NCL Nanotechnology Characterization Laboratory

NCI Alliance for Nanotechnology in Cancer

Preclinical Characterization of Nanomedicines: Lessons Learned from the NCL

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March 6, 2014

http://ncl.cancer.gov

NATIONAL® CANCER INSTITUTE

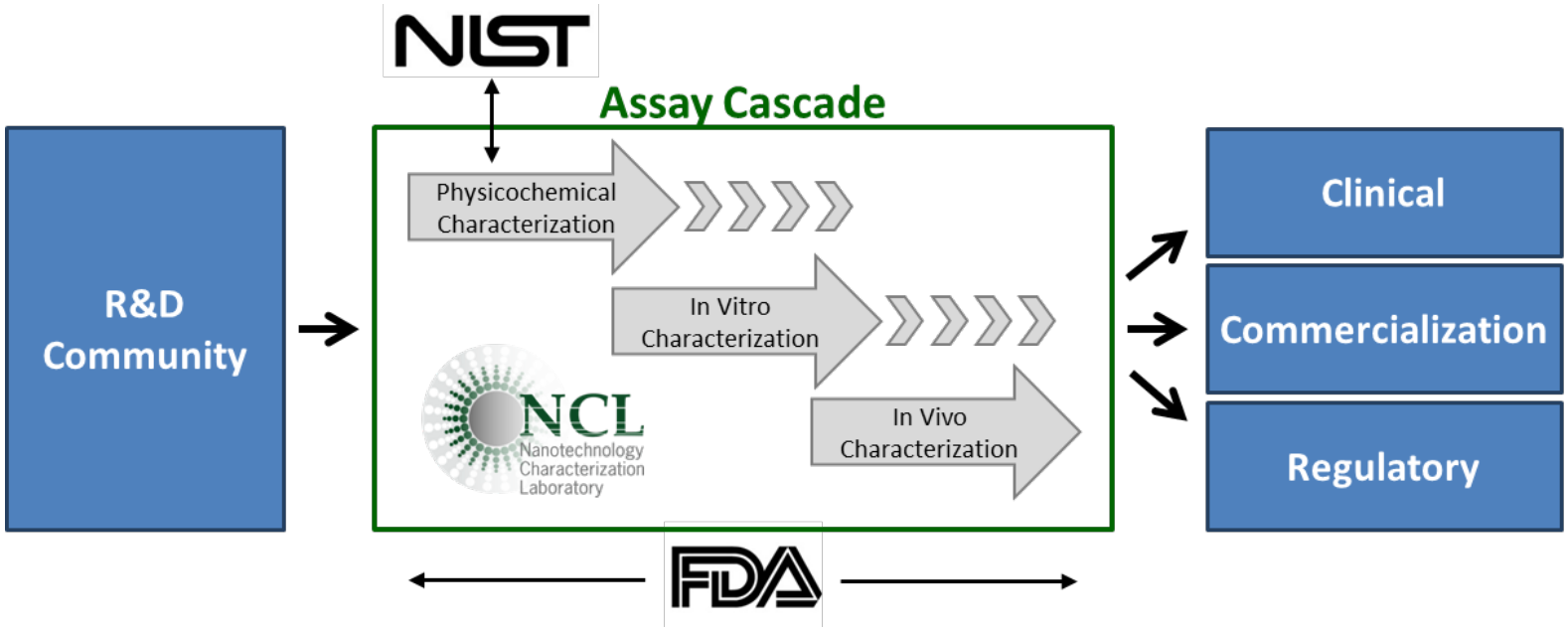
Frederick National Laboratory for Cancer Research

Agenda

- Overview of the NCL
- NCL Lessons Learned:
  - Physicochemical Properties Influence Biocompatibility
  - Know What you Have
  - Case Study in Nanomaterial Safety Testing

NCL Concept of Operations

The NCL was established in 2004 as an interagency collaboration among NCI, NIST, and FDA. The lab's mission is to accelerate the translation of promising nanotech cancer drugs and diagnostics.

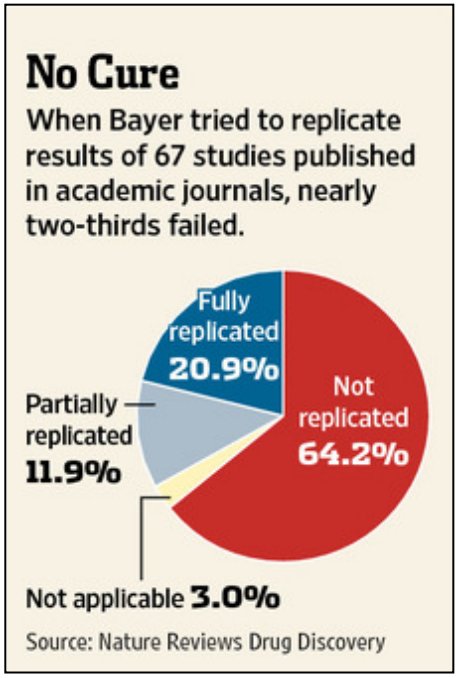


90% of NCL's efforts support the extramural community.

http://ncl.cancer.gov

NCL is a Translational Resource

- NCL provides independent verification of results → can help attract investment.
- Focus on questions related to "translatability":
  - Publication vs. commercialization
  - Manufacturing complexity
  - Economics (costs to produce, potential for return on investment)
  - Quality/regulatory requirements
  - Advantage over existing therapies
- Repeat player with FDA: NCL provides submitters a preview of what FDA may be concerned with based on past experience.



G. Naik, Scientists' Elusive Goal: Reproducing Study Results, Wall Street Journal, December 2, 2011

NCL provides independent validation of results, de-risks products.

NCL-FDA Relationship

- NCL allows FDA to preview what's in pipeline for nanotech INDs/IDEs.
- NCL is trusted source for preclinical data on nanomaterials.
- Scientific collaborations with FDA to address specific concerns for nanotech:
  - Immunological reactions to nanomaterials; dermal penetration of nanomaterials in sunscreens and cosmetics; endotoxin; methods of sterilization for devices.
- FDA provides input on NCL's assay cascade and is represented on NCL's scientific oversight committee.

Impact of NCL Data

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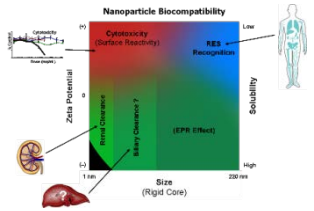
- Influencing the Field



- Informing Regulatory

FDA

- Assisting with Commercialization

- Collaborating with Pharma

- Basic Research

- Standardization

ASTM INTERNATIONAL Standards Worldwide

ISO

ANSI American National Standards Institute

The Motivating Force for NCL Creation

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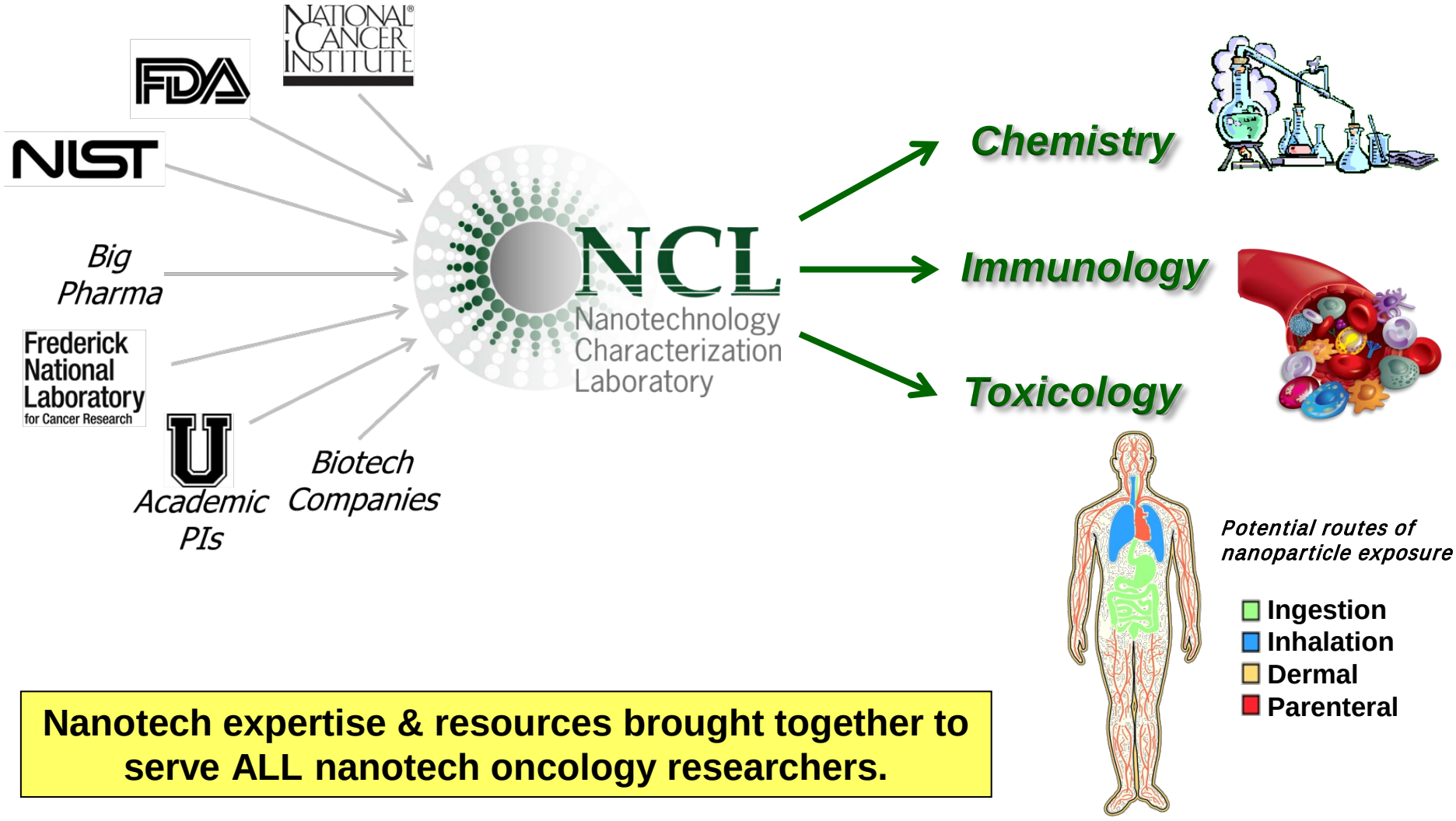

Nanotech expertise & resources brought together to serve ALL nanotech oncology researchers.

Stern & McNeil, Tox Sci, 2008, 101, 4-21.

