Characterization of Nanoparticles Intended for Cancer Therapeutics and Diagnostics

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Nanotech Characterization Laboratory

SAIC-Frederick

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SAIC-Frederick, Inc.
A Subsidiary of Science Applications International Corporation

NANOTECHNOLOGY CHARACTERIZATION LABORATORY

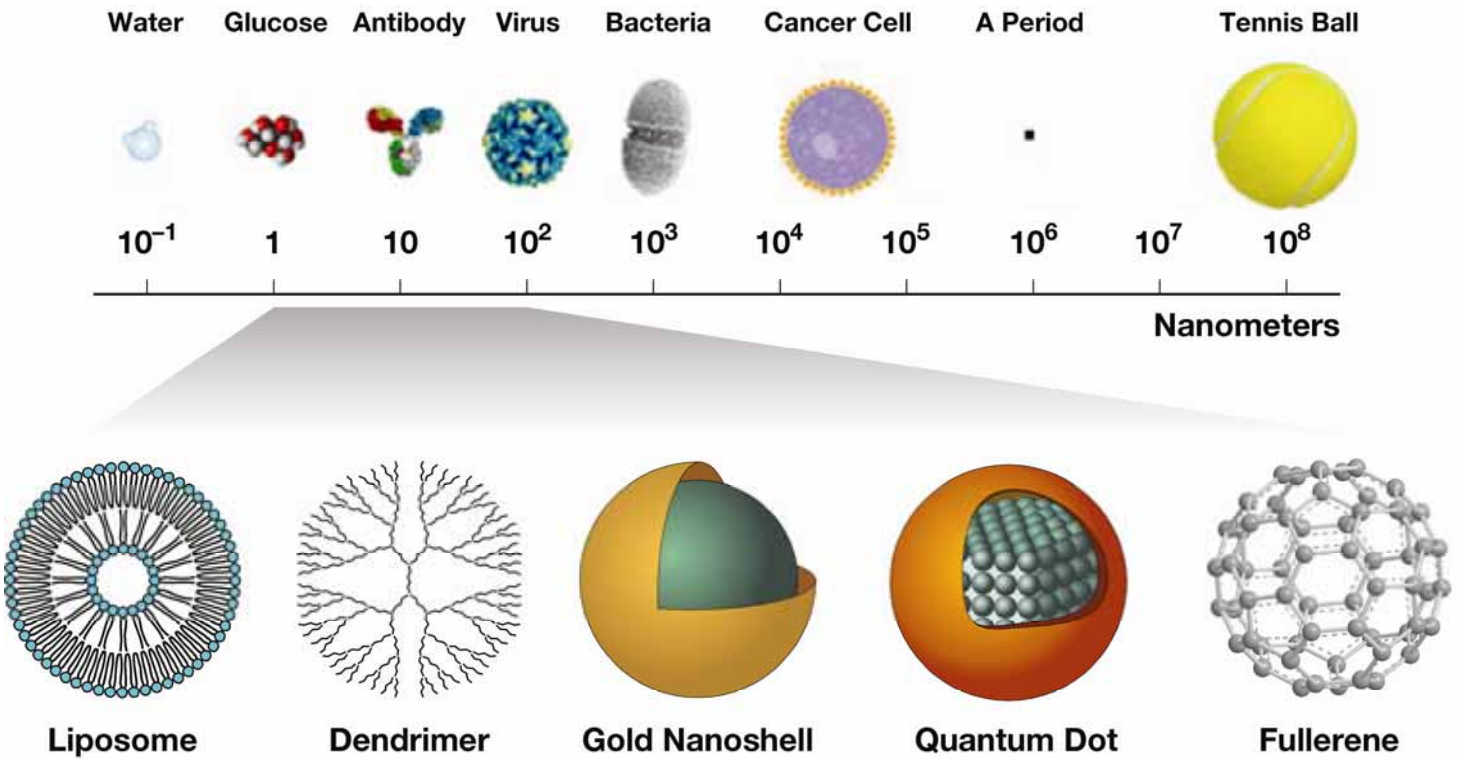
Outline

- Background on Nanotechnology
- NCI Efforts
- NCL Programmatics
- Characterization Data

