

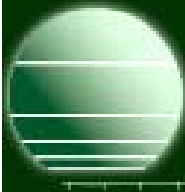
Characterization of Nanoparticles Intended for Cancer Therapeutics and Diagnostics

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Nanotech Characterization Laboratory
SAIC-Frederick

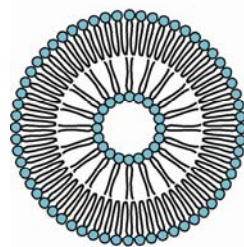
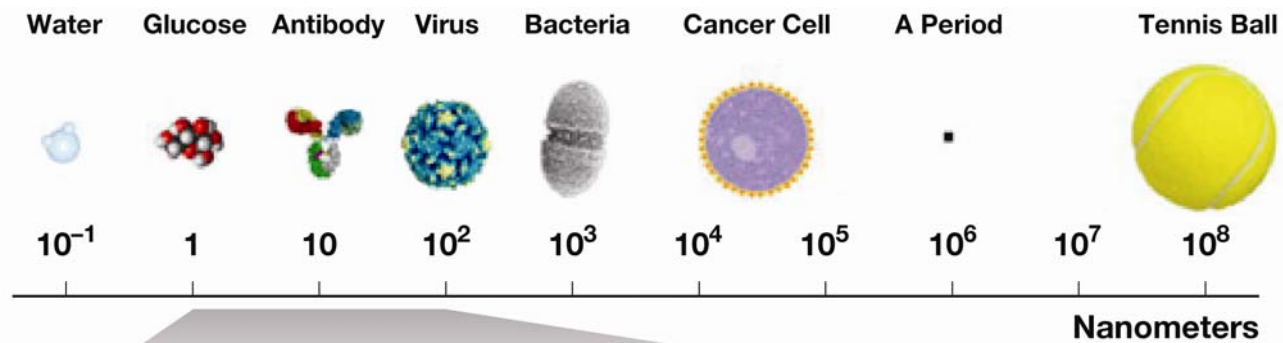
March 15, 2006



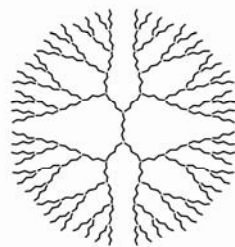


Definition

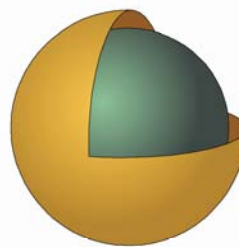
“Research and technology development at the atomic, molecular or macromolecular scale leading to the controlled creation and use of structures, devices and systems with a length scale of approximately 1 – 100 nanometers (nm).” (Source: National Nanotech Initiative)