NCL Assay Cascade

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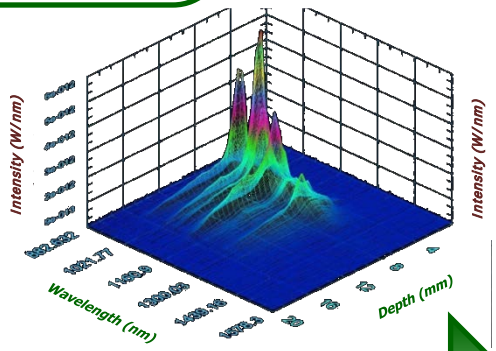
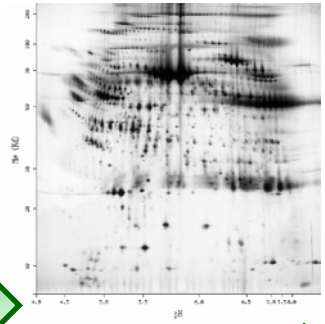
Prescreen:

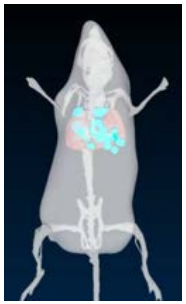
- ***Sterility***
- ***Endotoxin***
- ***Size/Size Distribution***
- ***Zeta Potential***



Physicochemical Characterization



In Vitro Characterization

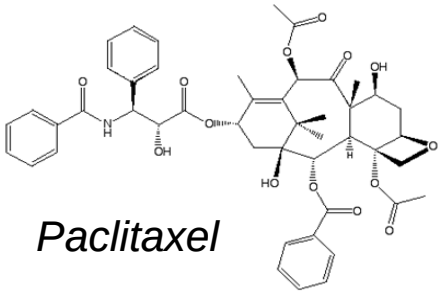


In Vivo Characterization

Characterization Parameters Required for New Drugs

Small molecules

- *Composition*
- *Physical Properties*
- *Chemical Properties*
- *Identification*
- *Quality*
- *Purity*
- *Stability*



Paclitaxel

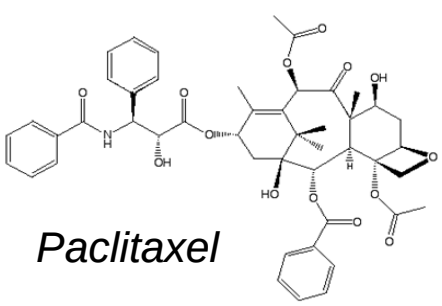
Traditional methods for the analysis of small molecules includes:

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

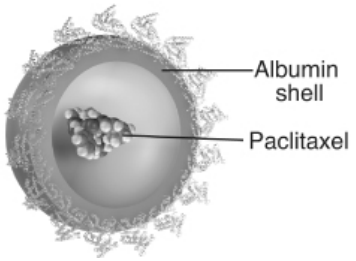
Characterization Parameters Required for New Drugs

- *Composition*
- *Physical Properties*
- *Chemical Properties*
- *Identification*
- *Quality*
- *Purity*
- *Stability*

Nanoparticles



Paclitaxel



Albumin-bound Nanoformulation

Abraxane

Nanoparticles need the same characterization parameters, but require different instrumentation

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

Physicochemical Characterization

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Size/Size Distribution

- Dynamic Light Scattering (DLS)
- Electron Microscopy (TEM, SEM, cryo)
- Atomic Force Microscopy (AFM)
- Field Flow Fractionation (FFF), SEC-MALLS

AFM

CryoTEM

Composition

- TEM with EDS
- Inductively coupled plasma-mass spec. (ICP-MS)
- Spectroscopy (NMR, CD, Fluorescence, IR, UV-vis)

Purity

- Chromatography
- Capillary Electrophoresis

ICP-MS

FFF

Surface Chemistry

- Biacore
- Zeta Potential

Stability

- Stability can be measured with any number of instruments with respect to time, temperature, pH, etc.

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

In Vitro Cascade

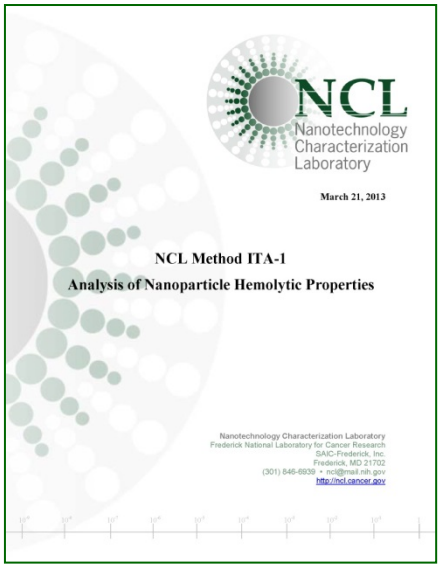
Sterility
- Bacterial/Viral/Mycoplasma
- Endotoxin

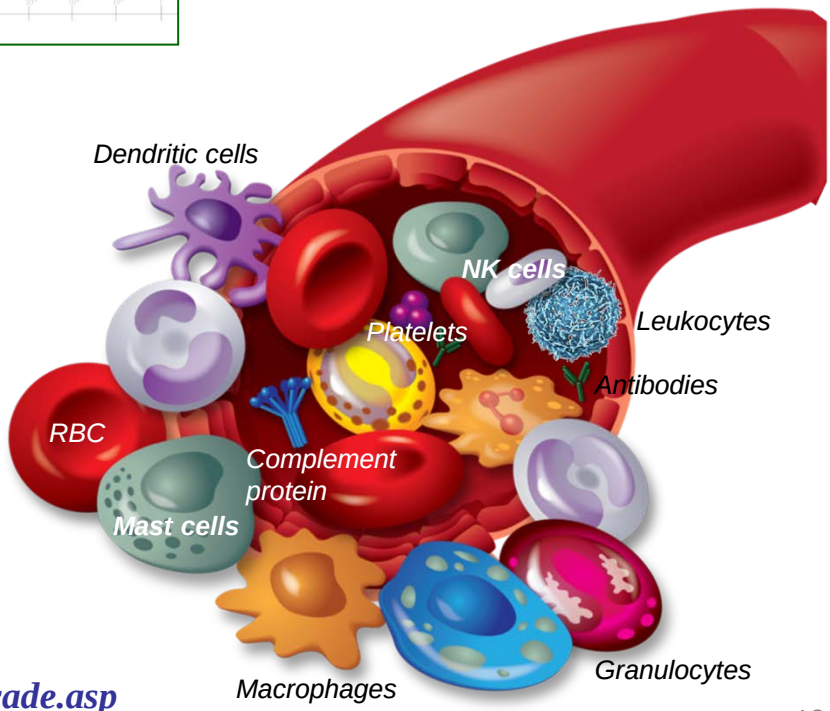
Cell Uptake/Distribution
- Cell Binding/Internalization
- Targeting

Hematology
- Hemolysis
- Platelet Aggregation
- Coagulation
- Complement Activation
- Plasma Protein Binding

Immune Cell Function
- Cytokine Induction
- Chemotaxis
- Phagocytosis
- Leukocyte Proliferation
- Leukocyte Procoagulant Activity

Toxicity
- Oxidative Stress
- Cytotoxicity
- Autophagy





http://ncl.cancer.gov/working_assay-cascade.asp

In Vivo Cascade

Initial Disposition Study

- Tissue distribution
- Clearance
- Half-life

Immunotoxicity

- Local lymph node proliferation assay
- T-cell dependent antibody response
- Rabbit pyrogen test

Single and Repeat Dose Toxicity

- Blood Chemistry
- Hematology
- Histopathology (42 tissues)
- Gross Pathology

Efficacy

- Therapeutic
- Imaging

Pharmacology

- Clinical Tx cycle
  - Schedule
  - Duration
  - Route
  - Formulation
- NP Quantitation methods
  - radiolabeled nanoparticle (scintillation)
  - Imaging
  - ELISA
  - ICP-MS
- PK Parameters
  - AUC, C_{max}, CL, $t_{½}$, $t_{max,}$ V_{ss}

Small Animal Imaging Program

Center for Advanced Preclinical Research

NCL Capabilities

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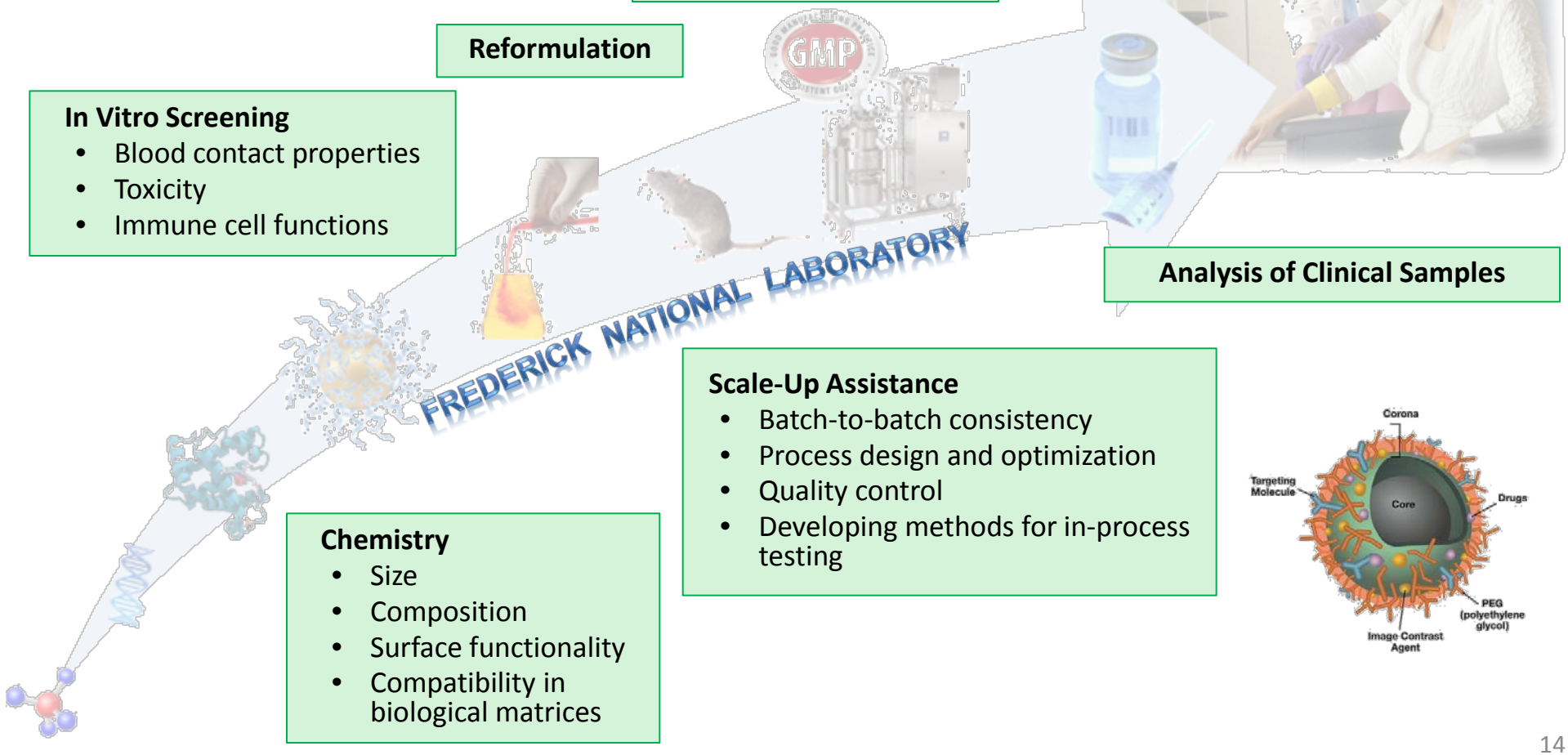
In Vivo Screening
- ADME-Toxicity
- Efficacy
- Pharmacokinetics
- Drug Metabolism
- Immunotoxicity