NANOTECHNOLOGY CHARACTERIZATION LABORATORY

Definition

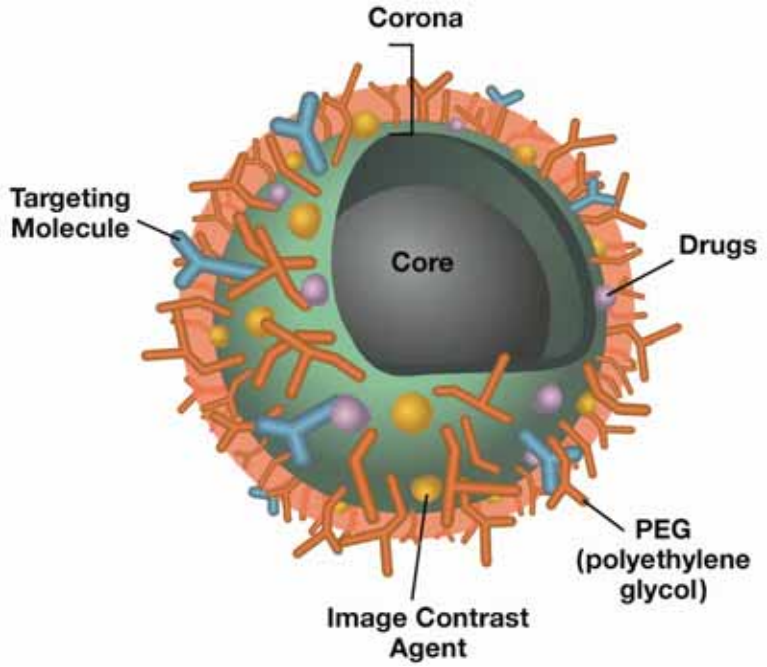
"Research and technology development at the atomic, molecular or macromolecular scale leading to the controlled creation and use of structures, devices and systems with a length scale of approximately 1 – 100 nanometers (nm)." (Source: National Nanotech Initiative)



Why Nano?

Therapeutic Benefits

- Solubility
  - Carrier for hydrophobic entities
- Multifunctional capability
- Active and passive targeting
  - Ligands; size exclusion
- Reduced toxicity



McNeil, (2005), J. Leuk. Biol., 78:585-594

↑Solubility ↑Stability ↑Specificity = ↓Toxicity ↑Efficacy

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Active Targeting

Free MTX

30 mg/kg total

Nanodevice MTX

3 mg/kg total MTX

Dr. James Baker, University of Michigan

Nanotech at NCI

Background

- NCI has funded exploratory work over the past 6 years on integrating nanotechnology into biomedical research
- Unconventional Innovations Program (UIP)
  - Diagnostics (Imaging)
  - Therapeutics
- Priority is to now transition that research into the clinical realm.

- Run by Office of Technology and Industrial Relations (OTIR)
  - Director: Dr. Greg Downing
  - Extramural Budget: $144M over 5 years
  - Launched on Sept 13th, 2004
  - Website: http://nano.cancer.gov/

- Consensus among cancer researchers that significant obstacles must be overcome in order to transition 'nano' to clinical realm
  - Critical lack of available standards
  - 1st principles characterization
  - Regulatory uncertainty