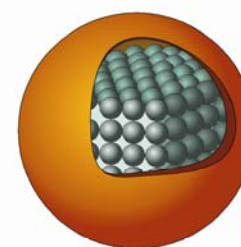
Liposome



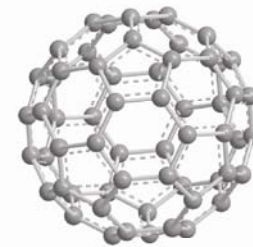
Dendrimer



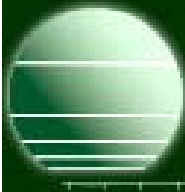
Gold Nanoshell



Quantum Dot

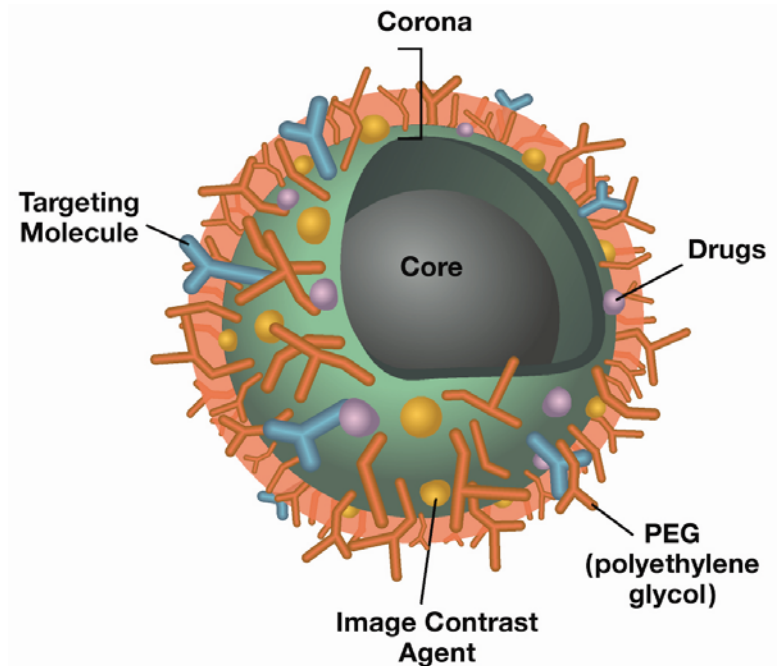


Fullerene



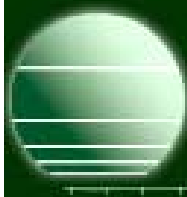
Therapeutic Benefits

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

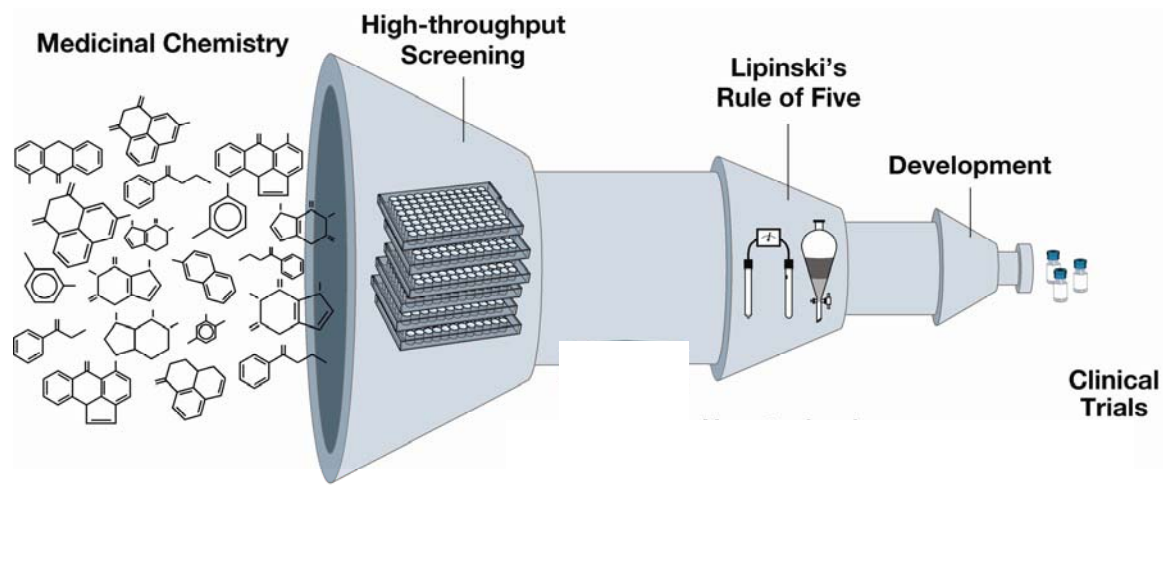


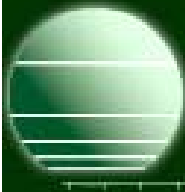
McNeil, (2005), J. Leuk. Biol., 78:585-594