Reformulation

In Vitro Screening
- Blood contact properties
- Toxicity
- Immune cell functions



Analysis of Clinical Samples

Scale-Up Assistance
- Batch-to-batch consistency
- Process design and optimization
- Quality control
- Developing methods for in-process testing

Chemistry
- Size
- Composition
- Surface functionality
- Compatibility in biological matrices

FNL Capabilities

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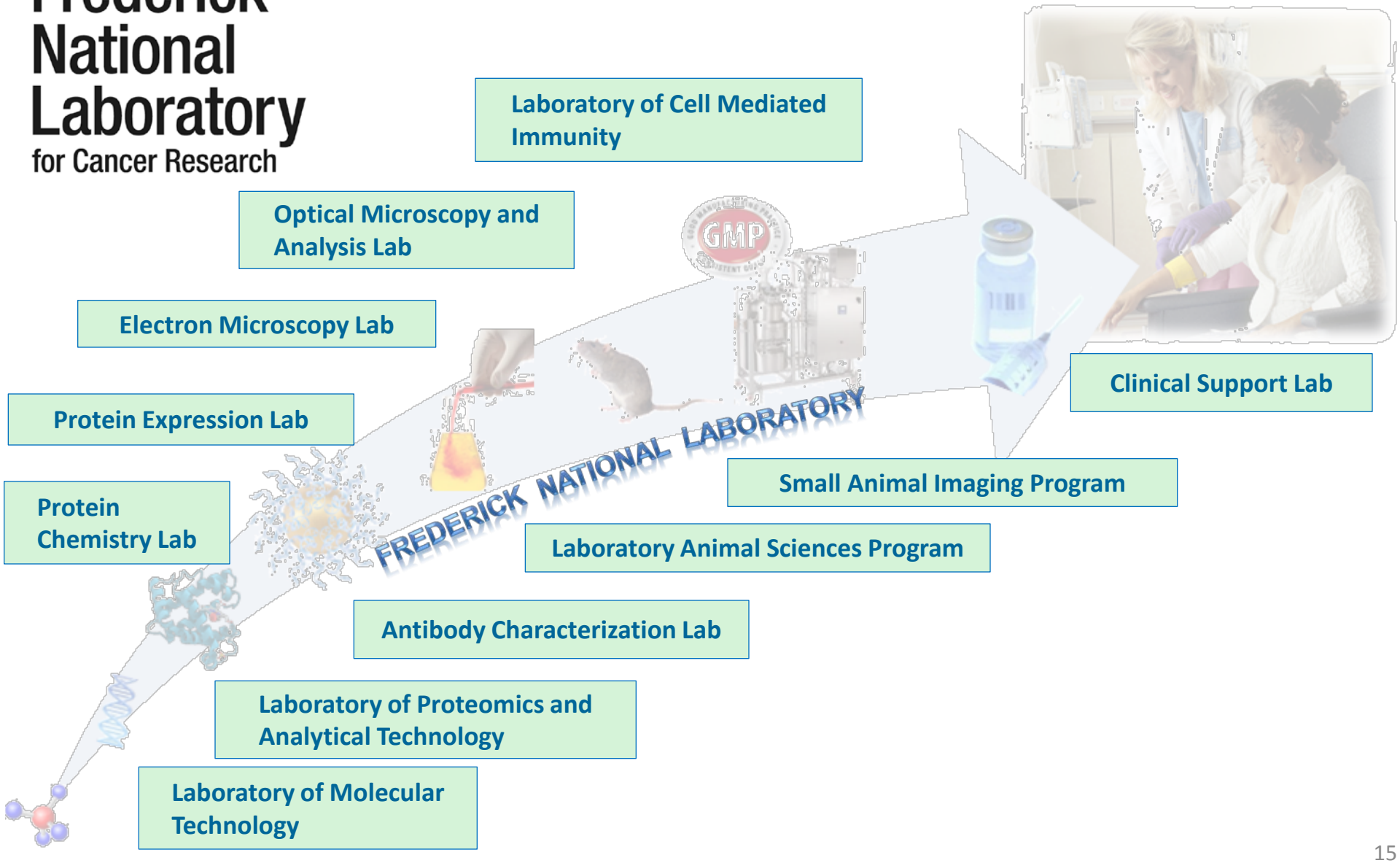
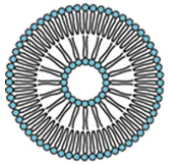
Frederick National Laboratory for Cancer Research

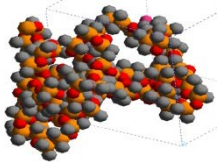


Materials NCL has Characterized

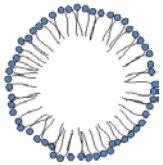
Dendrimers



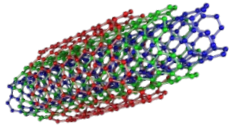
Liposomes



Polymers

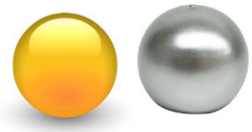


Nanoemulsions



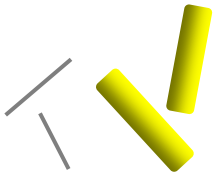
Carbon Nanotubes

Fullerenes

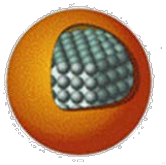


Gold & Silver Colloids

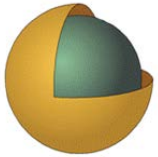
Metal Oxides



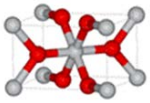
Nanorods & Nanowires



Quantum Dots



Core-Shell



Nanocrystals

More than 300 different nanoparticles have been submitted to the NCL.

NCL Collaborators

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Attracting Investment in Nanotech

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CYTIMMUNE AstraZeneca

CytImmune and AstraZeneca to Research Potential New Nanomedicine for Cancer

Rockville, MD and Waltham, MA –December 27, 2012-CytImmune and AstraZeneca have entered into an agreement to study the feasibility of a new cancer

Press Releases

BIND Therapeutics Announces Global Collaboration with Pfizer to Develop and Commercialize Multiple Accurins™

BIND Eligible to Receive Approximately $50 Million in Upfront and Development Milestone Payments Plus Additional Regulatory and Sales Milestones and Royalties for Each Accurin

Cambridge, MA, April 3, 2013—BIND Therapeutics, a clinical-stage biopharmaceutical

CELATOR® PHARMACEUTICALS RAISES $32.5 MILLION COMPLETING A $39.3 MILLION PRIVATE PLACEMENT FINANCING

Proceeds Will Support Phase 3 Study of CPX-351 in Acute Myeloid Leukemia

Princeton, NJ (April 30, 2013) Celator Pharmaceuticals, Inc., a clinical-stage biopharmaceutical

BIND THERAPEUTICS

CYTIMMUNE SCIENCES INC

Celator Pharmaceuticals

Pfizer

AMGEN

AstraZeneca

More than $1 billion in potential funding raised by NCL collaborators.

Success Stories: NCL Submissions Now in Clinical Trials

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Nanospectra

- *Silica-core gold-shell particle for photothermal ablation with NIR irradiation.*
- *Pilot safety study in head and neck cancers ongoing; efficacy study in lung tumors started in 2012.*

IDE 2008

AZAYA THERAPEUTICS™

- ATI-1123 PEGylated nanoliposomal formulation of docetaxel.
- Phase I safety study in patients with advanced solid tumors complete in 2012.

IND 2009

BIND THERAPEUTICS

- BIND-014 docetaxel-encapsulated PLGA nanoparticle-aptamer conjugates.
- Phase I safety study in patients with advanced or metastatic cancer ongoing.
- Phase II safety and efficacy studies in patients with metastatic prostate and NSCLC.

IND 2011

PDS Biotechnology

- PDS0101, a Versamune® HPV antigen nanoparticle.
- Phase I study ongoing for patients with human papilloma virus (HPV).

IND 2013

CYTIMMUNE SCIENCES INC

Phase 1 Complete in 2008

- AurImune® PEGylated colloidal gold nanoparticle-TNFa conjugates.
- Phase II study in combination with Taxotere to start soon.

ProNAi

IND 2010

- PNT2258 liposome-encapsulated oligonucleotide for breast and lung cancer.
- Phase I safety study in patients with advanced solid tumors ongoing.
- Phase II study for patients with Non-Hodgkin's lymphoma.