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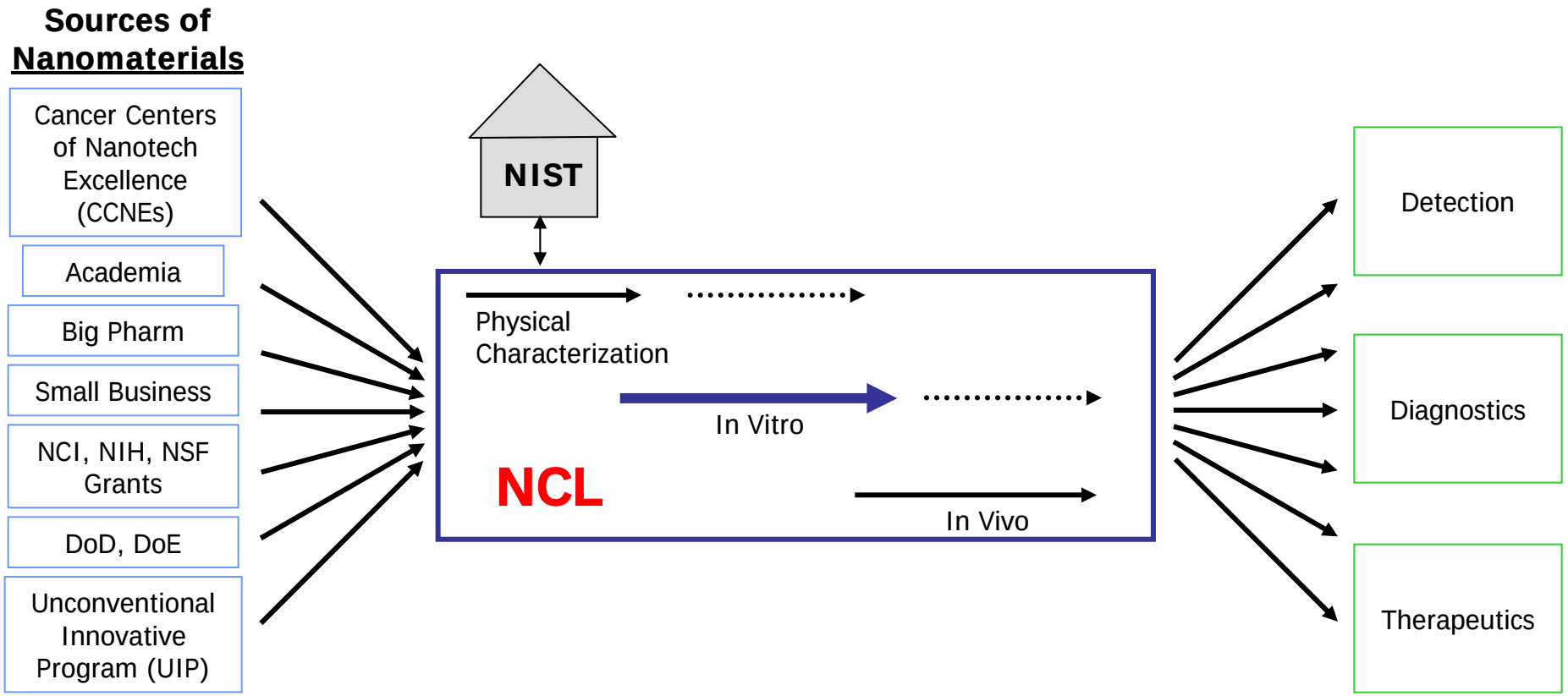
NCL Objectives

- Identify and characterize critical parameters related to nanomaterials' biocompatibility; structure-activity relationships.
- Establish and standardize an assay cascade for nanomaterial characterization.
- Examine the biological characteristics of multi-component/combinatorial platforms.
- Engage and facilitate academic and industrial-based education and knowledge sharing.

NCL Concept of Operations



NCL conducts pre-clinical characterization in support of an Investigative New Drug (IND) submission to the FDA

NCL Facilities

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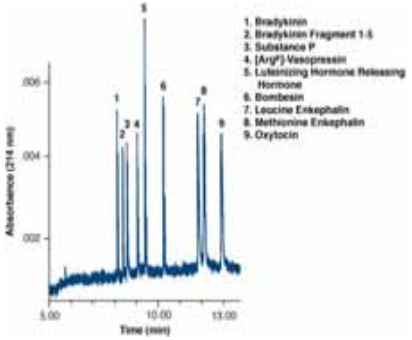
NCI/SAIC Research Technology Program

ABCC Advanced Biomedical Computing Center
A Center Devoted To Biocomputing

RTP SEPARATIONS TECHNOLOGY GROUP



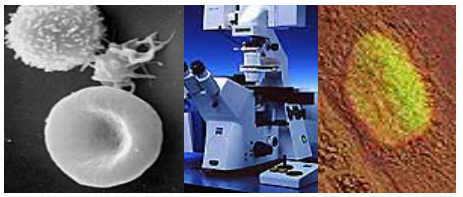
Physical Characterization

In Vitro

NCL

In Vivo

IMAGE ANALYSIS LABORATORY



Confocal and Electron Microscopy

LASP
Laboratory Animal Sciences Program

RTP MASS SPECTROMETRY CENTER

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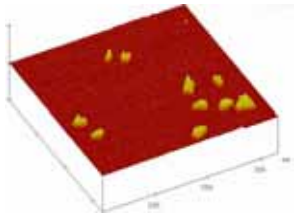
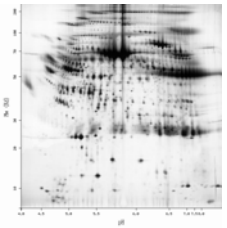
NCL Assay Cascade



Physical Characterization:

- Size
- Size distribution
- Molecular weight
- Morphology
- Surface area
- Porosity
- Solubility
- Surface charge density
- Purity
- Sterility
- Surface chemistry
- Stability



In Vitro:

- Binding
- Pharmacology
- Blood contact properties
- Cellular uptake
- Cytotoxicity

In Vivo:

- Absorption
- Pharmacokinetics
- Serum half-life
- Protein binding
- Tissue distribution
- Metabolism
- Excretion
- Safety

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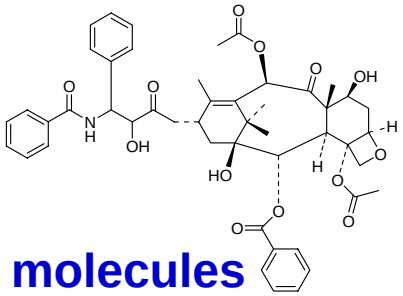
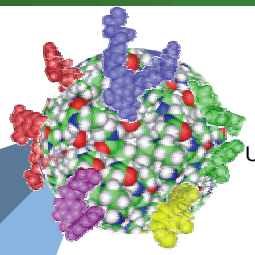
Core parameters to define physicochemical property of material



Small molecules



James Baker
University of Michigan

Nanomaterial

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

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

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

Same parameters – different/additional characterization methods

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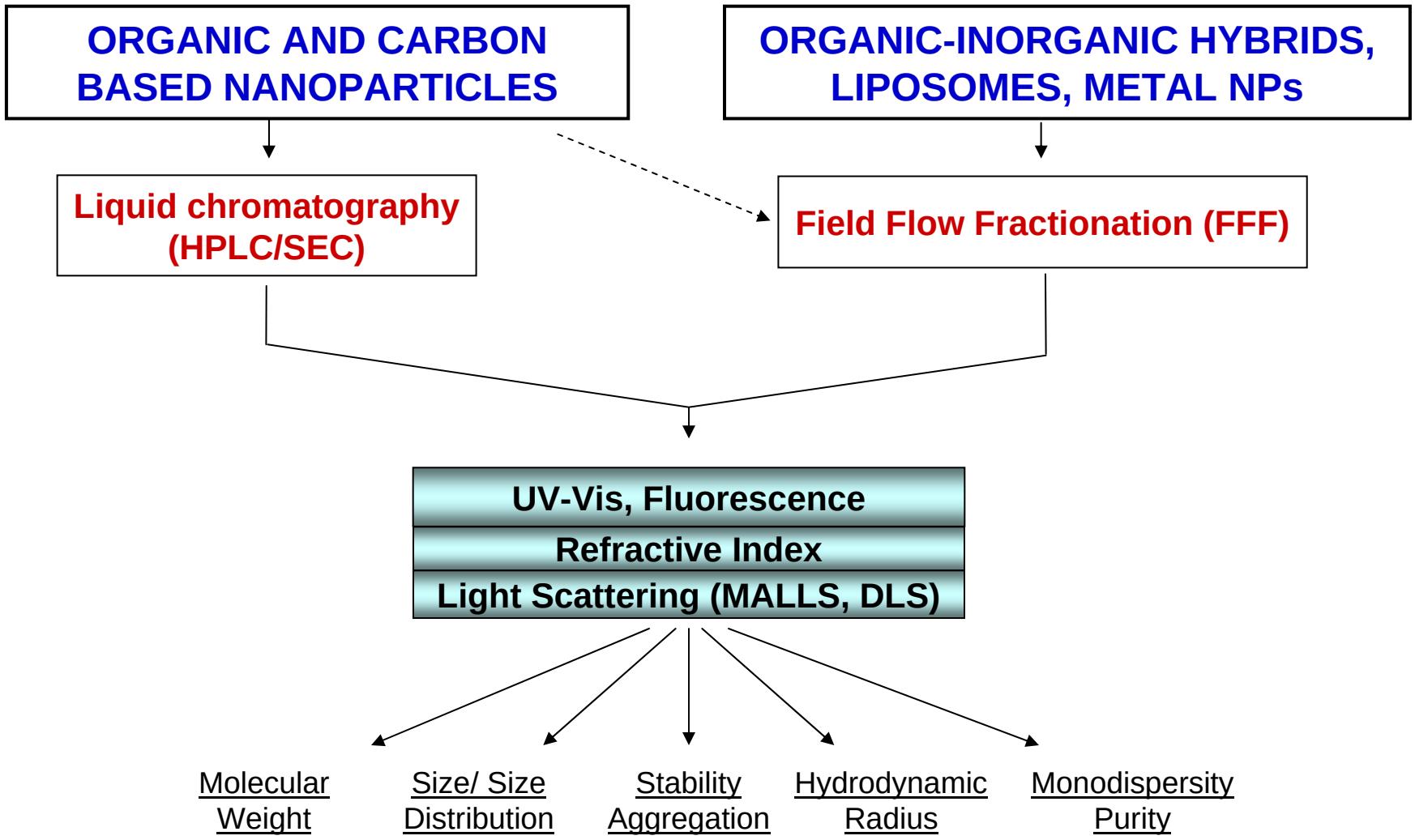
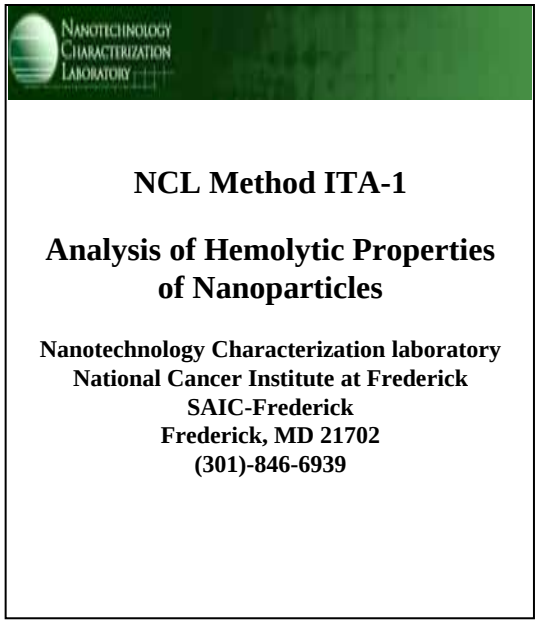
Flow mode analysis of Nanoparticles



