



Challenges in Preclinical Characterization of Nanoparticles

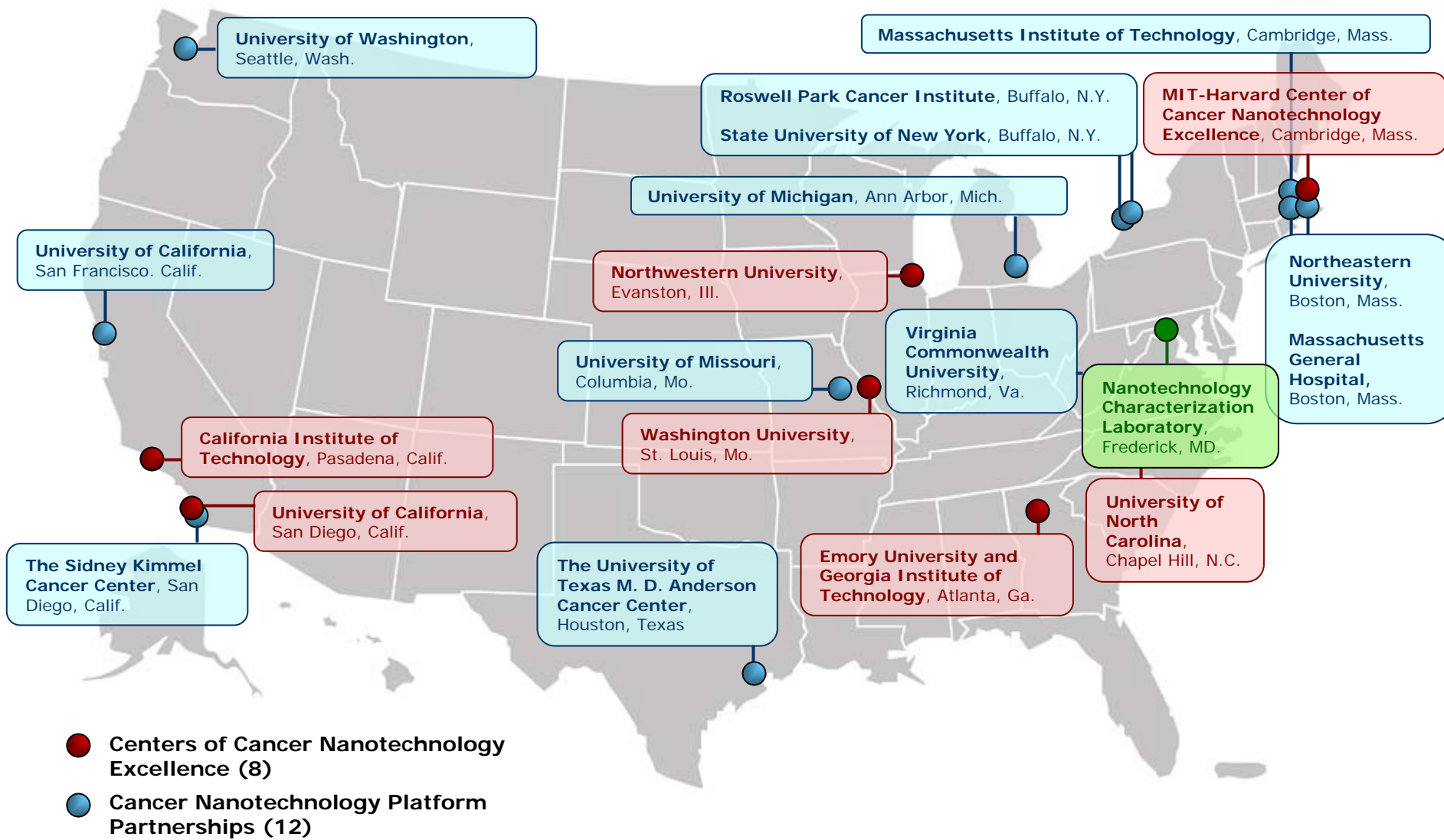
Presented to the
FDA/ANH Nanotechnology Initiative
March 10, 2007 4:45 PM



Advanced Technology Program

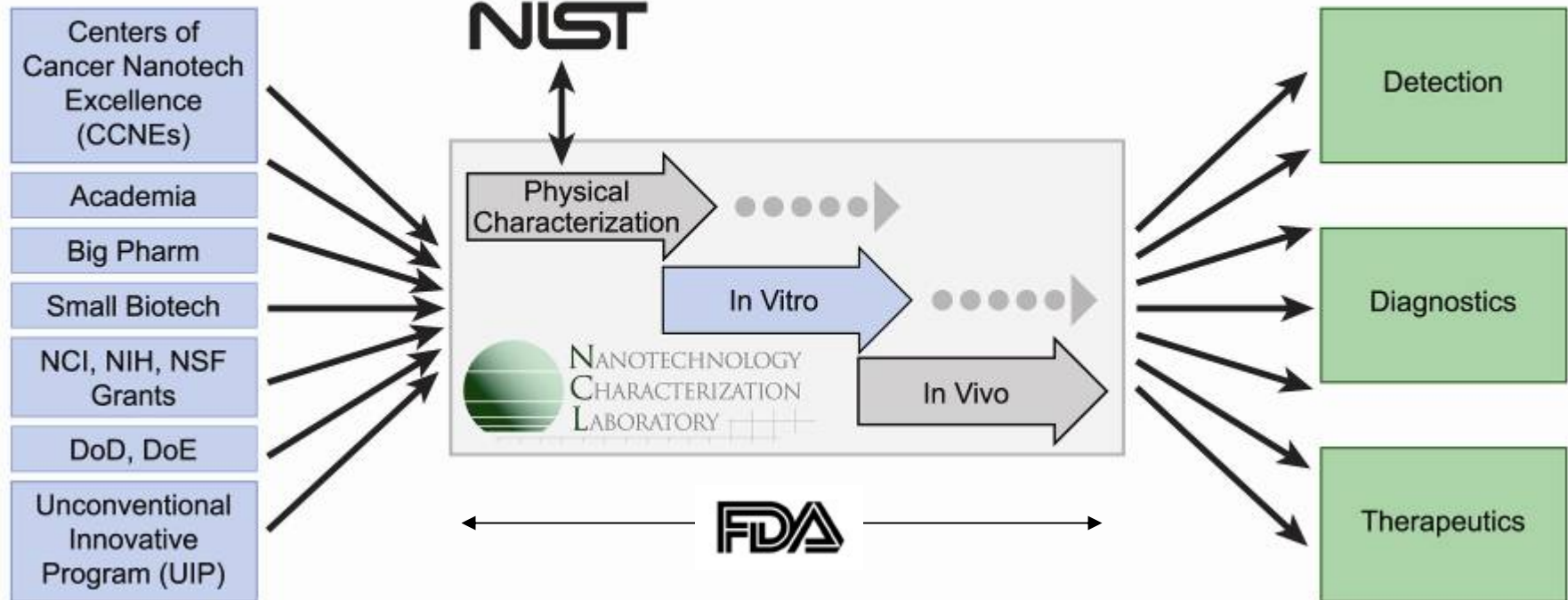
SAIC **SAIC-Frederick, Inc.**
From Science to Solutions[®] A subsidiary of Science Applications
International Corporation