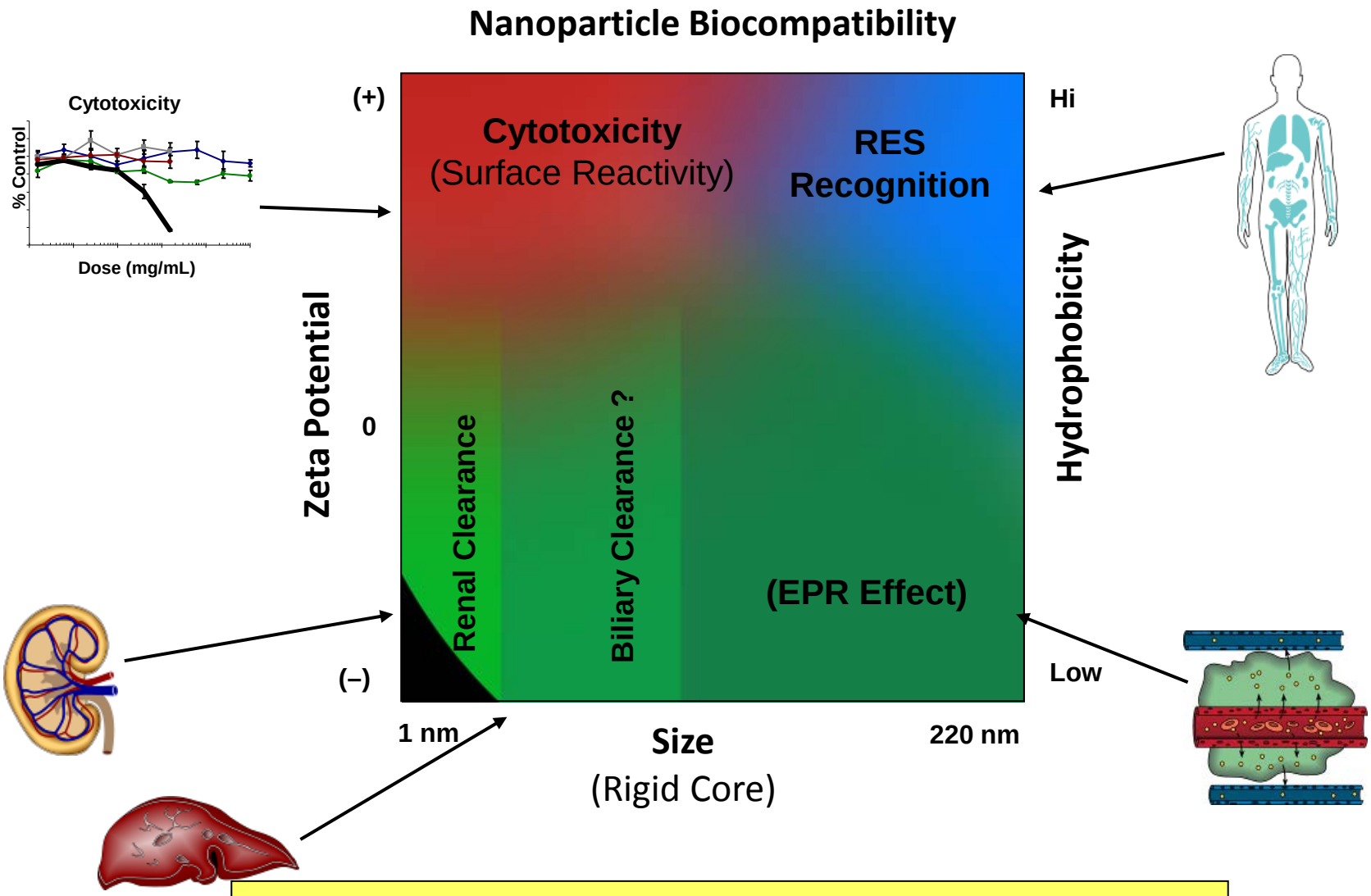
WE MAKE LIVES BETTER UT HEALTH SCIENCE CENTER™ SAN ANTONIO

- Rhenium nanoliposomes for intracranial glioblastoma treatment.
- Combined Phase I/II for patients with recurrent GBM.

IND 2014

NCL Lessons Learned – Physicochemical Attributes Influence Biocompatibility

Lessons Learned: Biocompatibility

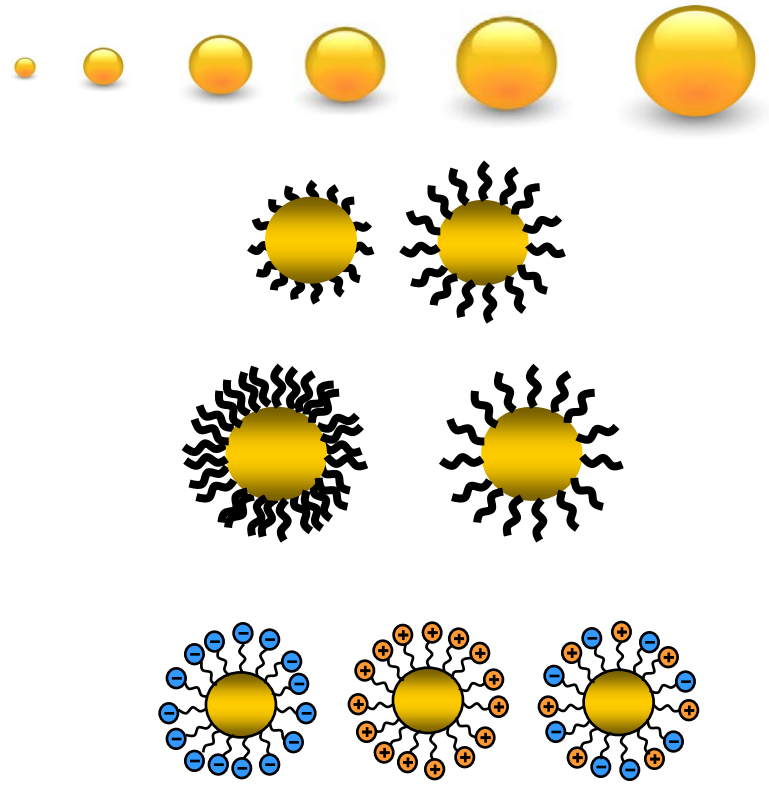


Physicochemical parameters contribute to toxicity.

McNeil (2009), Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, 1:264-271.
Nel et al. (2009), Nature Materials 8: 543-557.
Cover of Advanced Drug Delivery Reviews, June, 2009.

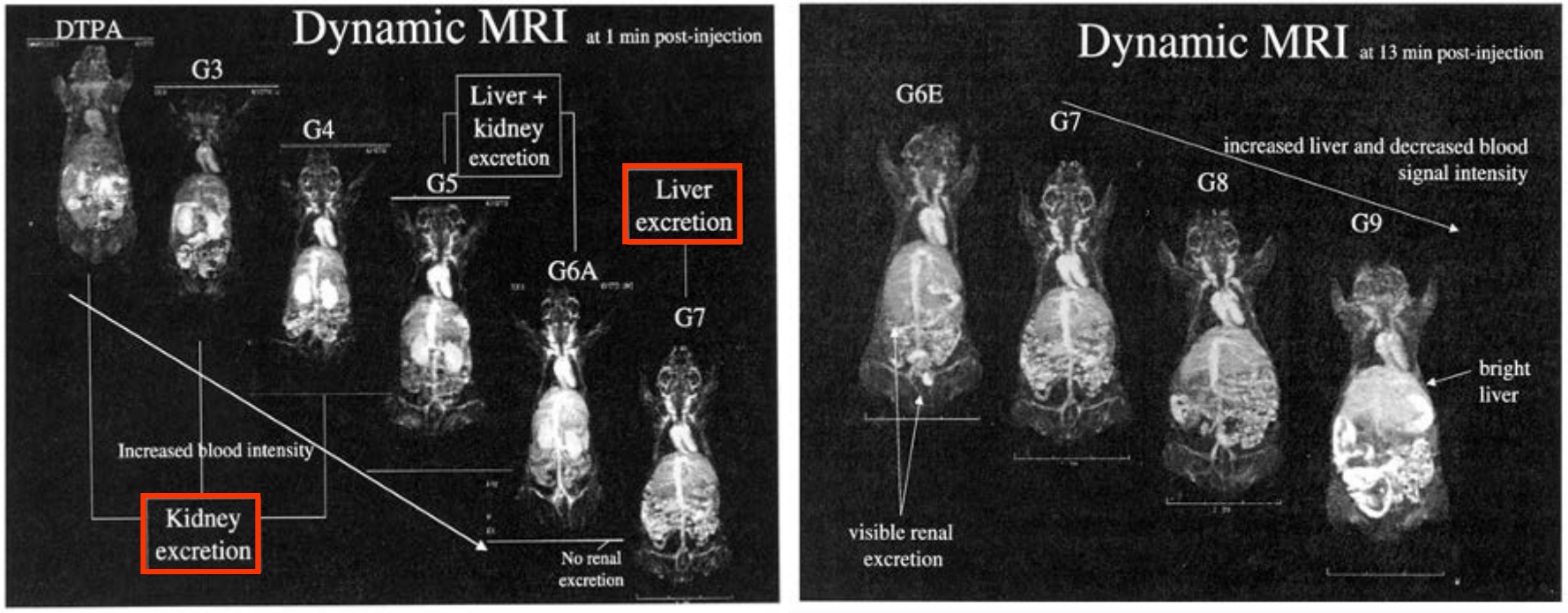
PCC Parameters to Monitor

- Size
- Surface ligand/coating
- Surface ligand density
- Surface charge
- Solubility
- Shape/Architecture
- Stability
- Purity



Importance of Size

Dendrimer-Based MRI Contrast Agents



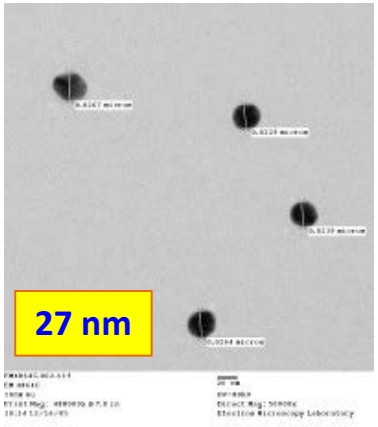
Kobayashi and Brechbiel, (2003), Molecular Imaging, 2:1-10.