In Vitro Cascade

In Vitro

- Sterility
  - Bacterial/Viral/Mycoplasma
  - Endotoxin
- Targeting
  - Cell Binding/Internalization
- 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)
- Metabolic Stability



VCS informs regulatory agencies and promotes commercialization of nanotechnology for medical applications

Industry, Academia, Government

E5620-06
STANDARD

Voluntary Consensus Standard (VCS) is established.

Consensus Ballot

Ballot of Standard is submitted to ASTM members for consensus.

Submit Draft Standard

Draft of Standard is submitted to ASTM E-56.20 Subcommittee for comment and revision

NCI
FDA
NIST

Solicit Comments on Protocol

Protocols are submitted to partners and collaborators for comment and revision.

Develop Protocols

Protocols are developed and validated at the NCL. Intent is to leverage existing methods and/or Standards when possible.

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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

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In Vivo Pharmacokinetics

- **Single/repeat-dose PK/TK/tissue distribution**

- **Clinical Tx cycle**
  -Schedule
  -Duration
  -Route
  -Formulation

- **Quantitation method**
  -radiolabeled nanoparticle (Scintillation)
  -Imaging
  -ELISA

- **PK Parameters**
  -AUC, Cmax, CL, t ½, tmax

Based on FDA Pre-clinical Guidance

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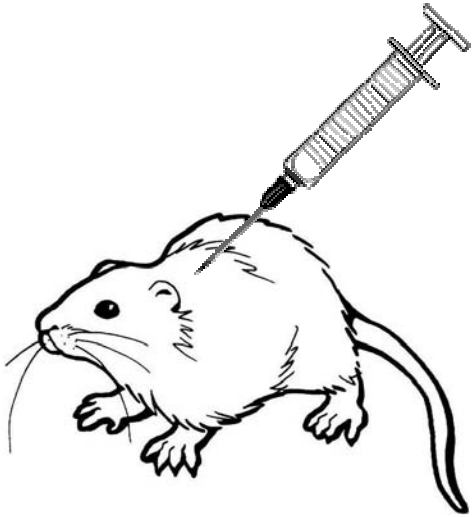
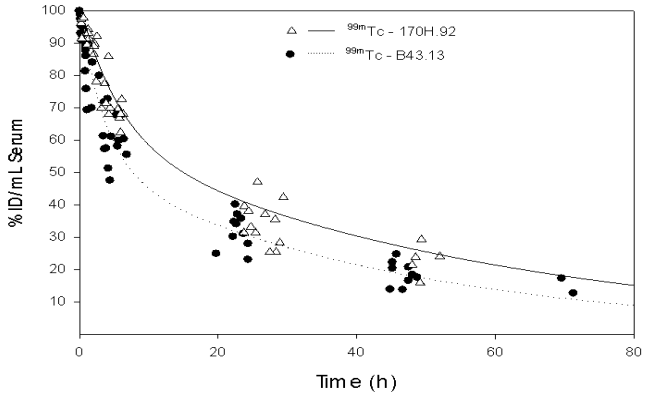
In Vivo Pharmacokinetics