Contract N01-CO-12400 - Funded by the National Cancer Institute



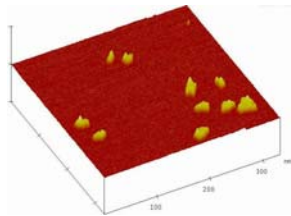
NCL Concept of Operations

Sources of Nanomaterials



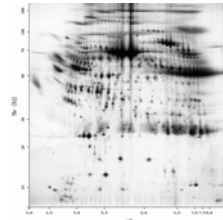
NCL is a formal collaboration between NCI, FDA and NIST

NCL Assay Cascade



Physicochemical:

- Size
- Shape
- Composition
- Molecular weight
- Surface chemistry
- Identity
- Purity
- Stability
- Solubility



In Vitro:

- Pharmacology
- Blood contact properties
- Immune cell function
- Cytotoxicity
- Mechanistic toxicology
- Sterility

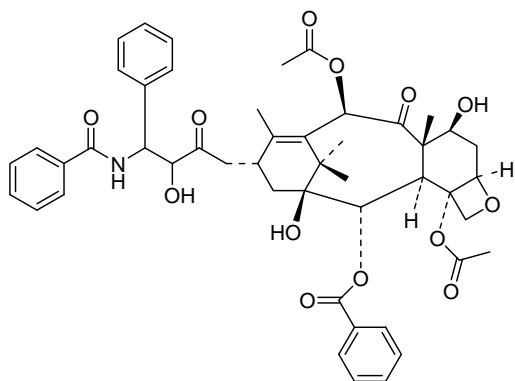


In Vivo:

- ADME
- Safety
- Efficacy

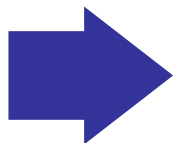
http://ncl.cancer.gov/assay_cascade.asp

Physicochemical Characterization



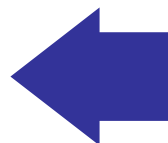
Small molecules

- Elemental analysis
- Mass Spec
- NMR
- UV-Vis
- IR
- HPLC
- GC
- Polarimetry

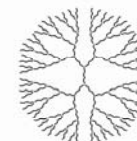


Physicochemical Parameters

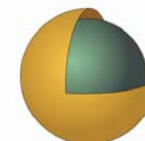
- Composition
- Physical properties
- Chemical properties
- Identification
- Quality
- Purity
- Stability



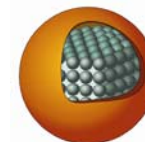
Liposome



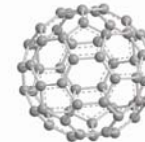
Dendrimer



Gold Nanoshell



Quantum Dot



Fullerene

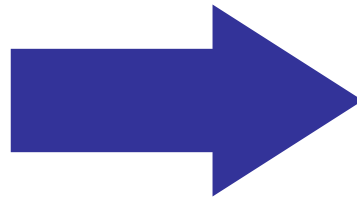
Nanomaterial

- Microscopy (AFM, TEM, SEM)
- Light scattering (Static, Dynamic)
- SEC, FFF
- Electrophoresis (CE, PAGE)
- Zeta sizer
- Fluorimetry

Same parameters – different/additional characterization methods

In Vitro Cascade

- **Sterility**
 - Bacterial/Viral/Mycoplasma
 - Endotoxin
- **Cell Uptake/Distribution**
 - Cell Binding/Internalization
 - Targeting
- **Blood Contact Properties**
 - Plasma Protein Binding
 - Hemolysis
 - Platelet Aggregation
 - Coagulation
 - Complement Activation
 - CFU-GM
 - Leukocyte Proliferation
 - Macrophage/Neutrophil Function
 - Cytotoxic Activity of NK Cells
- **Toxicity**
 - Phase I/II Enzyme Induction/Suppression
 - Oxidative Stress
 - Cytotoxicity (necrosis)
 - Cytotoxicity (apoptosis)



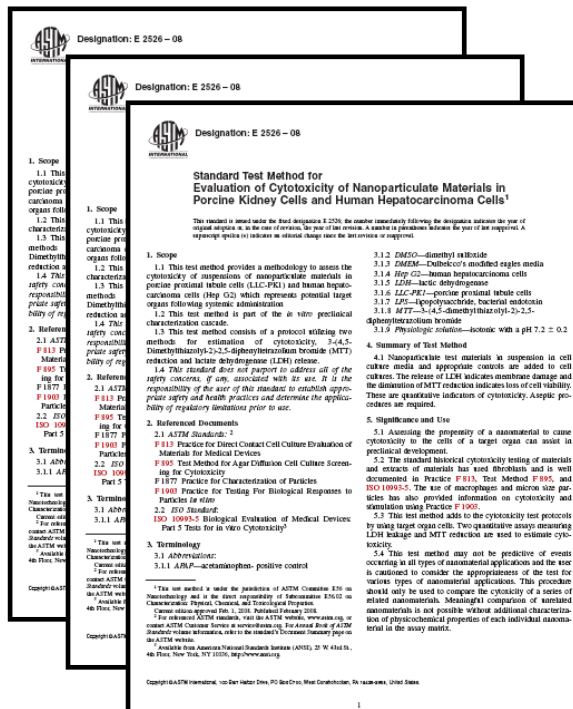
NCL Method ITA-1

Analysis of Hemolytic Properties of Nanoparticles

Nanotechnology Characterization Laboratory
National Cancer Institute at Frederick
SAIC-Frederick
Frederick, MD 21702
(301)-846-6939

ASTM Standards

- First voluntary consensus standards for biocompatibility-testing of nanomaterials intended for medical applications
- E2524 (hemolysis), E2525 (CFU-GM inhibition), E2526 (kidney and liver cytotox)

