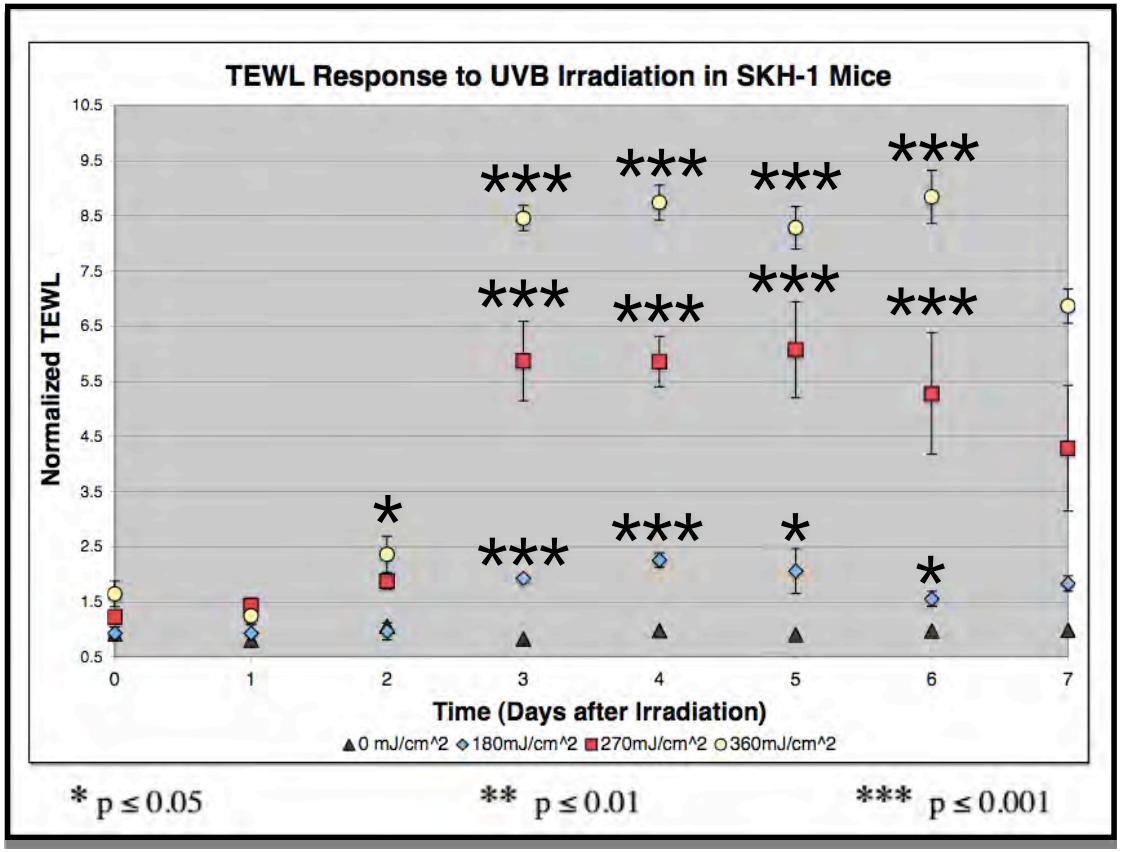


Fig. 3. Superposition of a transmission and fluorescent image, demonstrating the in vitro penetration of the dye-containing formulation into the hair follicles of porcine skin without massage. (A) Dye in particle form. (B) Dye in non-particle form.