Purpose	Duration	Time Point's	Groups	Tests	Comments
Plasma PK profile/ Tissue distribution (Liver, lungs, kidney, heart, spleen brain)	24 hrs	8	1X, 10X (5 F SD Rats/Tx)	scintillation counting of plasma and tissue samples (NCL)	Dosing, blood draws by Jugular catheter, cardiac puncture (final tp)





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In Vivo Toxicology Studies

- **Single/Repeat-Dose Acute/Subacute Toxicity**
  -Rats (determine STD10/NOAEL/Lethal dose)

- **Clinical Tx Cycle**
  -Schedule
  -Duration
  -Route
  -Formulation

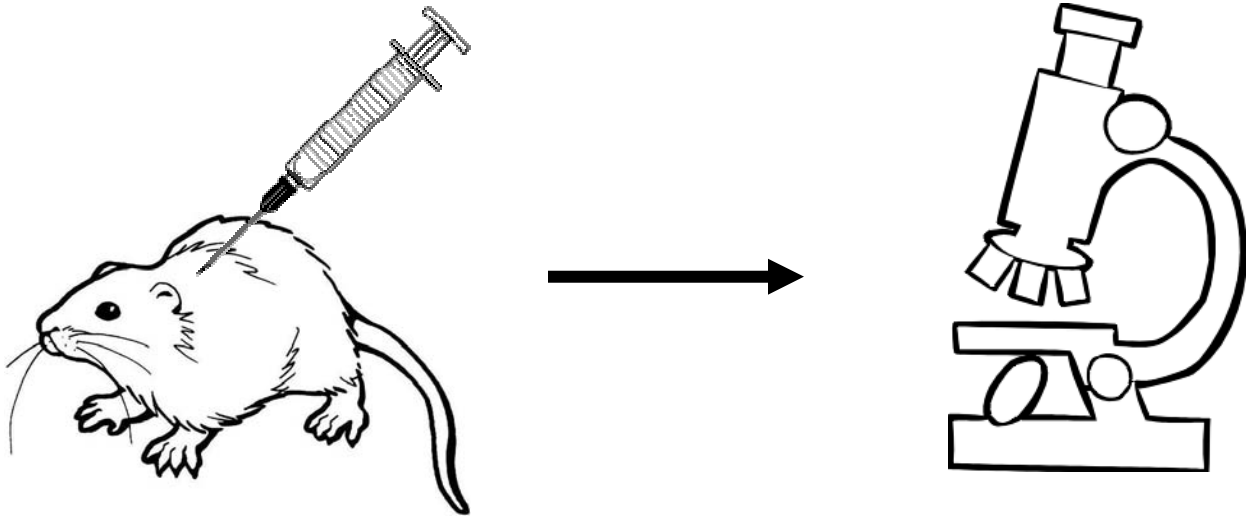
- **Endpoints monitored**
  -Hematology
  -Clinical chemistry
  -Gross pathology
  -Histopathology
  -Clinical signs

Based on NCI DTP toxicology protocols

In Vivo Toxicology Studies

Dose Range-Finding Toxicity Study

Purpose	Duration	Groups	Tests	Comments
determine dose at which toxicity is observed	14 days	ctrl, 10X, 50X, 100X (5 M+F SD Rats/Tx)	Clinical chemistries, histopathology, hematology, gross pathology, clinical observation (PHL)	BW measured daily, euthanasia criteria (decrease in body weight ≥ 20%)



Comprehensive Toxicology

Histopathology

Brain	Pancreas	Salivary gland
Lymph node	Esophagus	Parathyroid
Thyroid	Trachea	Adrenal
Pituitary	Heart	Kidney
Thymus	Gall Bladder	Liver
Spleen	Lung	Duodenum
Ileum	Rectum	Stomach
Cecum	Colon	Jejunum
Lymph node	Epididymis	Ovary
Prostate	Seminal vesicle	Testis
Urinary bladder	Uterus	Eye
Hardian gland	Nasal Sections	Femur
Femur	Vertebra	Spinal cord
Mammary gland	Skin/Subcutis	Tongue