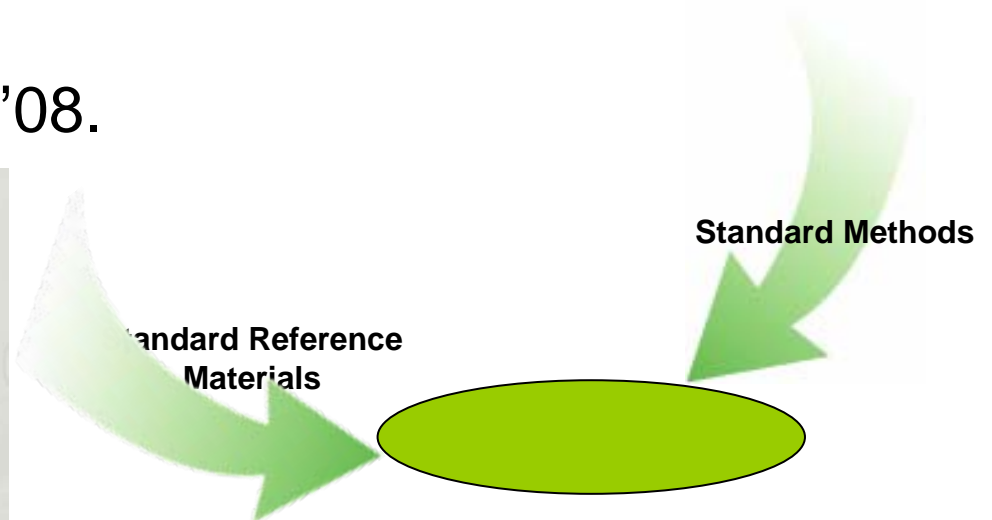


Standard Methods

ASTM ILS 166

Interlaboratory Comparison

- NCI supported the production of NIST's colloidal gold RM.
 - Au selected for calibration and biocompatibility
- 10nm, 30nm, and 60 nm diameters.
- Available as of January '08.

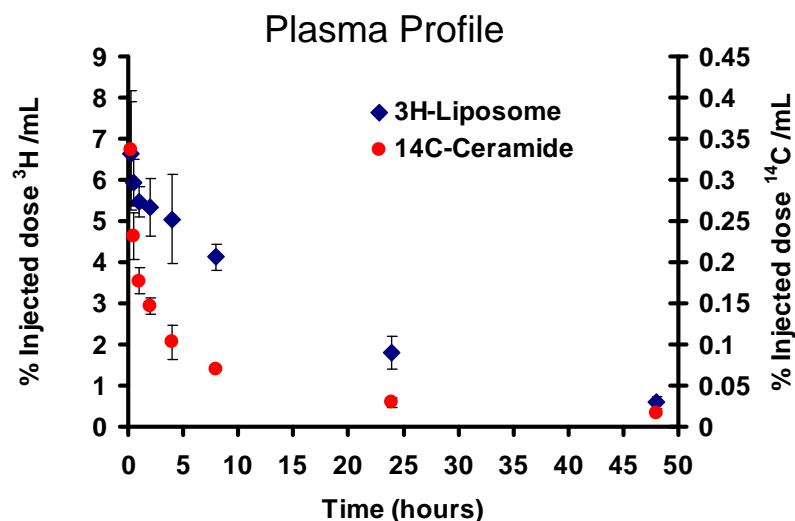


- **Initial Disposition Study**
 - Tissue Distribution
 - Clearance
 - Half-life
- **Immunotoxicity**
 - 28-day screen
 - Immunogenicity (repeat dose tox study)
- **Dose-Range Finding Toxicity**
 - Blood Chemistry
 - Hematology
 - Histopathology
 - Gross Pathology
- **Efficacy**
 - Therapeutic
 - Imaging



LASP
Laboratory Animal Sciences Program

Dual Radiolabels



NCL Extramural Collaborators



NCI Alliance for Nanotechnology in Cancer



Imperial College London



CEDARS-SINAI MEDICAL CENTER



SYNERGENE



UCLA Department of Chemistry & Biochemistry

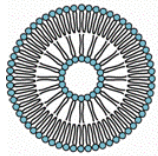


PURDUE



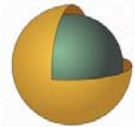
NANOPARTICLE BIOCHEM, INC.

Portfolio of Nanoparticles



Liposomes

- M. Kester (Penn State)
- E. Chang (Georgetown)
- R. Blumenthal (NCI)
- J. Nagy (Nanomed)
- J. Connor (Penn State)
- W. Zamboni (U. Pittsburgh)
- A. Miller (Imperial College London)



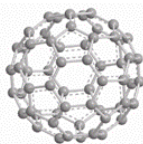
Gold nanoshells

- NanoSpectra (Rice)



Colloidal gold

- CytImmune Sciences
- TedPella Inc.
- A. Wei (Purdue)
- K. Katti (U. Missouri)



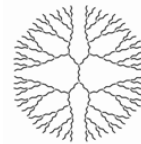
Fullerenes

- Tego Bioscience



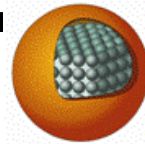
Iron Oxide

- Alnis



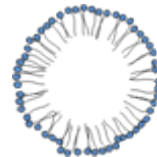
Dendrimers

- Dendritic Nanotechnologies (DNT)
- Avidimer Therapeutics
- M. Brechbiel (NCI)
- E. Simanek (Texas A&M)



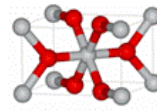
Quantum Dots

- S. Weiss (UCLA)
- Evident Technologies
- J. Barchi (NCI)



