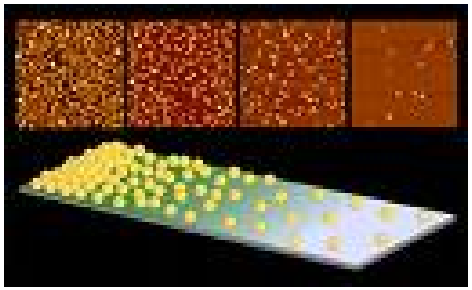
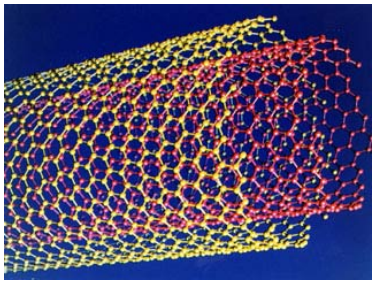
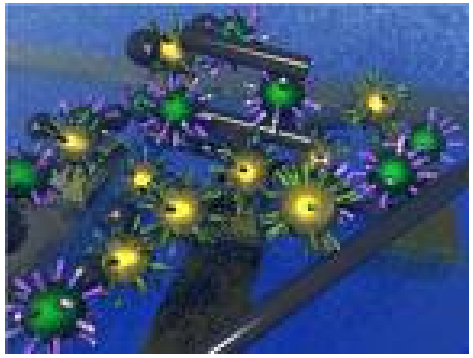
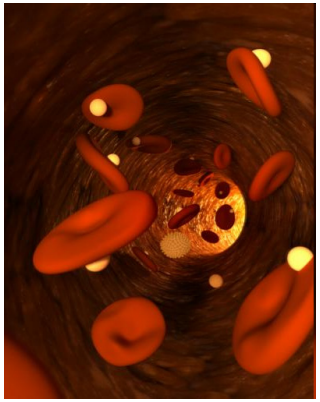
The NIST NanoBio/NanoTox Working Group

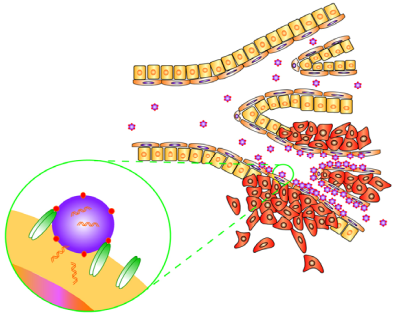


Current and Future Directions…





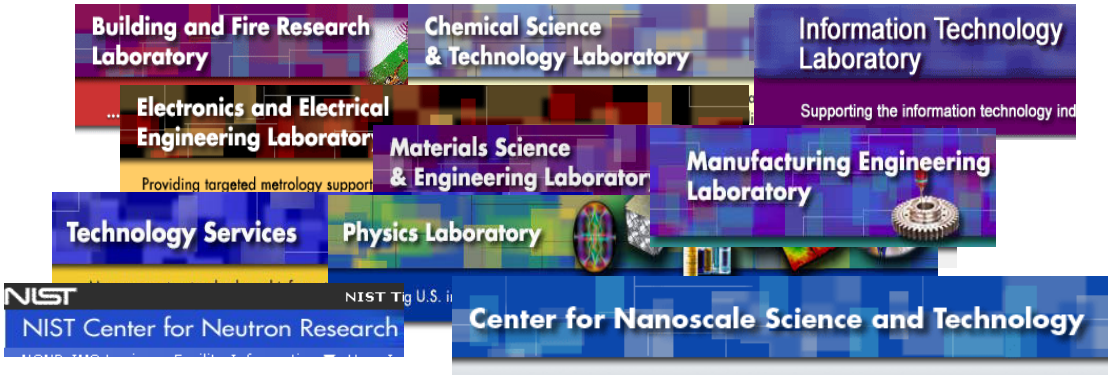




John T. Elliott
Biochemical Sciences Division/
Cell Signaling Systems
NIST
Gaithersburg, MD 20902

ERC Teleseminar Aug 20, 2009

What is NIST?

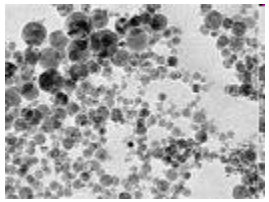


- Department of Commerce
  - **NIST's mission:** To develop and promote measurement, standards, and advanced technology to enhance productivity, facilitate trade, and improve the quality of life.
- Addressing measurement infrastructures
  - **Sources of measurement variability**
    - Analysis of Measurement Chain
    - Standards for interlaboratory comparability
  - **New measurement technologies**
    - Confirmatory and improved measurements- “gold” standards
    - Multi-disciplinary group of scientist
  - **US commerce agenda**
    - Relevant to advanced new technologies
    - Standards and Reference Data
    - Independent 3rd party
    - International standards committees and interests

Nanotechnology in 2009

Types of nanomaterials:

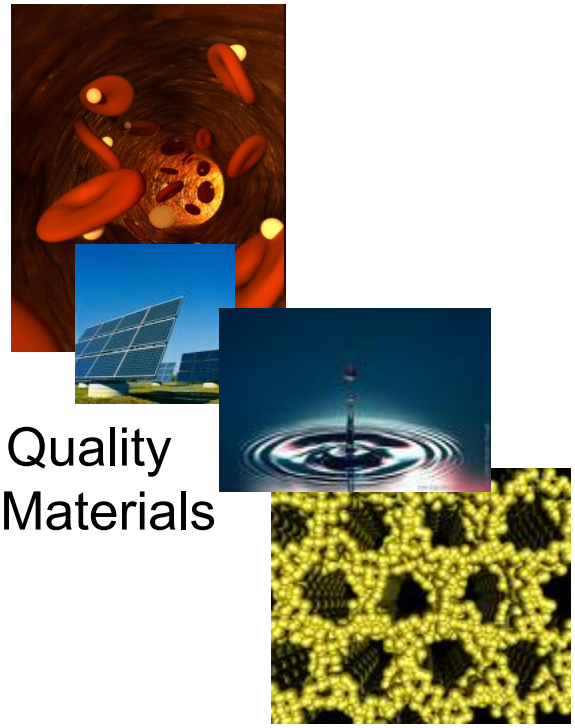
1) *Carbon based nanomaterials*
2) *Nanocomposites*
3) *Metals & alloys*
4) *Biological nanomaterials*
5) *Nano-polymers*
6) *Nano-glasses*
7) *Nano-ceramics*



•Nanoparticles are in 1-100 nm range
•Unique properties

Impact:

Health
Energy
Water Quality
New Materials



- Nanotechnology is revolutionary
- Advanced instrumentation and procedures are available
- Are there any unexpected consequences?
  - Environmental issues
  - Health/biology hazards

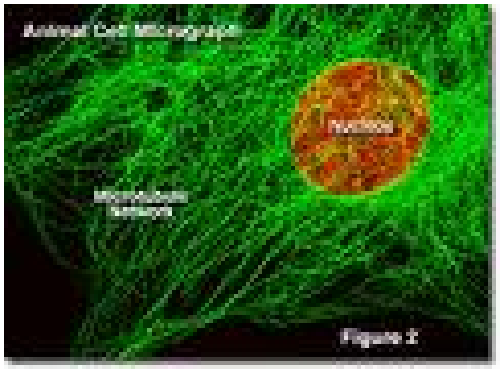
Why is Nano-Bio Important?

Cell size: 30-100 um
Proteins: 2-20 nm
Cell membrane: 4 nm thick
DNA: 2 nm wide
Tubulin: 25 nm wide

Proteins

http://tbn2.google.com/images?q=tbn:KfNefBE2W688FM:http://upload.wikimedia.org/wikipedia/commons/e/e6/Spombe_Pop2p_protein_structure_rainbow.png