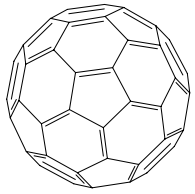
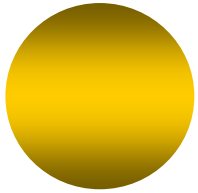
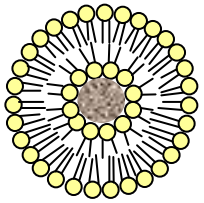
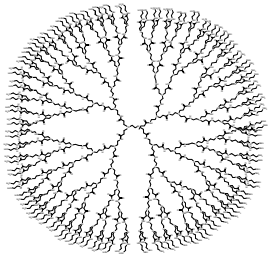
Comprehensive Toxicology

Hematology

Erythrocyte count (RBC)
Hemoglobin (HGB)
Hematocrit (HCT)
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)
Platelet count (Plate)
Reticulocyte count (RETIC)
Total leukocyte count (WBC)
Differential leukocyte count
Nucleated red blood cell count

Nanoparticles by type for medical applications

- **Organic Nanoparticles (e.g.: Polymers, Dendrimers)**
- **Inorganic Nanoparticles (e.g.: Iron oxide, gold nanoparticles)**
- **Organic/Inorganic hybrids (e.g.: Nanocomposites, core-shell type, Gd-chelates)**
- **Carbon based (e.g.: Functionalized fullerenes)**
- **Liposomes (e.g.: Functionalized, inclusion complexes)**
- **Biological nanoparticles (e.g.: Protein and peptide based nanoparticles with other biological components)**

Environmental Aspects

Studies Applicable to Environmental Risk Assessment

- **General Cytotoxicity Assays-** determining concentration-response relationships.
- **Mechanistic Studies-** Identifying apoptosis, oxidative stress and cytochrome P450 induction/suppression as potential mechanisms
- ***In Vivo* Toxicology Studies-** Identification of target organs
- **General ADME-** define t1/2, clearance mechanisms (i.e. metabolism, biliary excretion, renal clearance, etc.)

NCL Data

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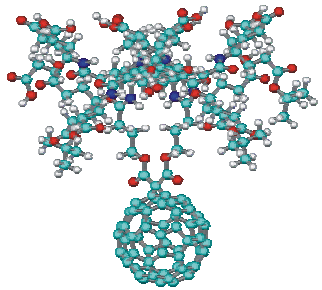
NP Structure-Activity Relationship

I. Surface hydrophobicity



- Hydrophobic surface- taken up by RES system

- Hydrophilic surface- Increased systemic half-life, enhanced permeability and retention in tumors (EPR)

II. Surface Charge

- Toxicity: cationic>anionic>neutral

III. Surface reactivity

- More reactive (↑ROS)= ↑Toxicity

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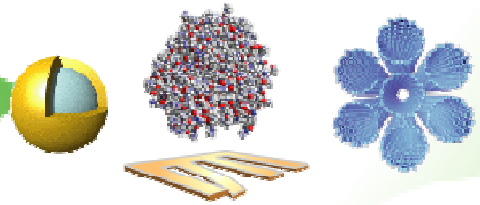
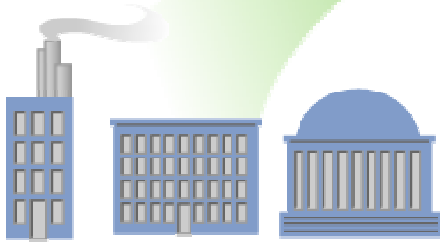
Summary



Nanotechnologies



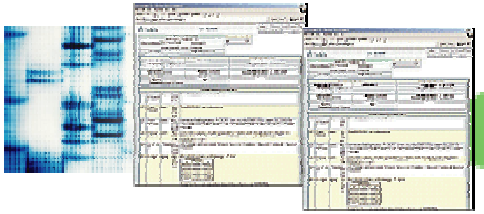
- University and Medical Centers
- Technology Centers of Excellence
- National Labs
- NCI Technology
- Development Programs

Clinical Applications

Nanotechnology Characterization Laboratory

NCI

NIST FDA



Protocols and Data

Questions/Comments

http://NCL.cancer.gov

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