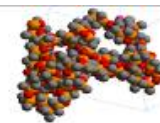
Nanoemulsions

- M. Amiji (Northeastern)
- Nanoscan



TiO₂

- US FDA

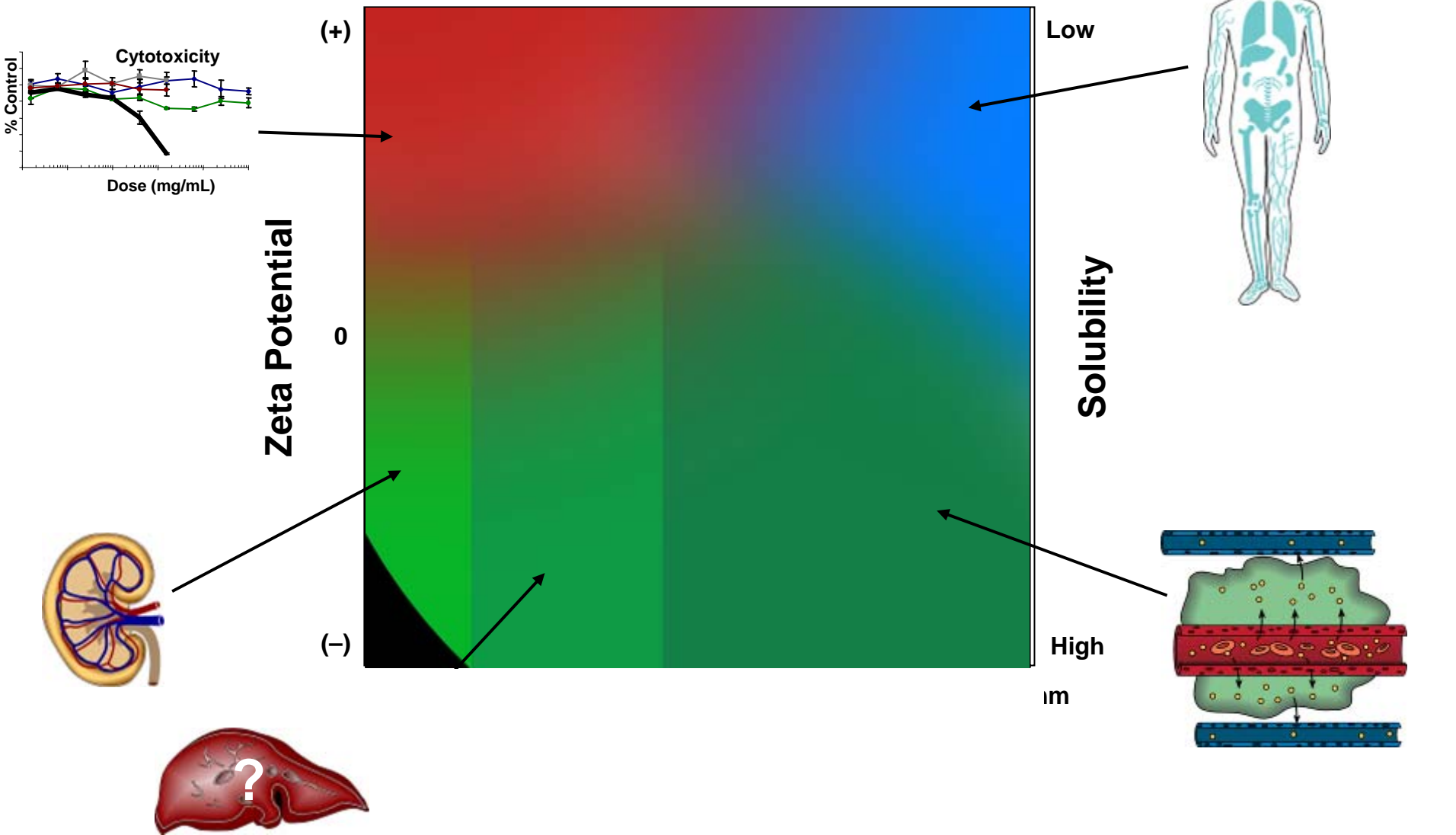


Polymers

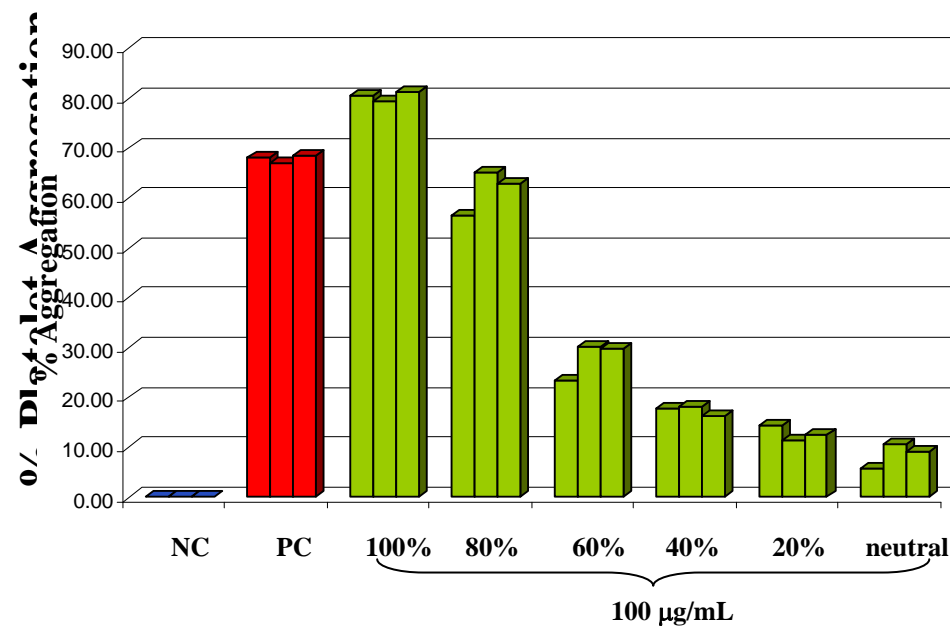
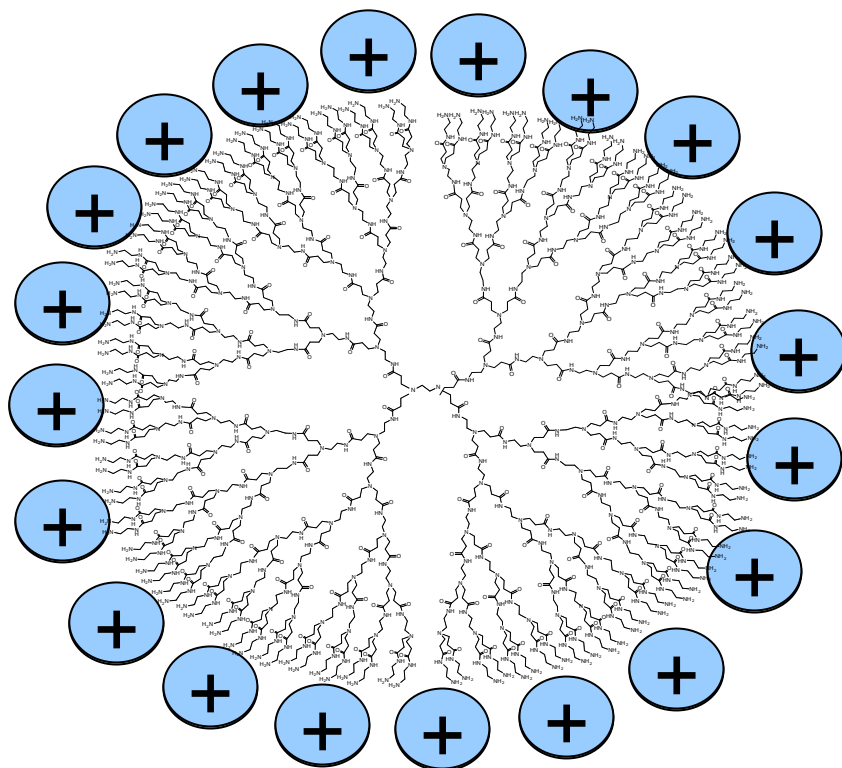
- J. Ljubimova (Mt. Sinai)
- M. Amiji (Northeastern)
- N. Tarasova (NCI)
- D. Ferguson (U. Wisc)
- V. Torchilin (Northeastern)
- Carigent Therapeutics