Stained Cell



Microtubules in green

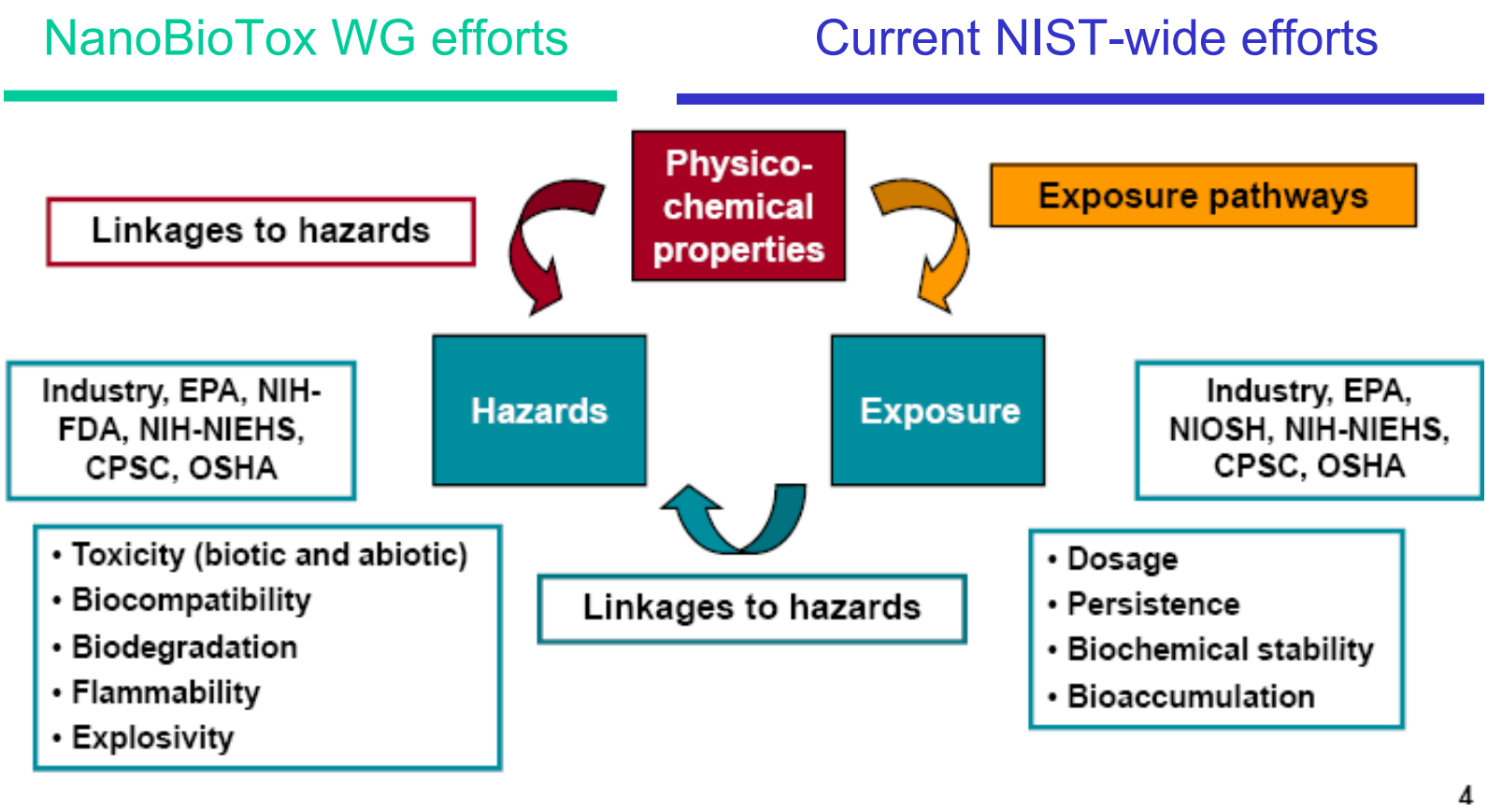
Biology is based on nanomaterials

Addressing the Health and Safety Issues-

- Nanotoxicology- toxicology in animal systems
  - Data most relevant to human toxicology
- Nano-cytotoxicology- toxic effects in cellular systems
  - Can provide molecular mechanism details and systematic studies
  - May not predict animal toxicity effects

NIST “nano” footprint (2009)

NanoEHS-Measurements and Needs



•All NIST laboratories have nanomaterial-based projects

Measurement issues in nano-cytotoxicity

- Problems are similar to the cytotoxicity testing of chemicals

- Main Objective: Develop predictive rules that link nanomaterial features to particular cellular effects

For example:

- Your nanomaterial has shape and surface coating similar to a carboxylated gold NP. It aggregates in aqueous buffer.
- Looking in a **predictive database**, we find that 50 nm carboxylated gold NP influence cell permeability, motility and accumulation in cells.
- Data provides leads for assessing cytotoxic effects.
- May have knowledge of how to change the NP to be less toxic!!

A Nano-cytotoxicology Testing Process

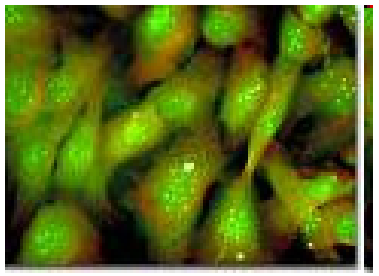
Nano-materials

•Reference materials
•measurements

Dispersion in cell culture media

•Protocols
•Highly controlled processes

Add to cells



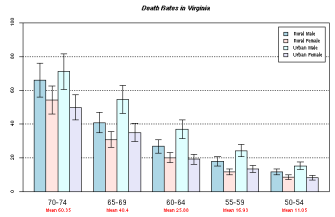
•Quality control in cell culture

•New measurements
•Protocols

•Instrument validation stds
•calibration standards stds

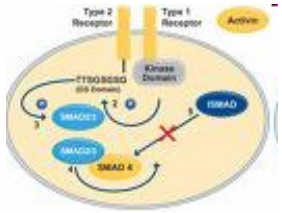
Data collection

•Appropriate statistics
•Procedures



Statistical data analysis

•Model development
•Database infrastructure



Knowledge/Meaning

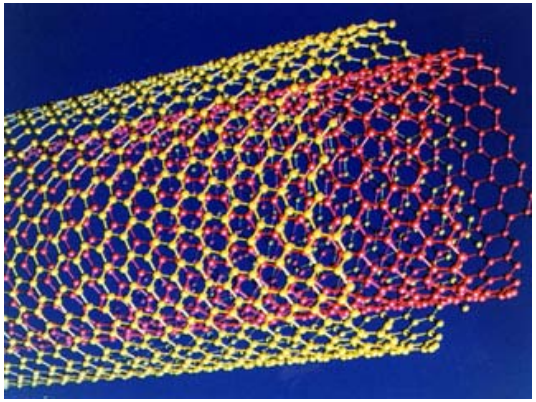
measurement infrastructure details

Who are NanoBio NanoTox Working Group?