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

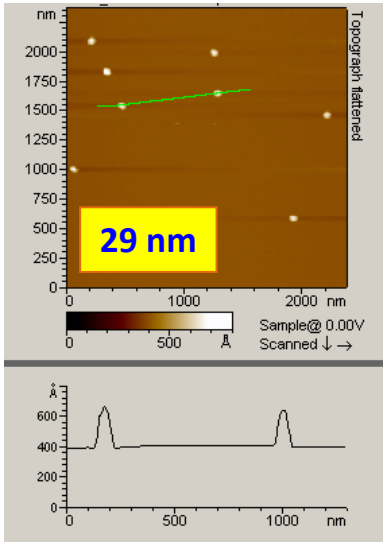
Size in a Biological Context

30 nm Gold colloids in PBS

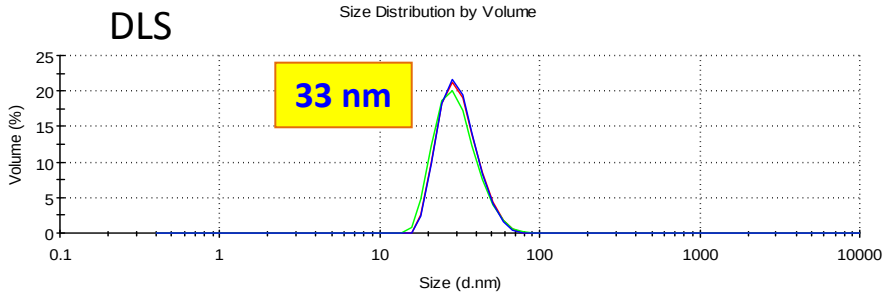
TEM



AFM

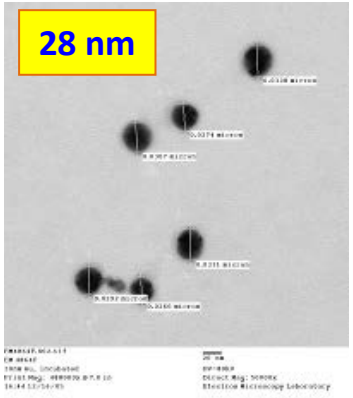


DLS

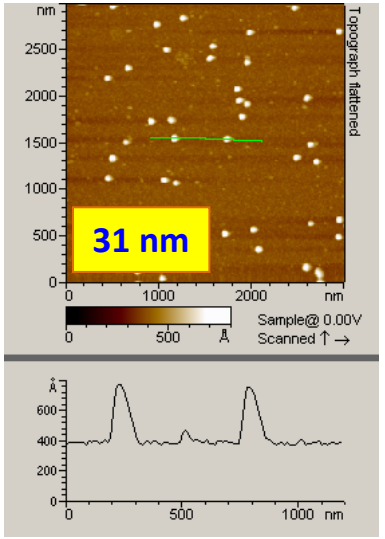


30 nm Gold colloids incubated in plasma

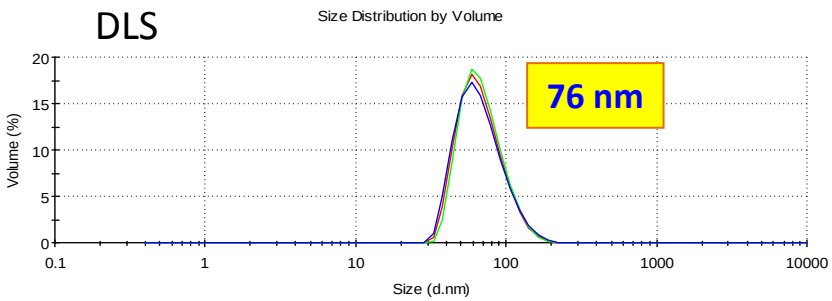
TEM



AFM



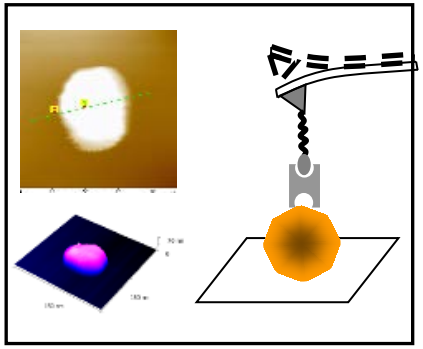
DLS



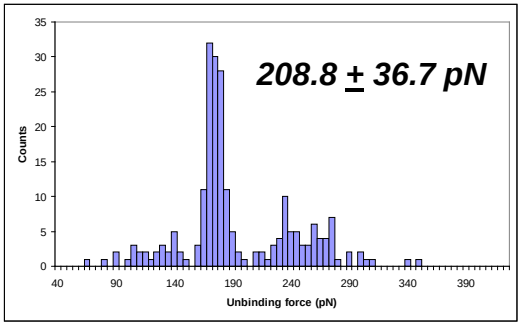
Multiple orthogonal methods needed to characterize size.

Dobrovolskaia et al, (2009), Nanomed. Nanotechnol. Biol. Med., 5:106-117.

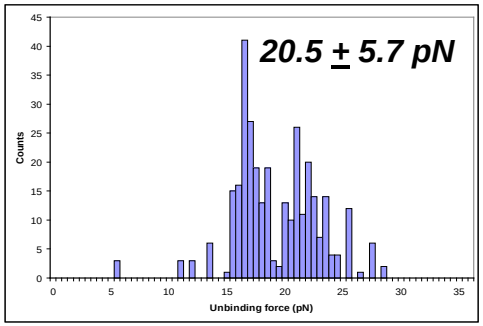
Importance of Surface Ligand/Coating

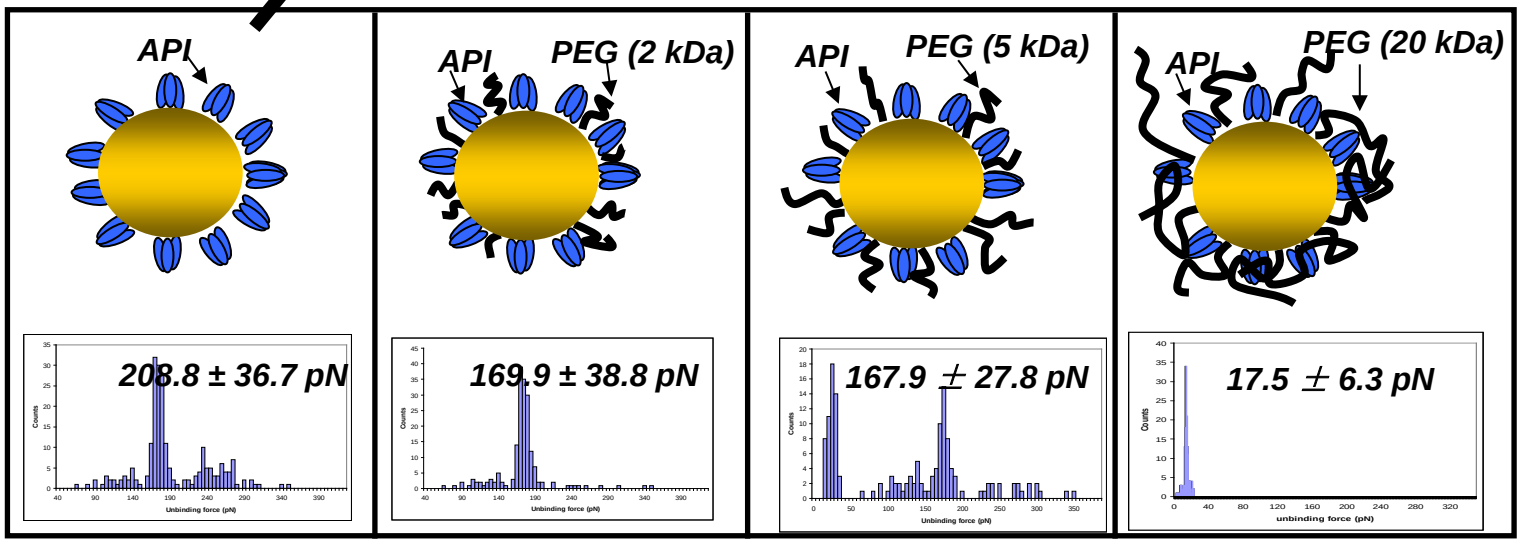


IgG – API Binding



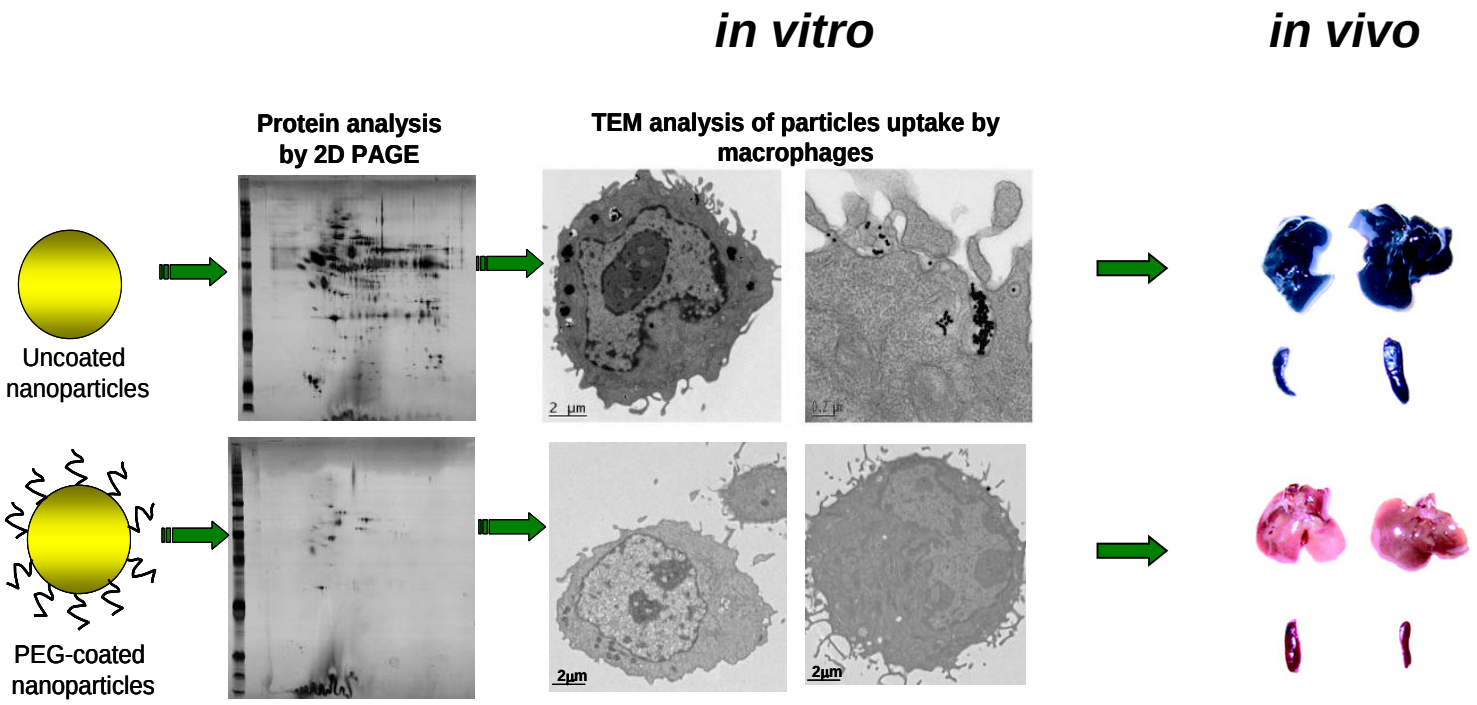
Control – IgG Blocking





PEG masks API recognition; PEG molecular weight is critical.

Importance of Surface Ligand Density

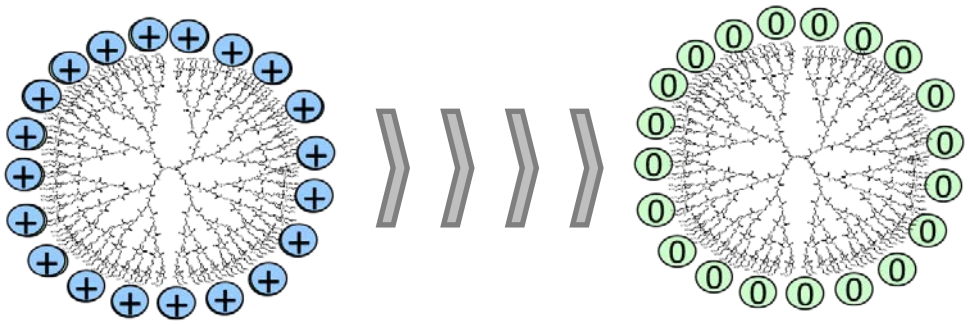


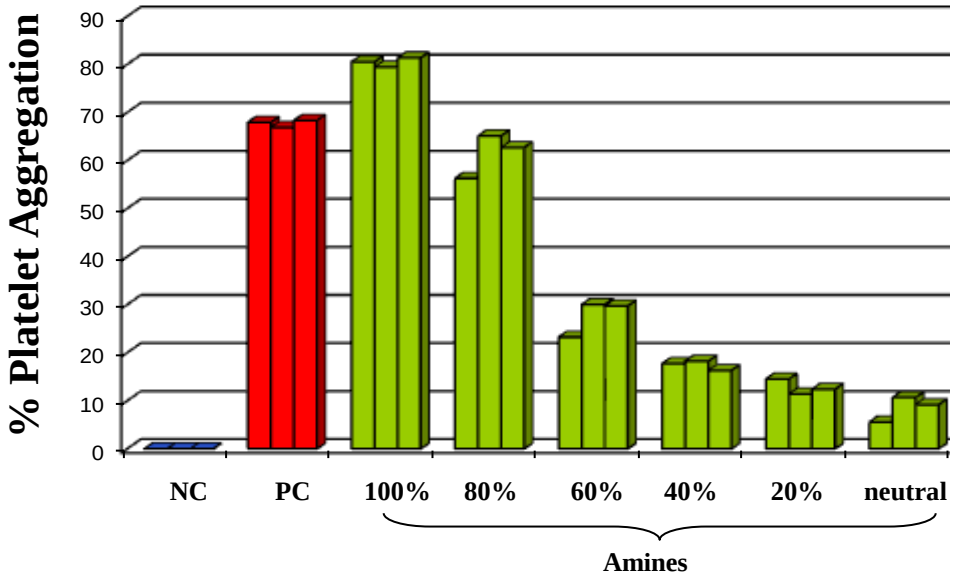
Dobrovolskaia et al., (2008), Mol.Pharm., 5:487-495.