Trends: Biocompatibility

Nanoparticle Biocompatibility



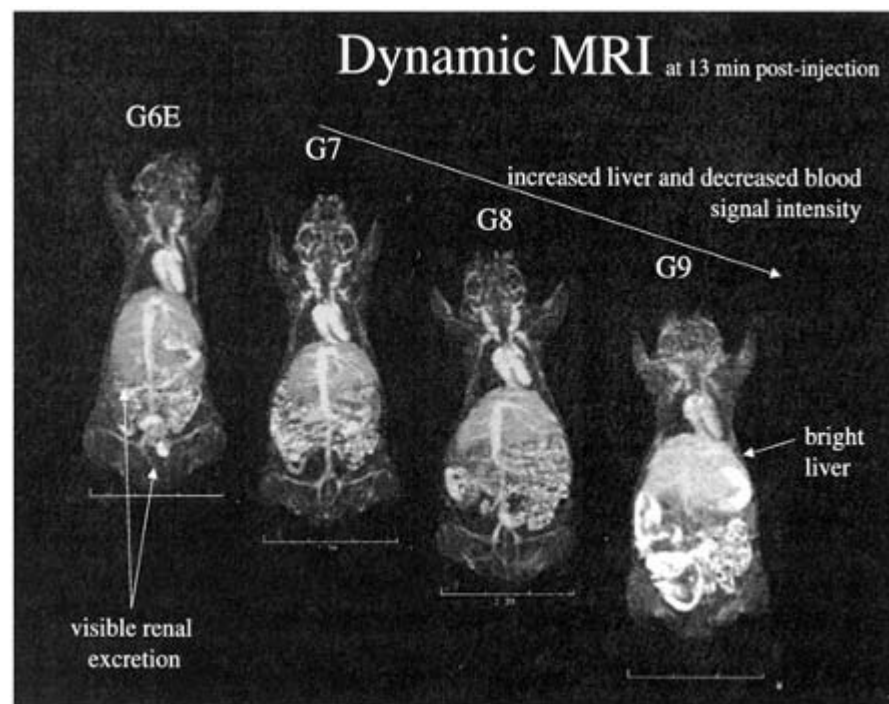
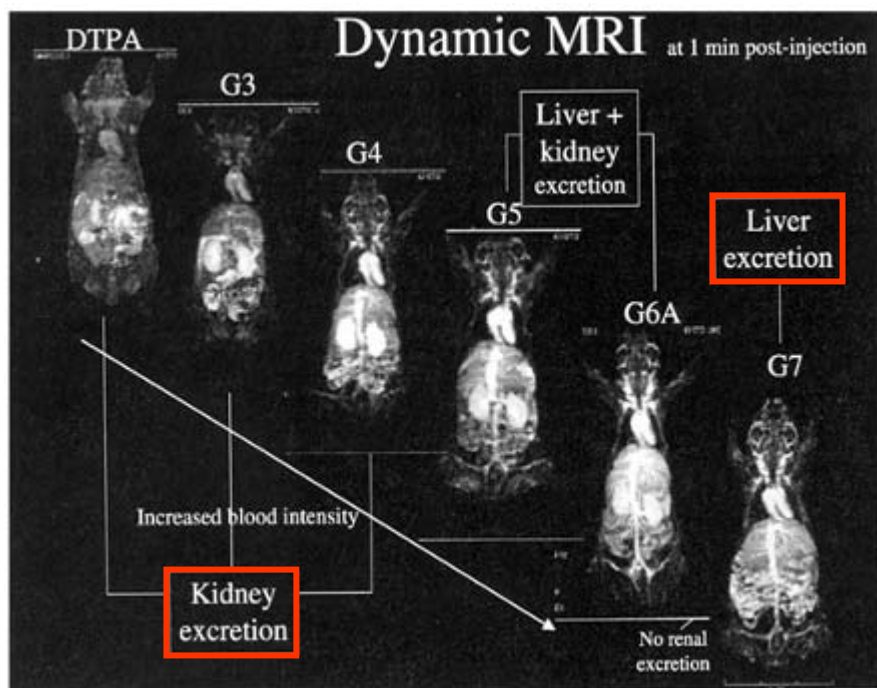
Trends: Charge



Biocompatibility depends on surface charge.

Size Matters

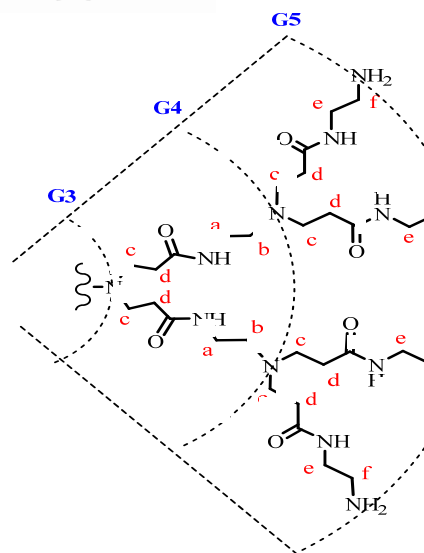
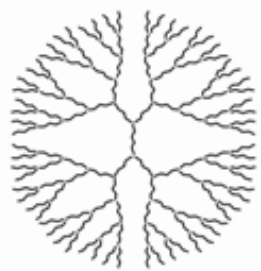
Dendrimer-Based MRI Contrast Agents



Kobayashi and Brechbiel, *Molecular Imaging*, 2:1-10, 2003.

A difference in size as little as 2nm can influence route of clearance

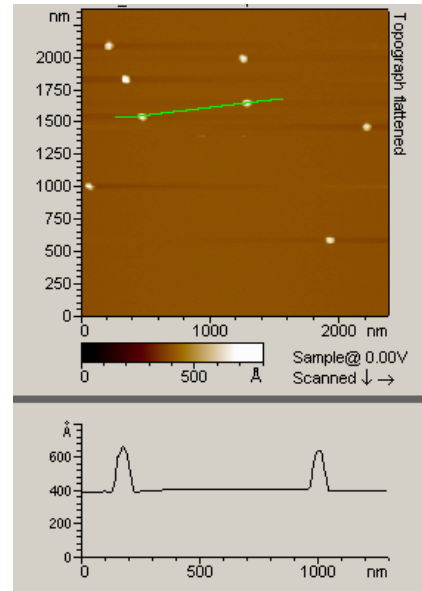
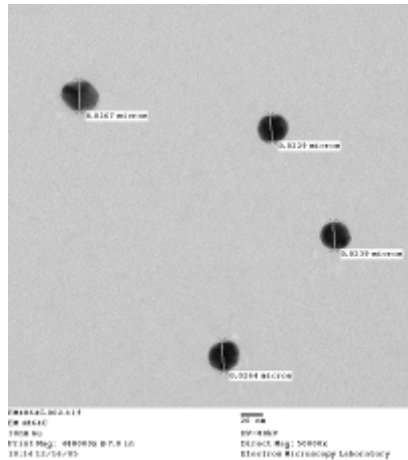
Size effects *In Vitro*