- Started in 2007
- Approximately 60 NIST and external scientist in Nanobio NanoTox research
- Chemists, physicists, materials and surface scientist, biologists, biotechnology and medical.
- Discuss measurement issues, new technology and facilitate collaboration
- Members are associated with national and international standards groups

- ISO TC229: Laurie Locascio, Angela Hight Walker
- ASTM F04.46 Cell Signaling: John Elliott, Anne Plant
- APL, Univ of MD, NCI

Nano Reference materials



Currently available from NIST:
Polystyrene NP (down to 60 nm)
Gold/citrate NP (10 nm, 30 nm and 50 nm)

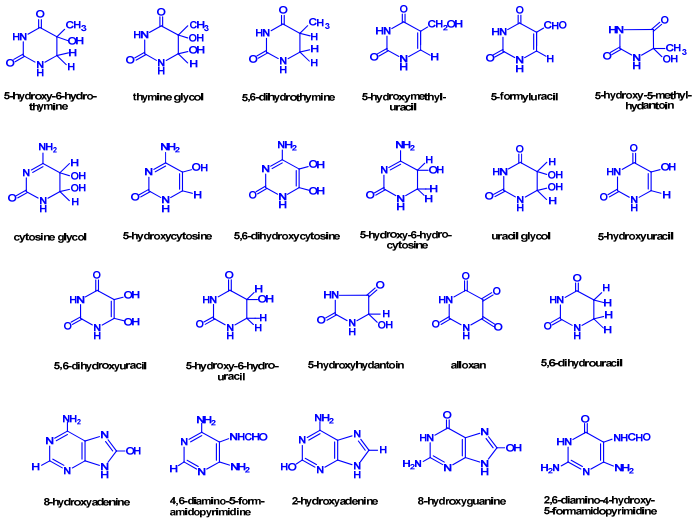
In process:
SW carbon NT (in 2% deoxycholate)

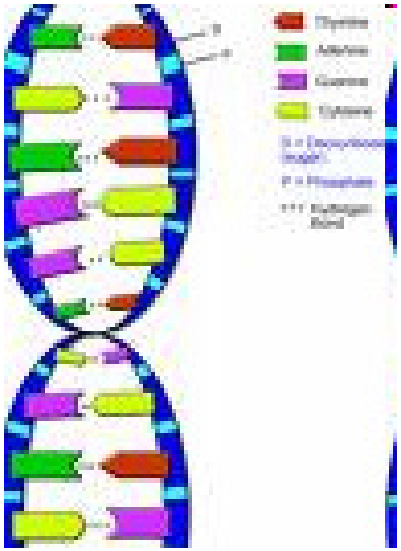
Future:
Si NP (4 nm, fluorescent)
TiO2 NP
Ag NP

•Metal, polymer, ceramic, etc particles available from NanoBioTox members

Genotoxicity

Do nanoparticles induce DNA lesions?

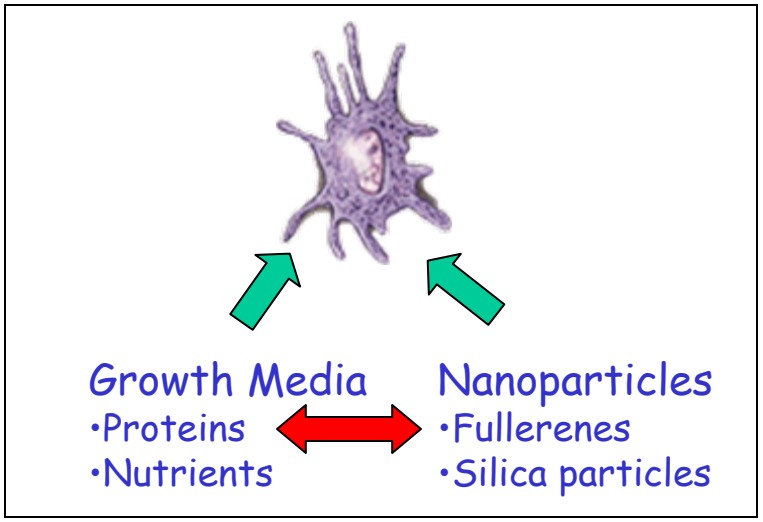


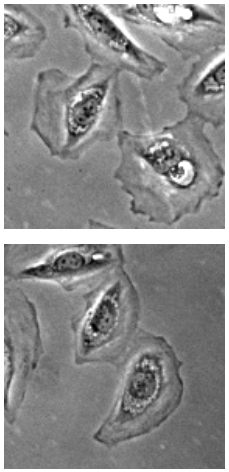


Contact: Bryant Nelson

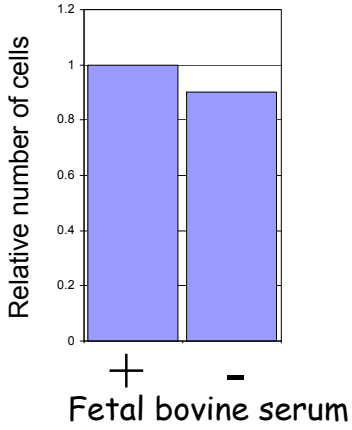
- LCMSMS existing technology for detecting DNA damage
- Sensitivity to 1 lesion in 1 million to 100 million bases