Paciotti J. et al.,(2004), Drug Delivery,11:169-183.

Difference in surface characteristics can cause dramatically different in vivo outcomes.

Importance of Surface Charge

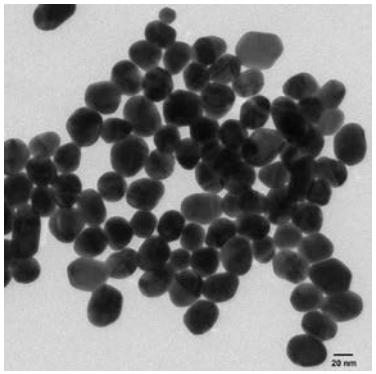


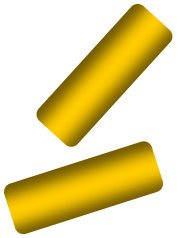


Biocompatibility depends on surface charge.

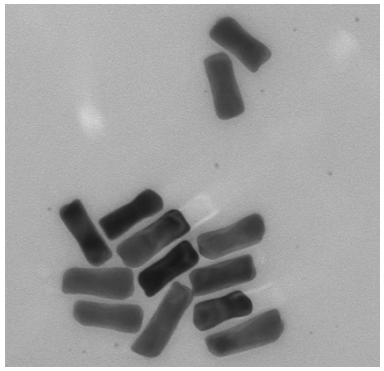
Importance of Shape/Architecture

Gold Spheres





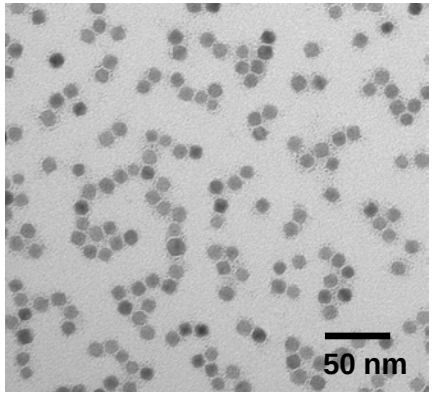
Gold Nanorods

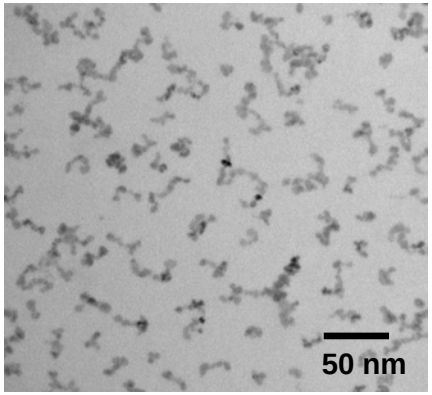


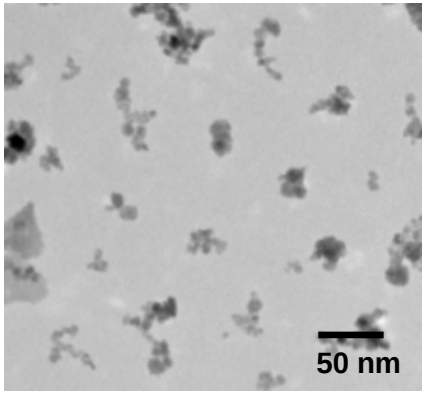
Shape and size can vary widely.

Iron Oxide

TEM *Three different sources of iron oxide nanoparticles*





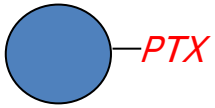


DLS

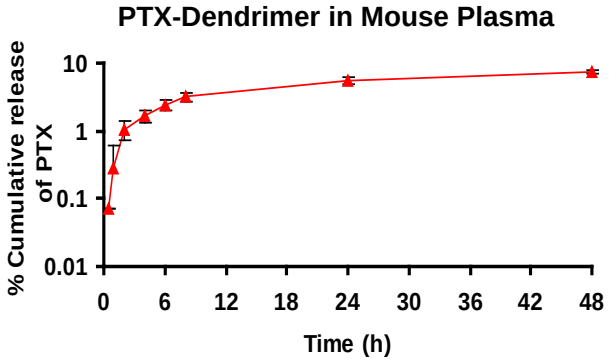
Z-Avg:	*55.3 nm*	*46.2 nm*	*82.9 nm*
PdI:	*0.058*	*0.113*	*0.124*

Importance of Stability

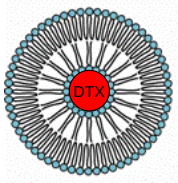
Too Stable: *<10% release after 48 hours in vitro*



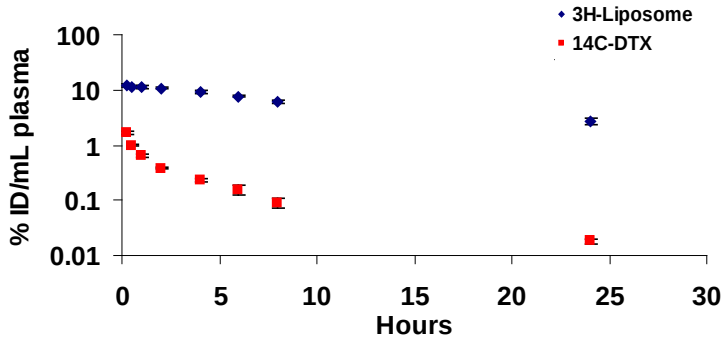
Triazine dendrimer conjugated to paclitaxel (PTX)

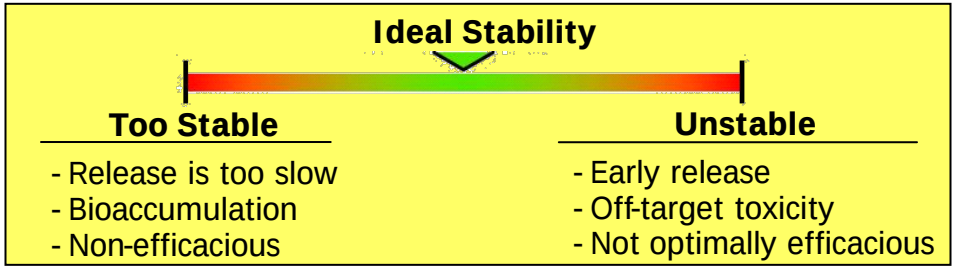


Unstable: *Different rates of clearance from plasma indicate the particle comes apart within 15 min*

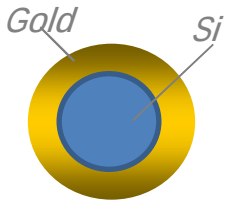


Liposomal formulation of docetaxel (DTX)

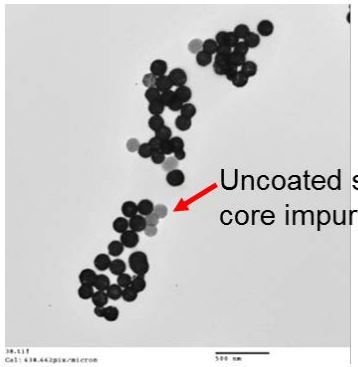


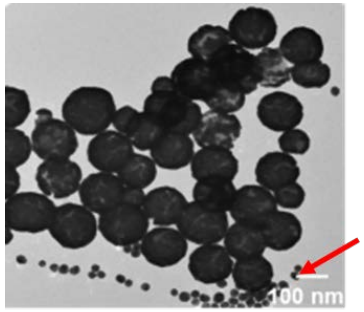


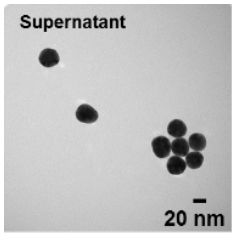
Importance of Purity

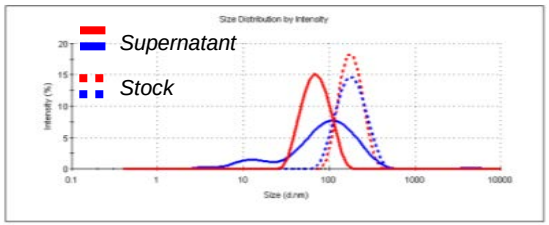


Gold-Coated Silica

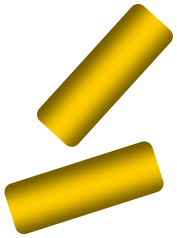


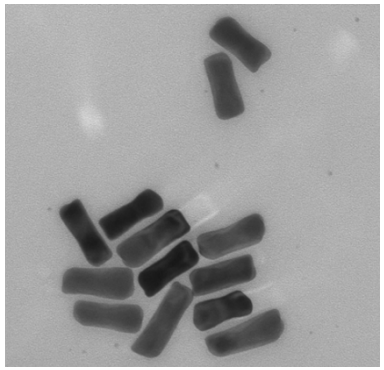


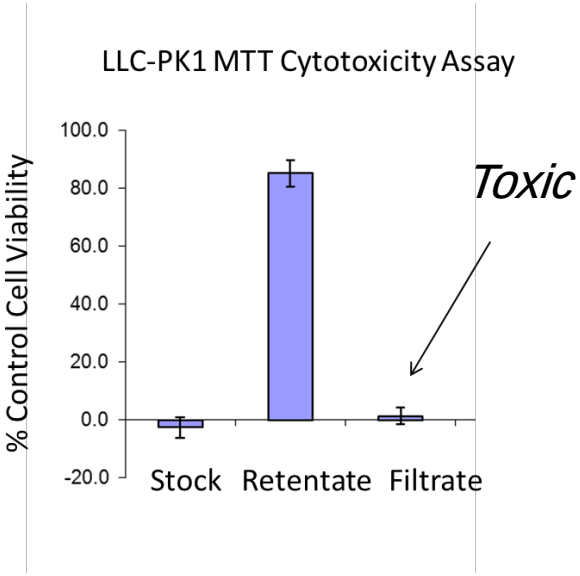




Gold Nanorods







Impurities can be separated, characterized for batch-to-batch consistency.