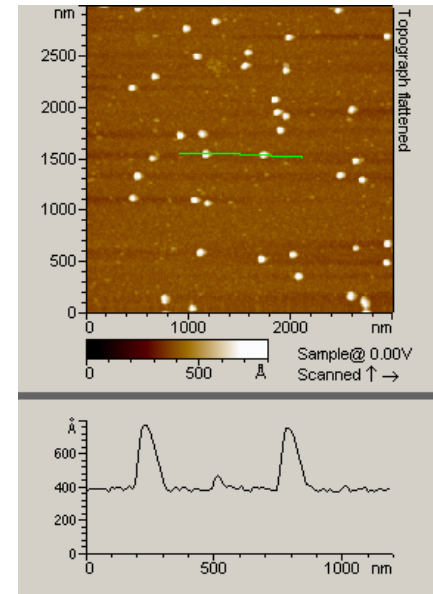
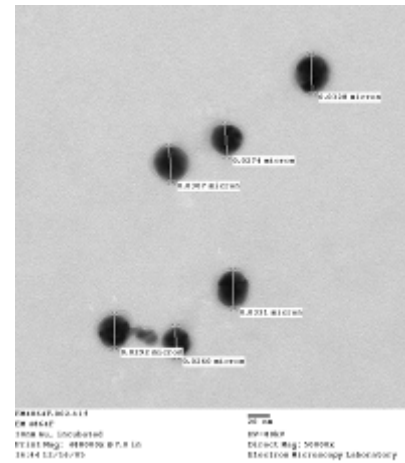
Biocompatibility depends on particle size.

Size in a Biological Context

30 nm Gold colloids



30 nm Gold colloids incubated in plasma



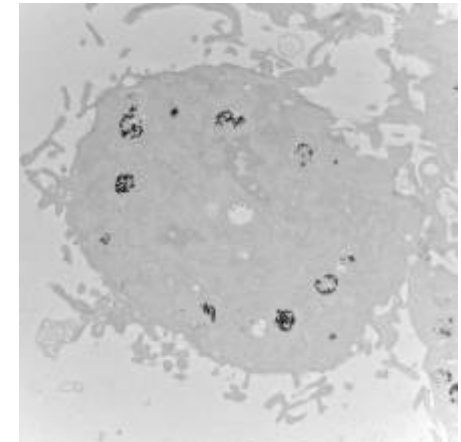
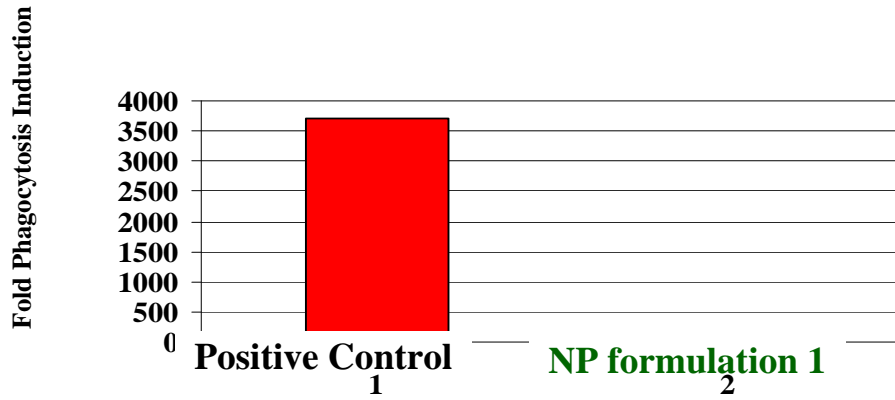
31 nm

69 nm

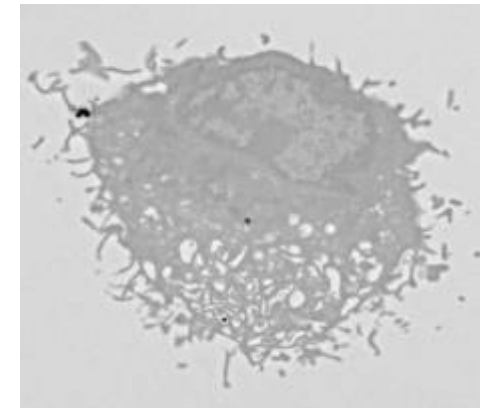
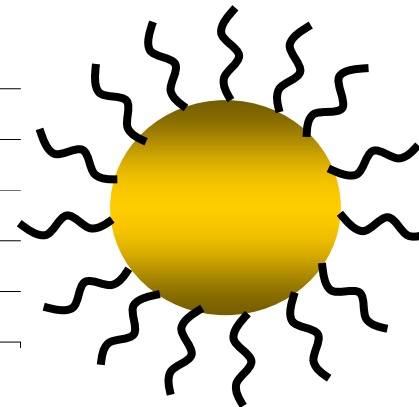
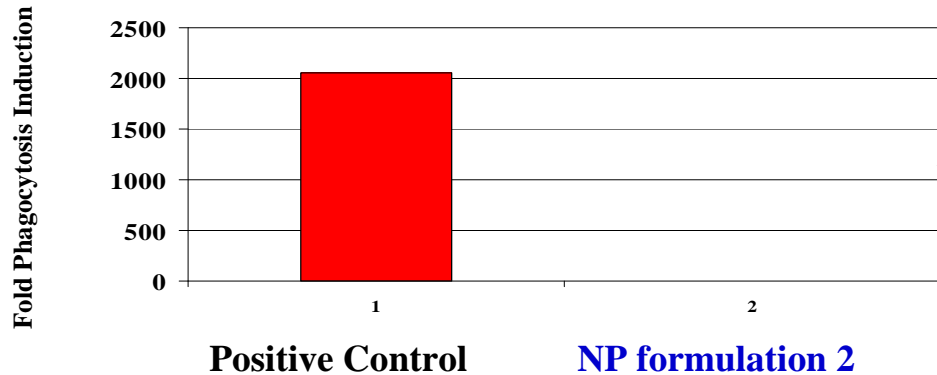
Solubility