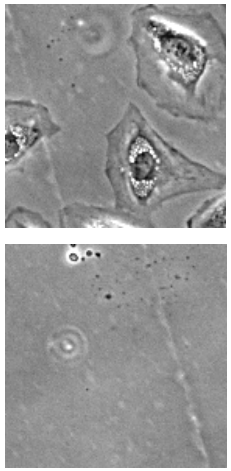
Nanoparticles and Cells



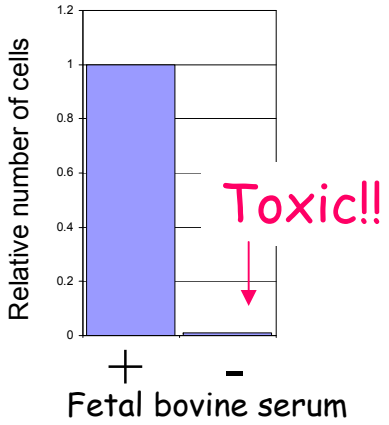


Fullerenes





50 nm Silica NP



- Cellular effects depend on NP/NP/protein/media effects

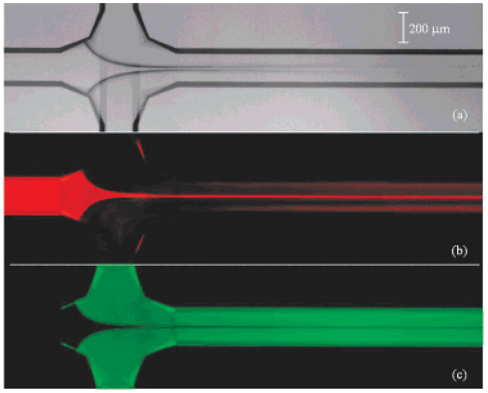
Controlled Mixing and NP dispersion

High quality nanomaterials

Disperse in cell culture media

? Results depend on dispersion process

Microfluidic mixer

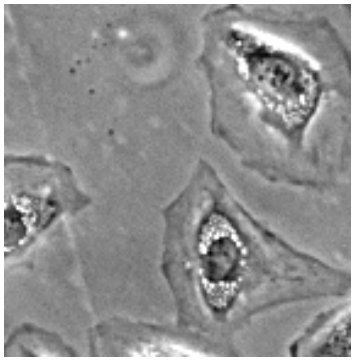


Control of mixing
- Flow
- Concentration
- Mixer design

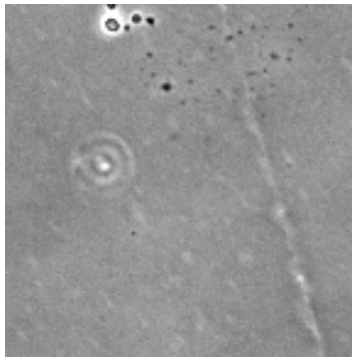
Also, there is a significant need for NM characterization in aqueous solutions

- Take advantage of microfluidics to produce a highly controlled mixing environment.
- Nanotox tests require NM and specified mixing conditions.

Cell-based assays- Mechanism



Silica NP + serum

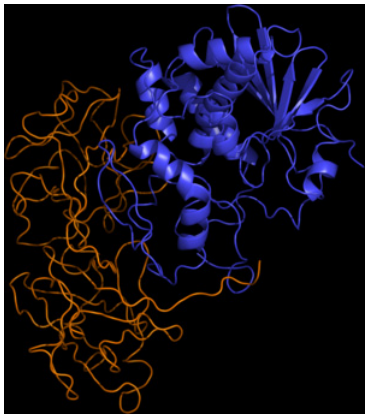


Silica NP - serum

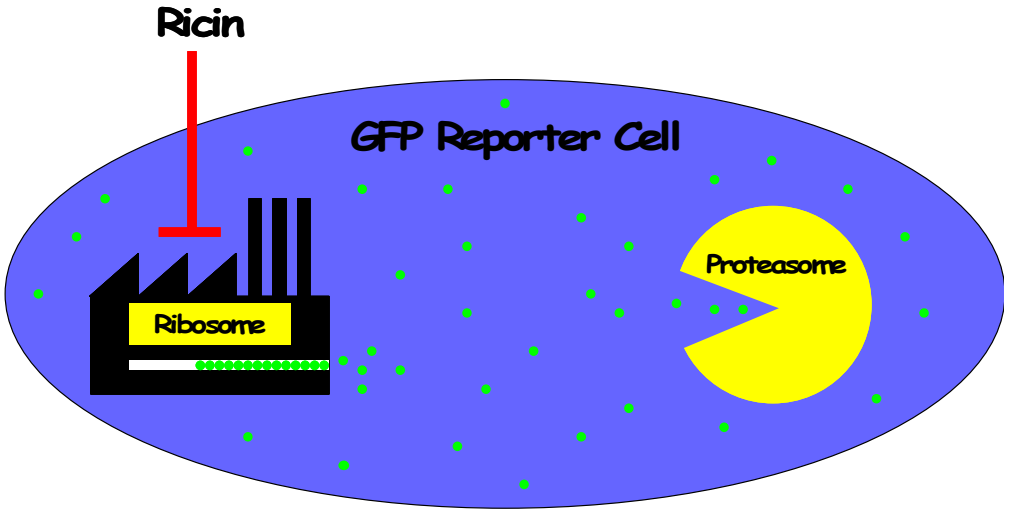
Many conventional assays measure cell number.