Characterization Challenges – Properties Affect Biocompatibility

Physicochemical properties greatly affect biodistribution, efficacy and toxicity profile

- Small changes in any of these parameters can dramatically influence biocompatibility
- Importance of characterization:
  - *Batch-to batch variability; which assays are critical for monitoring*
  - *Are adequate analytical methods available?*
  - *In process analytical (at intermediate stages)*
  - *Homogeneity and inhomogeneity in ligand distribution*
  - *Free components/impurities*
  - *Quantitation and activity of individual components*
  - *Image contrast agents, drugs, targeting ligands*
  - *Surface component characterization*
  - *Stability assessment*

Know What You Have

Cerium Dioxide

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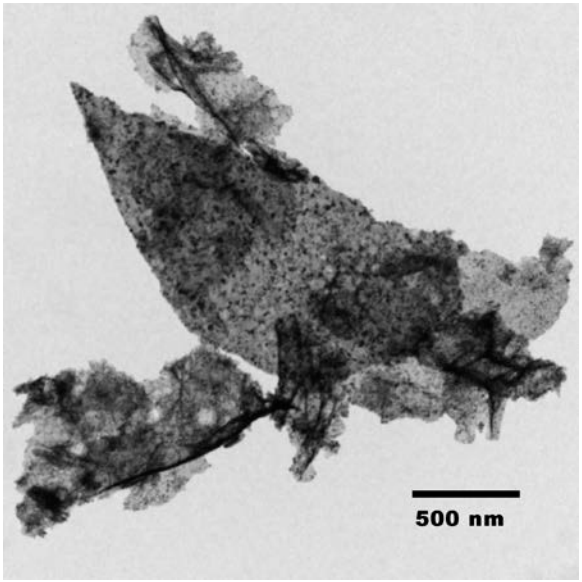
NIEHS National Institute of Environmental Health Sciences

Manufacturer-Stated Specs:

Formula: CeO_2
Purity: 99.5% minimum (based on rare earth oxide impurities)
Formula Weight: 172.12 g/mol
Melting Point: 2600°C
Density: 7.132 g/mL
Form: 15-30 nm average particle size, powder

What the Material Actually Looks Like:

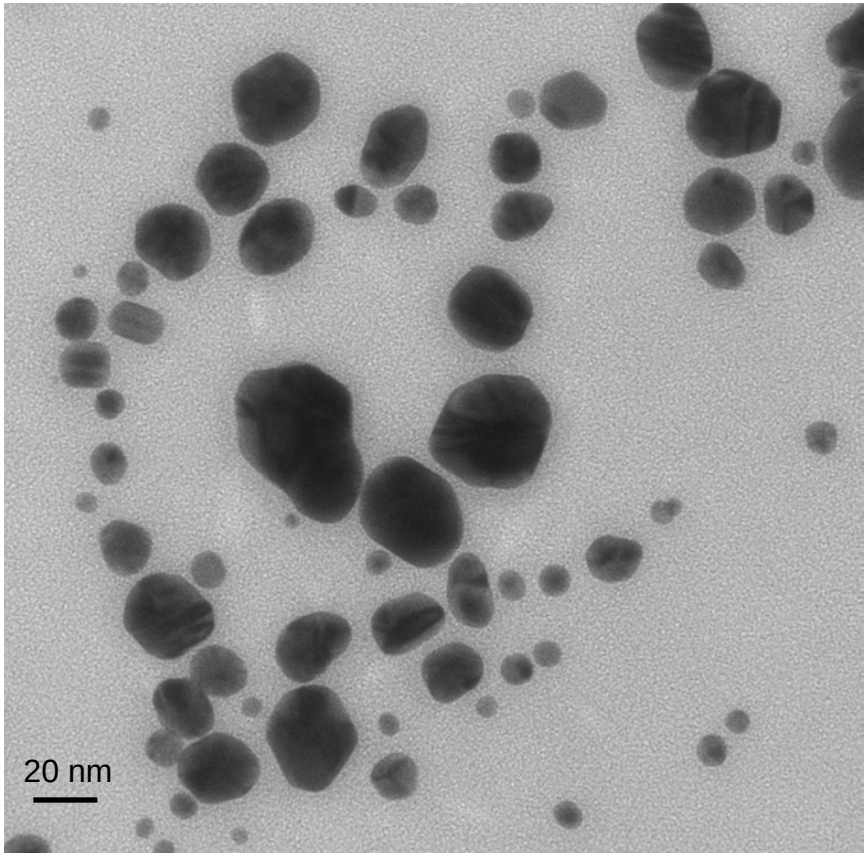
- *Micron-sized aggregates/agglomerates*
- *Largely insoluble in aqueous media*



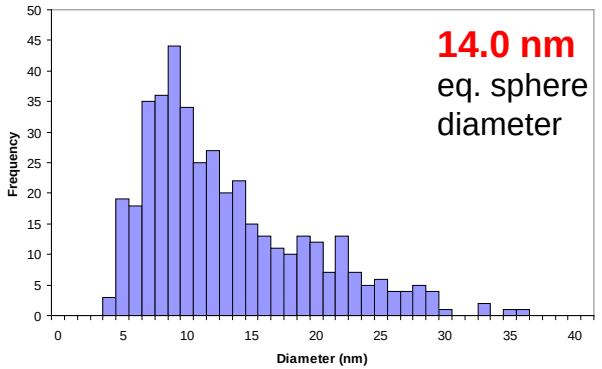
**Vendor used BET for measuring size –
BET is a surface area measurement,
not accurate for size measurements**

BET = Brunauer, Emmet, and Teller

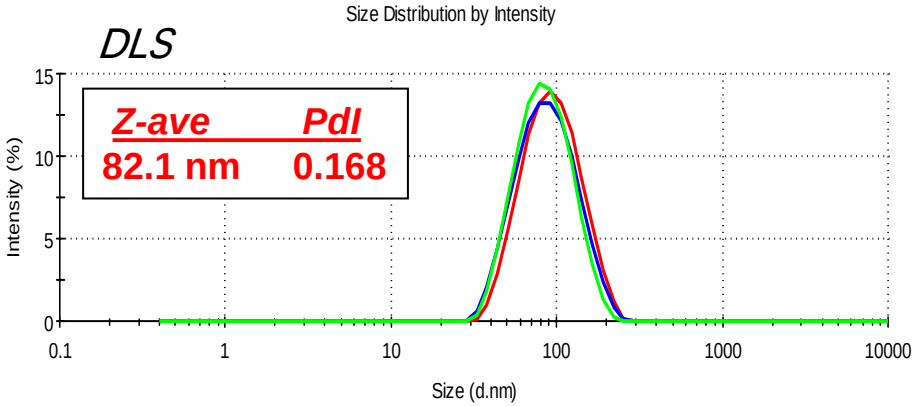
Gold Nanoparticles



TEM



DLS



Different techniques are sensitive to different size/shape populations. Different size/shape particles may have different biodistribution and toxicity.

Carbon Nanotubes

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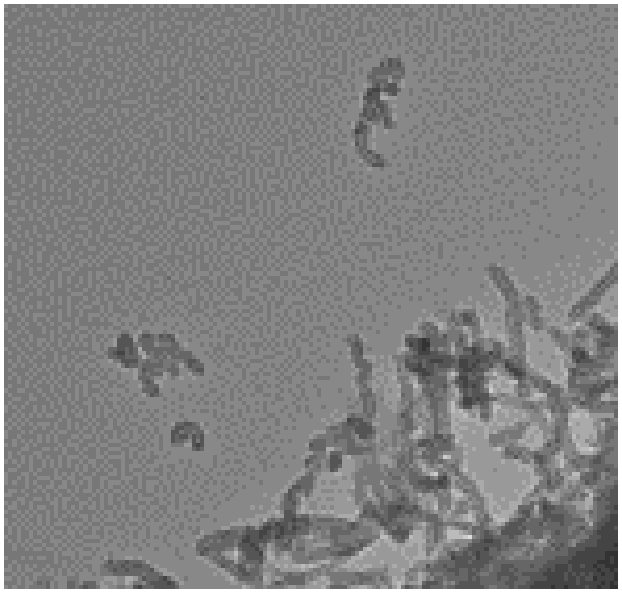
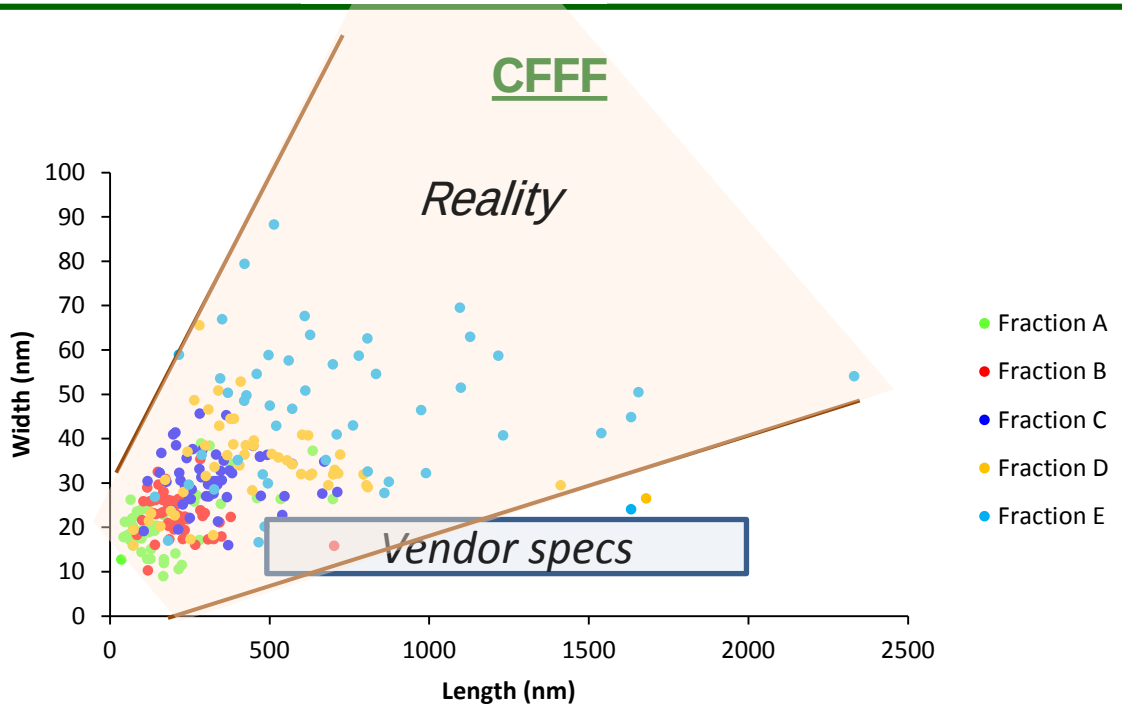
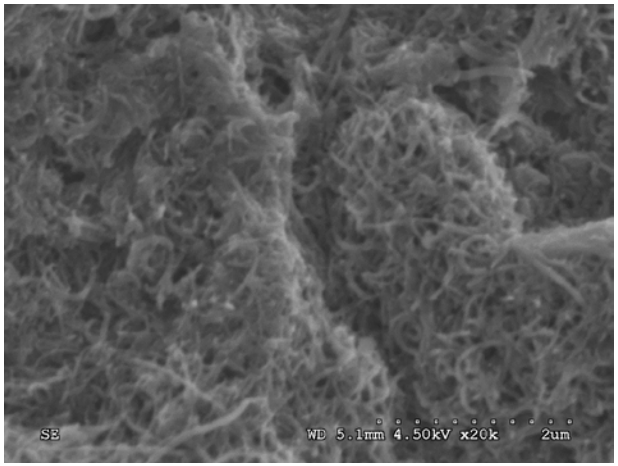
TEM



CFFF



SEM



Vendor specs: OD 10-20 nm, length 0.5-2 µm

CNTs will exist in a variety of sizes, shapes, and agglomeration states.