Phagocytosis

TEM



NP formulation 1

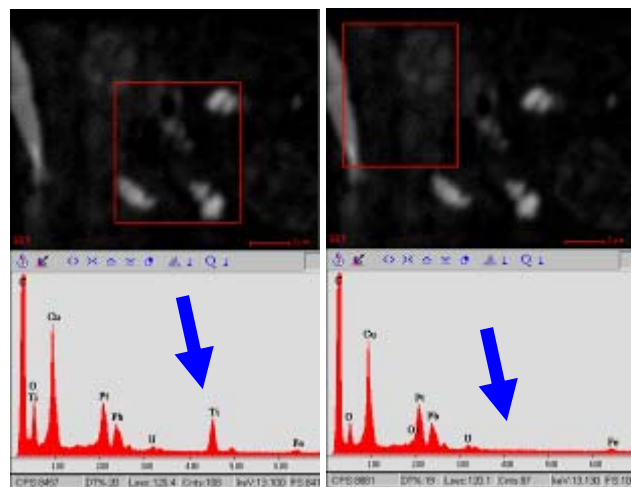
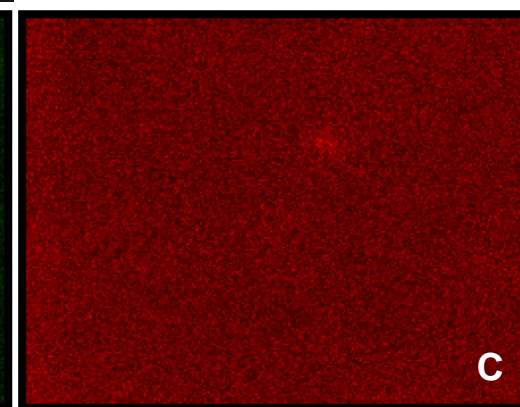
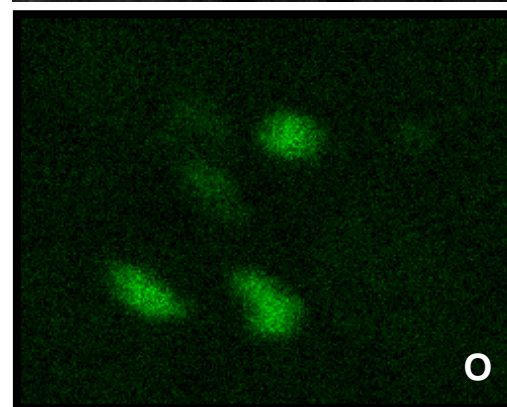
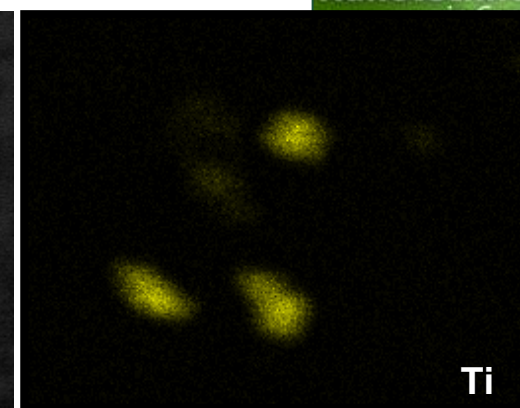
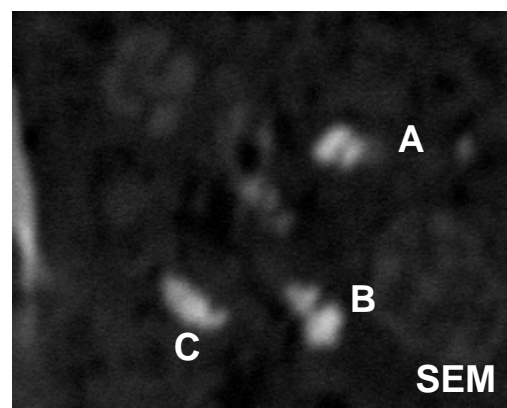
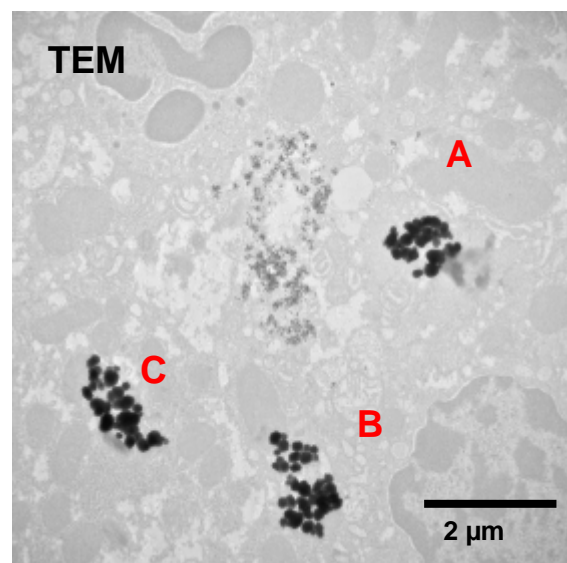


NP formulation 2

Fluorescence assay

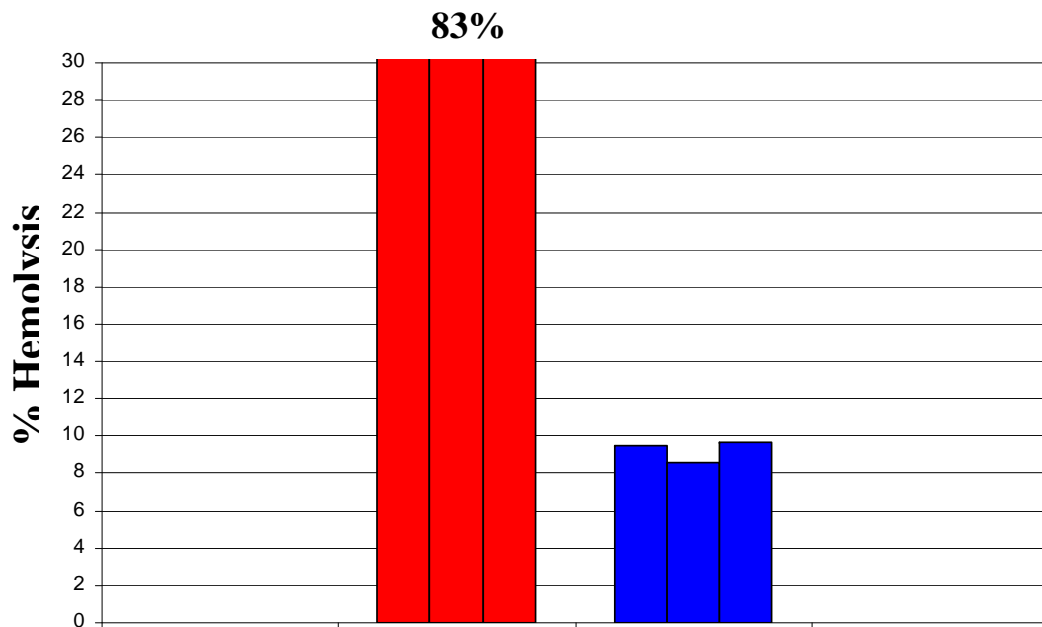
More than one method is often required to draw conclusions