- Use assays that report specific information about cell response to NM
  - •Cell membrane permeability
  - •Protein expression/degradation
  - •mRNA production machinery
  - •Signaling pathway interaction

Indicator Cell: CMV-dsGFP vero cells

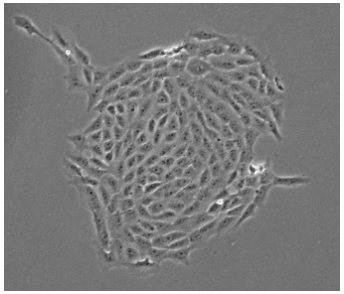


Ricin is a protein toxin that catalytically inhibits ribosomes

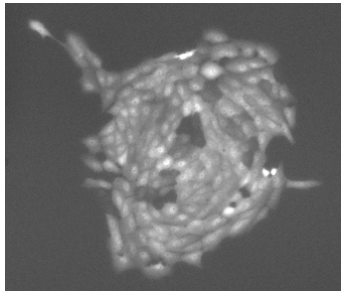


CONCEPT: The inhibition of GFP synthesis by ricin is detected as a loss of GFP intensity

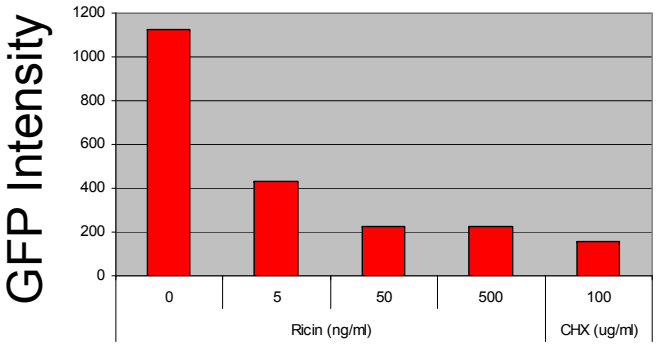
Vero GFP reporter cells



phase



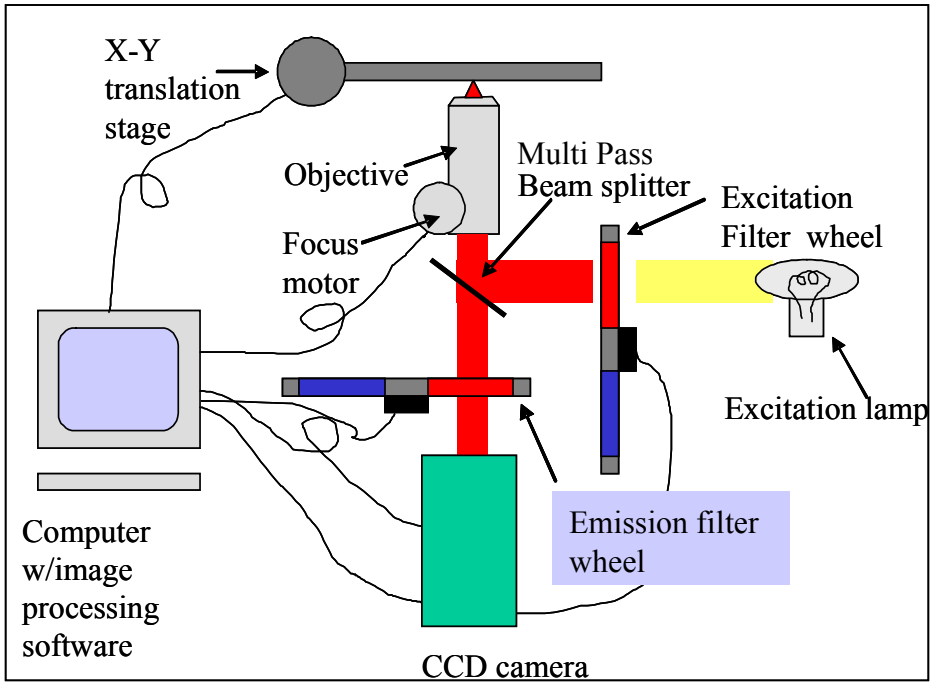
GFP



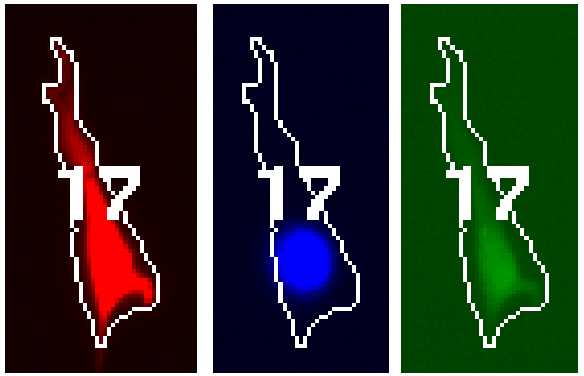
Ricin concentration

Halter, Almedia, et al.