TEWL - UVB Induced Barrier Damage



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

State of the Science – Models

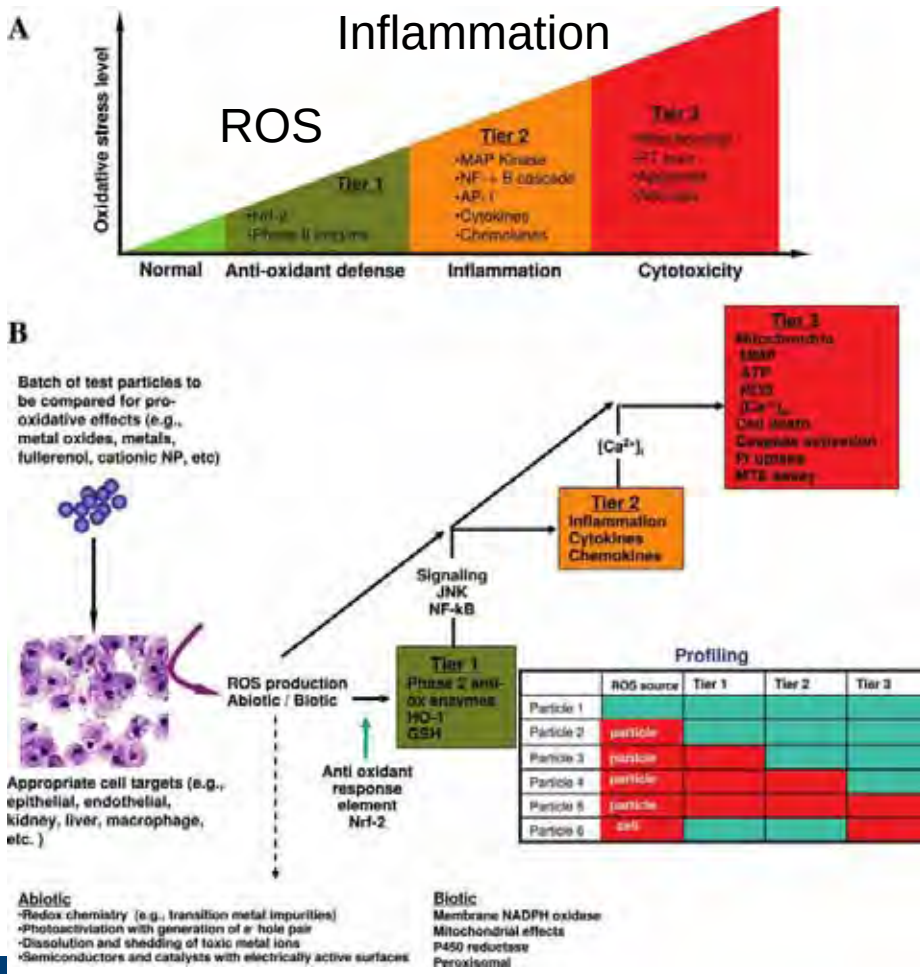
In vitro cell culture ubiquitously used to:

- Screen NM cytotoxicty
- Uptake mechanisms

Are the results useful are these results?

Nel's "Hierarchical Oxidative Stress Paradigm"

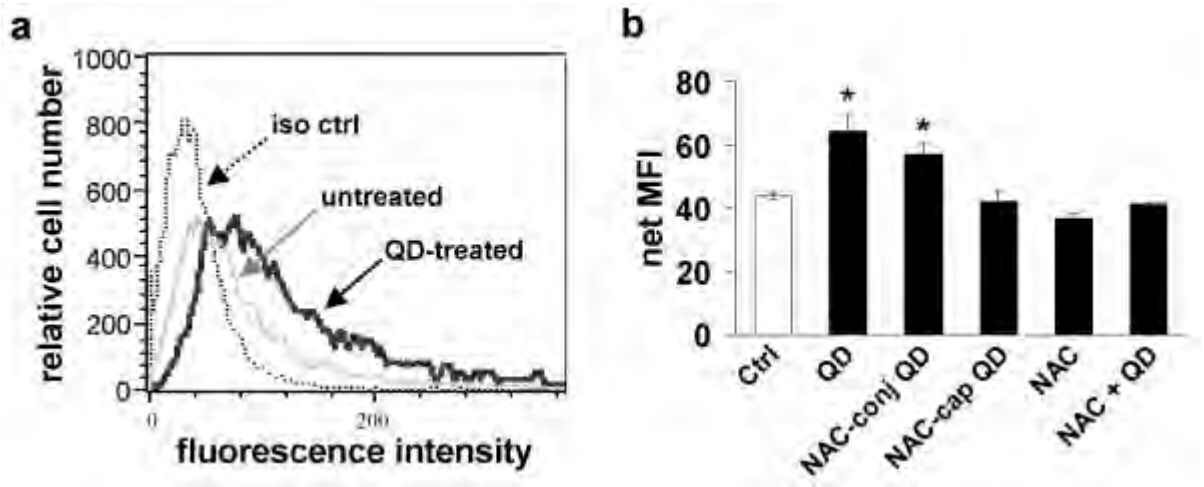
If a link exists between an in vivo disease (eg. allergic airway inflammation) and a mechanistic pathway at the cellular level (eg. oxidative stress) one can use a cell line (bronchial epithelial cells, macrophage) for NM high-throughput screening.



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

FACS - Limitation

Flow cytometry used to quantify NM uptake using fluorescence,



Choi *et al. Journal of Nanobiotechnology* 2007 **5**:1

But….uptake or associated?