EDX Spectral Mapping



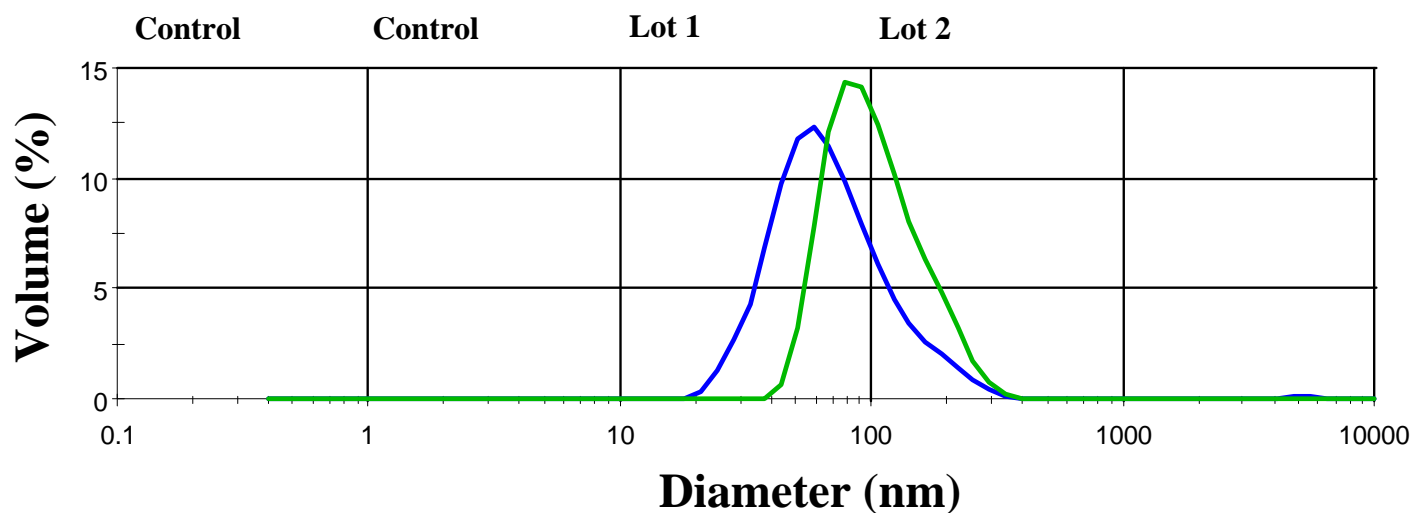
Spectral mapping confirms the agglomerated particles contain Ti and O.

Batch to Batch Variability



Hemolytic properties of two lots of the same nanomaterial appear different

DLS size measurement shows that nanoparticles in the two lots are not the same size.

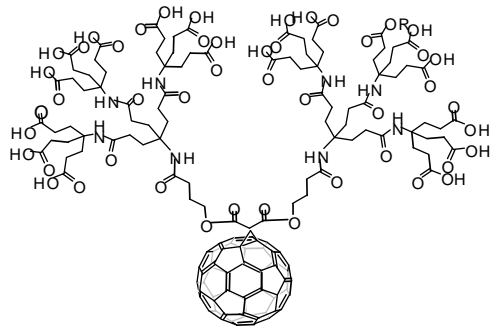
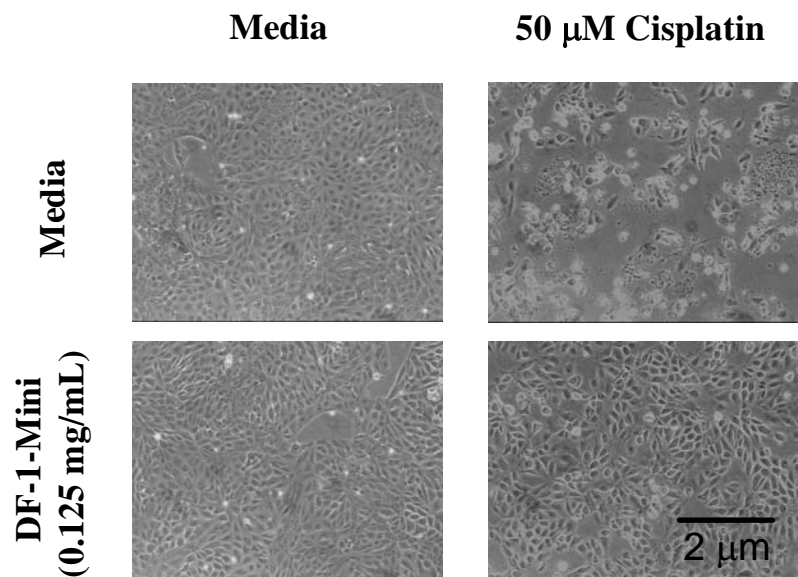
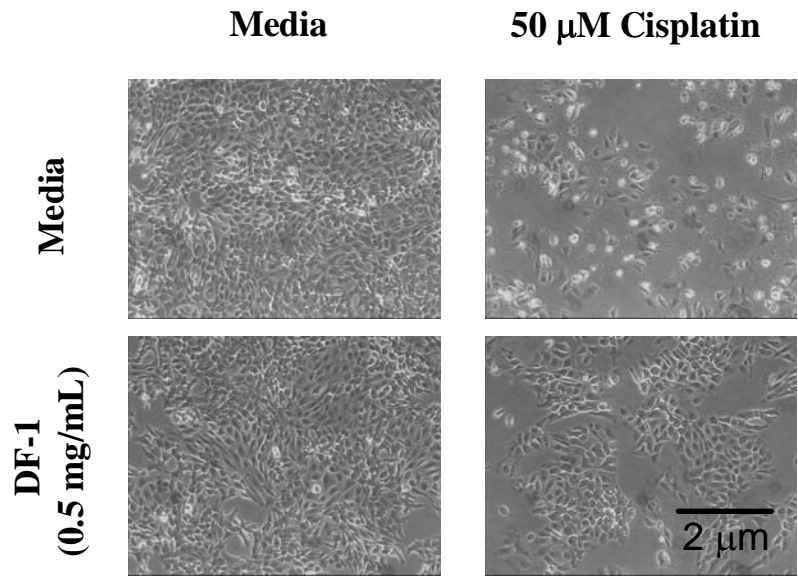


Size by DLS

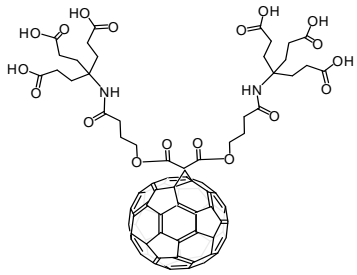
— Lot 1 in PBS

— Lot 2 in PBS

Fullerene Chemoprotection



DF-1



DF-1 Mini

Characterization Challenges

- Parameters that influence biocompatibility
 - Surface Charge
 - Size
 - Hydrophobicity/solubility
 - Stability
- Importance of characterization
 - Batch to batch variability
 - Physical parameters greatly affect ADME/Tox
 - Reference material, ASTM standards
- Each nanoparticle is unique!

Acknowledgements

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Nader Ayub

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Advanced Technology Program

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