Automated Quantitative Microscopy



Multi-fluorophore imaging



Cell Shape Nucleus 3rd marker

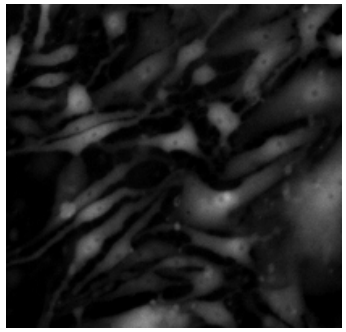
Advantages:

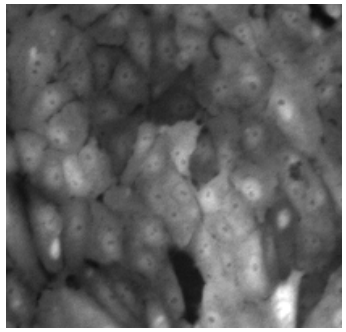
- -Unbiased data collection
- -Sample large number of cells
- -Multi-fluorophore imaging
- -Live cell imaging
- -Evaluate cells in real culture conditions

Characterizing a Indicator Cell Line

- Images say a lot…High-content

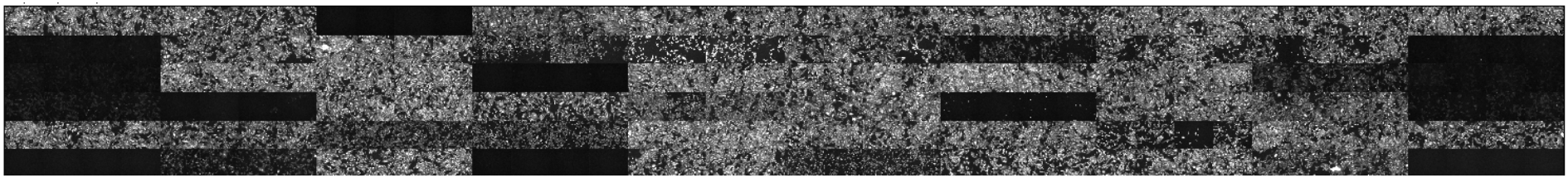
+treatment -treatment





44-MEIC toxins screen

EGFP Image

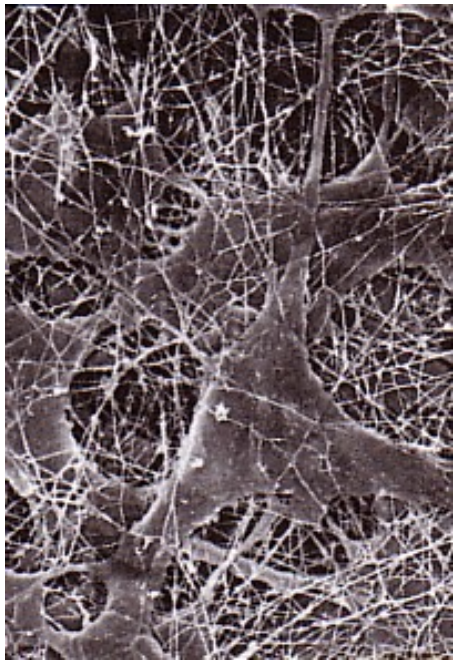


Hoechst (nuclear) image

We measure:

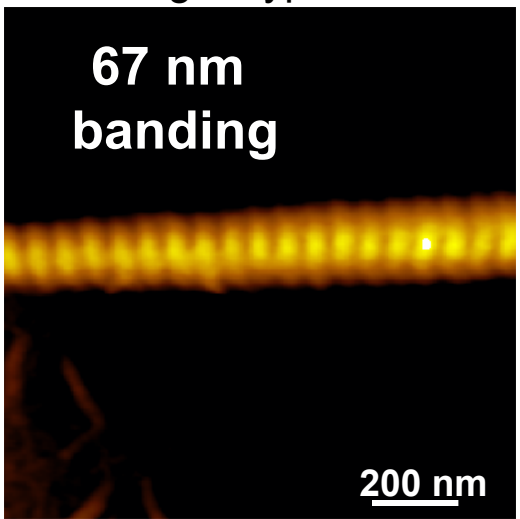
- Cell deadhesion (relative cell number)
- Change in protein expression/degradation (dsEGFP)
- Morphology change (multiparametric)
- P-glycoprotein pump activity (Hoechst intensity)
- Stress granules (texture in cell)