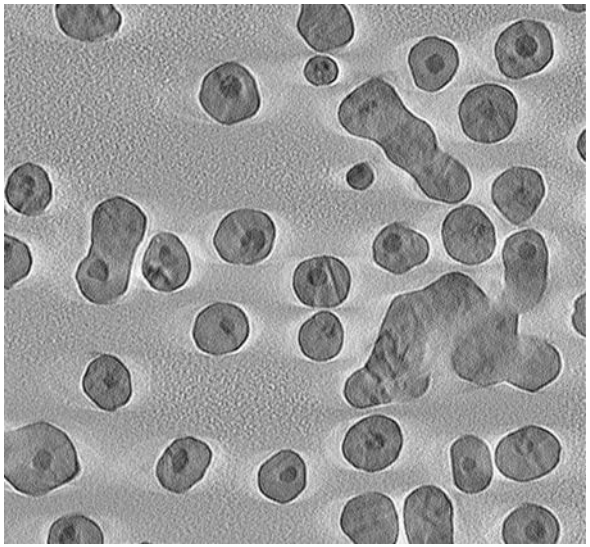
Silver

Advertised as:

unagglomerated, monodisperse, spherical silver nanoparticles

Vendor reported: TEM diameter size distribution, Ag concentration, UV-vis spectral properties

However…

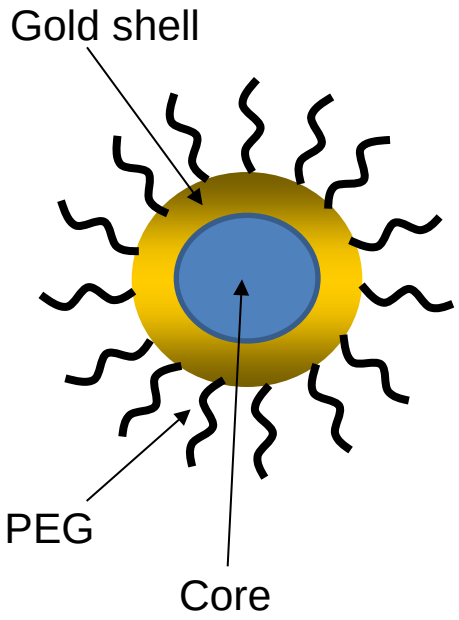


Silver nanoparticles were manufactured using a gold core.

Range of sizes and shapes present.

Case Study in Nanomaterial Safety Testing

Core Shell Nanoparticles



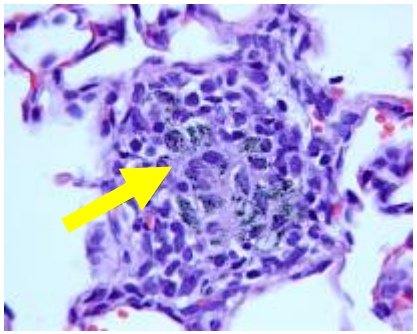
- Two batches of core shell nanomaterials appeared identical to physicochemical characterization.
- In tox studies, 1st batch caused extensive lung lesions, 2nd batch was largely benign.
- What's causing the dramatically different safety profiles of seemingly identical batches?

Dramatic Difference *In Vivo*

14-day ADME-Tox Study in Rats

Gold nanoparticle Batch 1

Extensive pigmentation in liver, spleen, lungs, ovaries, muzzles.
Treatment-related granulomous lesions in lungs.



Pyogranulomatous Inflammation-Lung- H&E-40x

There was some difference between the batches of nanoparticles not apparent by physicochemical characterization…

Gold nanoparticle Batch 2

Much less pigmentation. Few, statistically insignificant, mild lung lesions.

PCC: No Difference in Size, Zeta Potential

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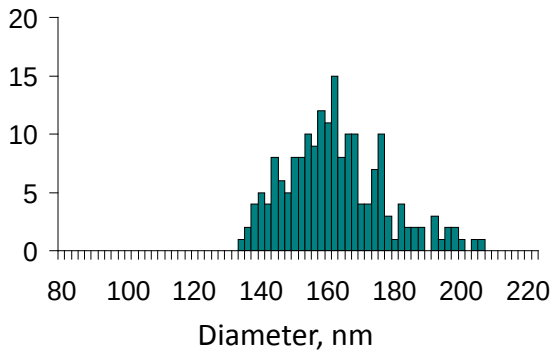
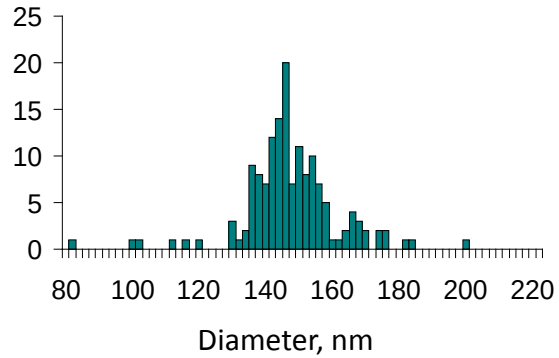
TEM

Batch 1: 157 ± 16 nm



Batch 2: 147 ± 14 nm



DLS

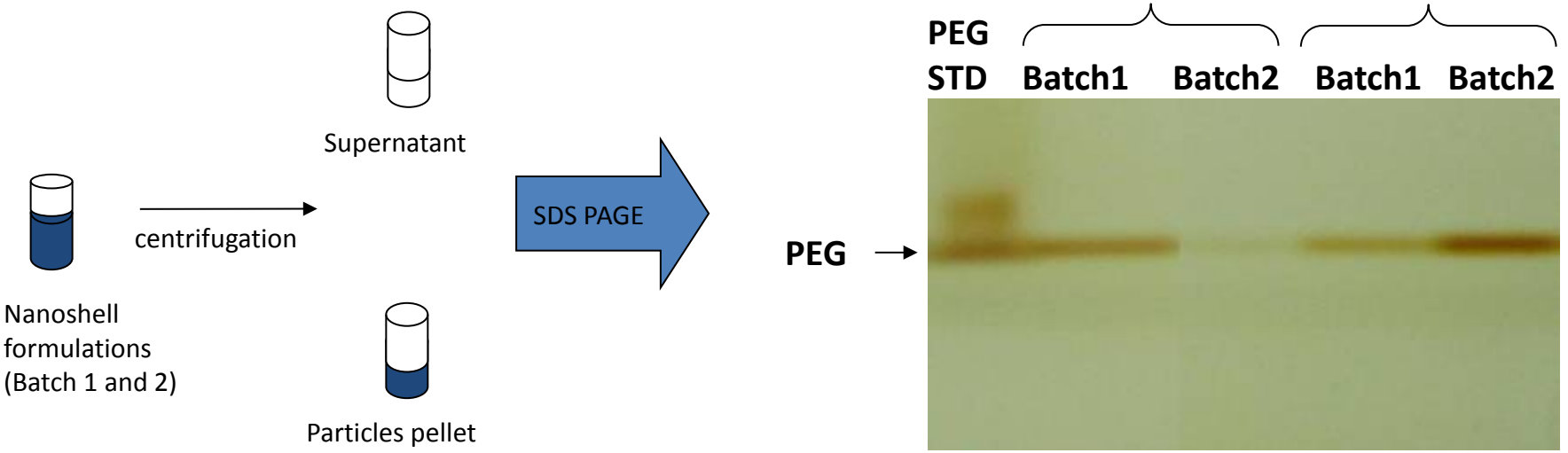
Sample	Z-Avg (nm)	PdI	Vol-Peak (nm)	%Vol
Batch 1	165 ± 1	0.114 ± 0.013	176 ± 2	100 ± 0
Batch 2	171 ± 1	0.060 ± 0.022	180 ± 2	100 ± 0

Zeta Potential

Sample	Zeta Potential (mV)
Batch 1	-7.2 ± 0.5
Batch 2	-8.0 ± 0.7

No significant difference between batch 1 and batch 2 in terms of size, charge, or polydispersity.

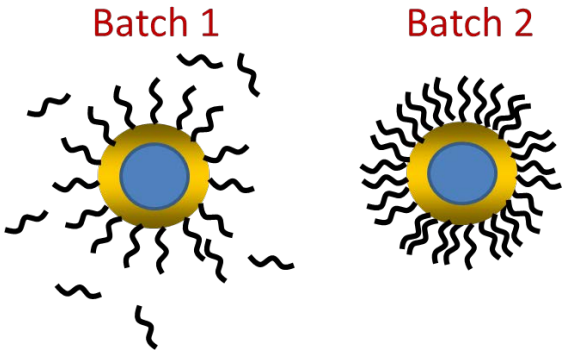
Difference in PEG Coatings



Barium Iodine Gel Staining

The PEG was dissociating from the particles over time, ending up in solution.

This difference in coatings was subtle enough not to be detected by routine PCC.



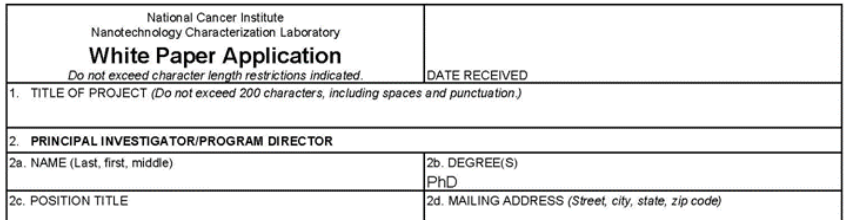
Summary

- **Physicochemical Characterization Matters!**
  - **Physical and chemical properties contribute to a nanomaterial's biocompatibility**
- **Know What You Have**
  - **Manufacturer's specifications may not always be right**
  - **Perform characterization under relevant conditions**
- **Interdisciplinary Nature of Nanomaterial Safety Testing**
  - **Combination of physicochemical, *in vitro*, and *in vivo* testing to understand results**

How to Apply for NCL Characterization

The NCL has a two-phase application process. For detailed information on submitting a proposal, please visit http://ncl.cancer.gov/working_application-process.asp.

- Brief (3 page) White Paper
- Quarterly deadlines (next: June 2nd)
- Specific questions from review committee
- Part II presentation & discussion with NCL scientists - in person or via webex
- 50% acceptance rate for qualifying applications



National Cancer Institute
Nanotechnology Characterization Laboratory
White Paper Application
Do not exceed character length restrictions indicated.

DATE RECEIVED

1. TITLE OF PROJECT *(Do not exceed 200 characters, including spaces and punctuation.)*

2. **PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR**

2a. NAME (Last, first, middle)	2b. DEGREE(S) PhD
2c. POSITION TITLE	2d. MAILING ADDRESS *(Street, city, state, zip code)*

Thinking of applying?
Have questions?
Email: ncl@mail.nih.gov
Ph # 301-846-6939

- NCL resources are FREE !

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