The nanoscale-extracellular matrix

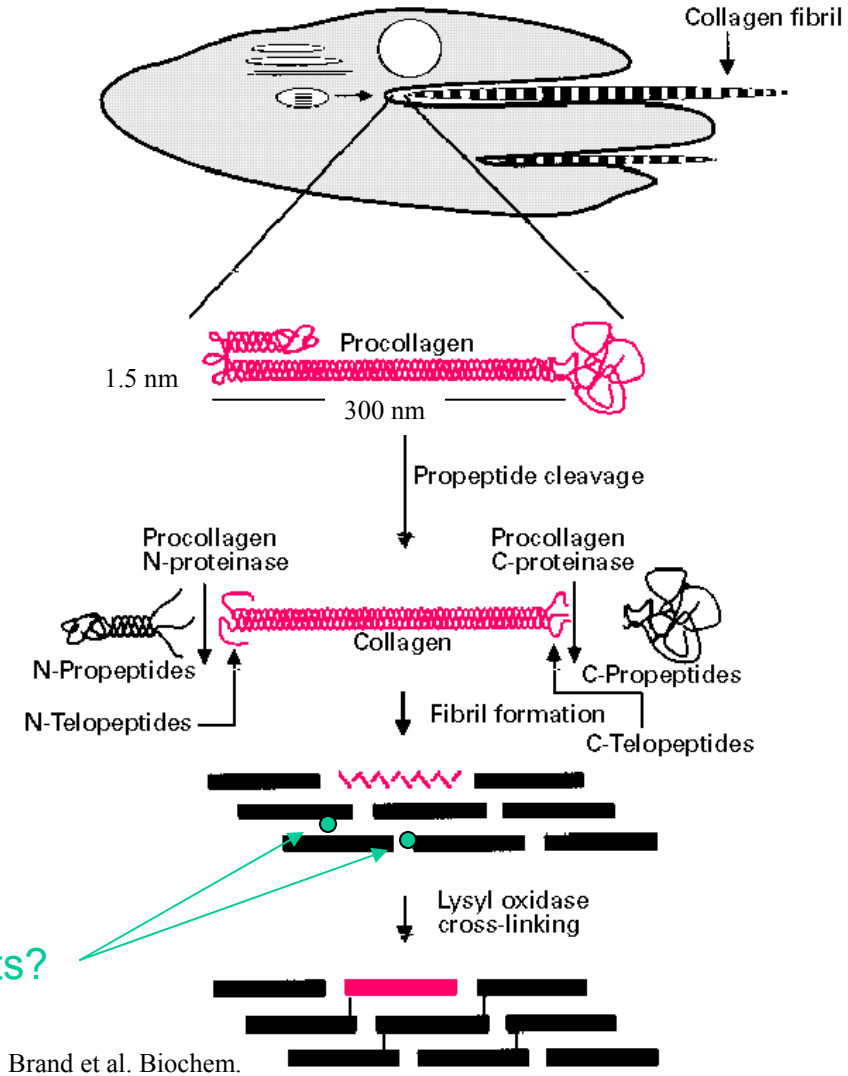


Cells on a fibrillar extracellular matrix

Collagen type 1 fibril



Collagen Synthesis



NP effects?

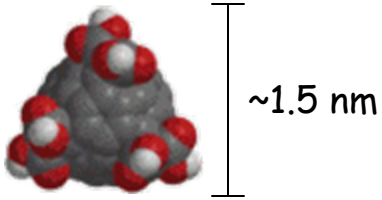
Brand et al. Biochem. J. 2001

Effects on Extracellular Matrix

Collagen gel pellet after centrifugation

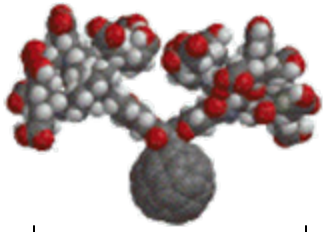
C Sixty Inc.

Fullerene Derivatives

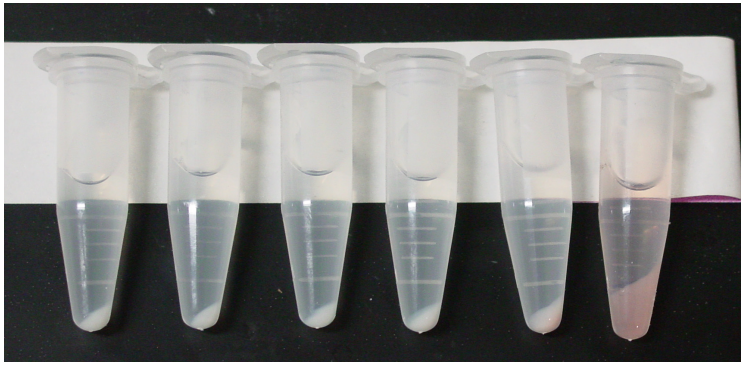


C3

DF-1



C3 np



1 2 3 4 5 6

Did not pack as well suggesting stiffer gel?

Tube #	Col:np mol ratio
1, 7	10000:1
2, 8	1000:1
3, 9	100:1
4, 10	10:1
5, 11	1:1
6, 12	1:10

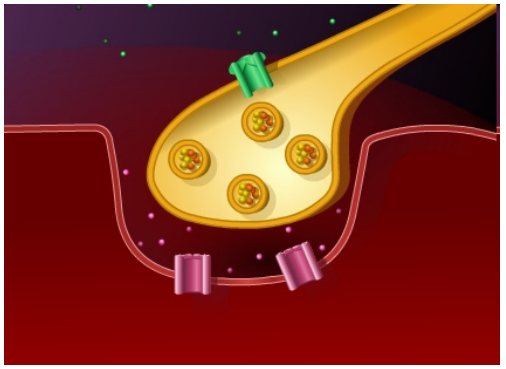
DF-1 np

7 8 9 10 11 12

Conclusions so far…

- Nanomaterials can influence biological systems
- NM designed to facilitate measurement are good
- Reference materials are available
- Need control and measurements for dispersion process
- Need characterization measurements in cell culture media
- Use well-defined mechanistic assays to generate detailed information about mode-of-action
- Need a NP vocabulary and database for storing results

Future Interests…



http://biol1114.okstate.edu/study_guides/scenarios/4-chemical_defenses/images/Neuromuscular%20junction.jpg

- **More Complex Biology Models-**
  - In vitro tissue models
  - Tissue-on-a-chip
- **Reference cell-based assays**
  - Report well known mode-of-action info
- **Participation in nanomaterial rules database development**
  - Vocabulary for describing nanomaterials
  - Vocabulary for describing biological responses

Acknowledgements:

NIST NanoBio NanoTox working Group!!