↑Solubility ↑Stability ↑Specificity = ↓Toxicity ↑Efficacy

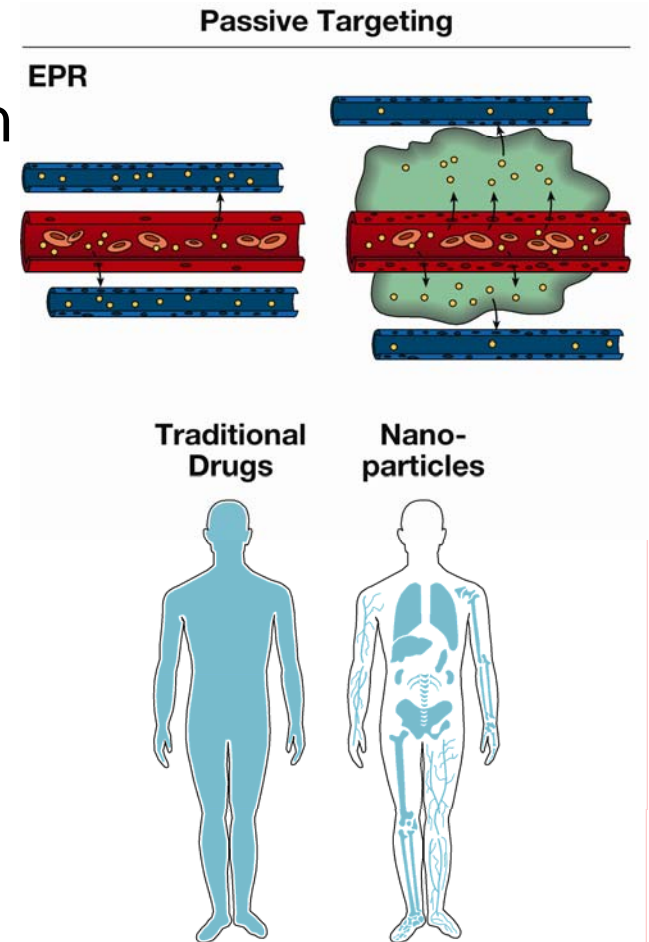


- Drug development
 - 3M compounds synthesized → 30 developed
 - Candidates assessed based on ‘rule of 5’
 - Filter for ‘chemical lead’ and development
 - H-bonding, MW, partition coefficient



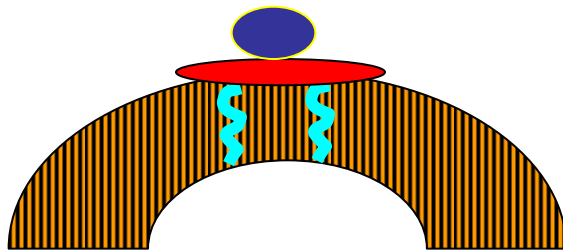


- **Passive Targeting**
 - Enhanced Permeation and Retention (EPR)
 - Targeting of RES cancers
 - Size and surface chemistry
- **Active Targeting**
 - Surface chemistry allows functionalization w/ targeting molecules
 - Antibodies, Herceptin attached to QDs.
 - Folic acid attached to dendrimers
 - Carbohydrates attached to GNP & QDs.

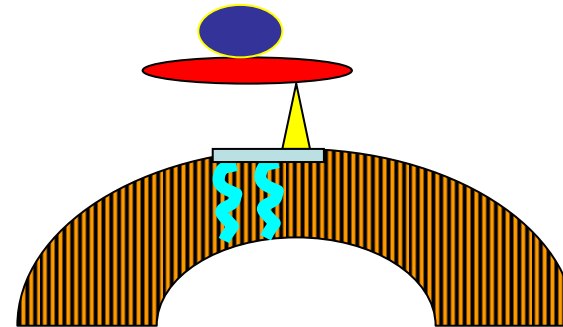


McNeil, (2005), *J. Leuk. Biol.*, 78:585-594

Dr. Greg Lanza, Washington University



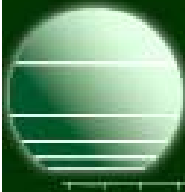
Gd-DTPA-BOA



Gd-DTPA-PE

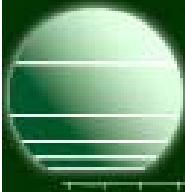
Magnetic Field	Paramagnetic Chelate	Ion-Based Relaxivity (s*mM) ⁻¹		Particle-Based Relaxivity (s*mM) ⁻¹	
		r ₁	r ₂	r ₁	r ₂
0.47 T	Gd-DTPA-BOA	21.3 ± 0.2	23.8 ± 0.3	1,210,000 ± 10,000	1,350,000 ± 20,000
	Gd-DTPA-PE	36.9 ± 0.5*	42.3 ± 0.6*	2,710,000 ± 40,000*	3,110,000 ± 50,000*
1.5 T	Gd-DTPA-BOA	17.7 ± 0.2	25.3 ± 0.6	1,010,000 ± 10,000	1,440,000 ± 30,000
	Gd-DTPA-PE	33.7 ± 0.7*	50 ± 2*	2,480,000 ± 50,000*	3,700,000 ± 100,000*
4.7 T	Gd-DTPA-BOA	9.7 ± 0.2	29.4 ± 0.3	549,000 ± 9,000	1,670,000 ± 20,000
	Gd-DTPA-PE	15.9 ± 0.1*	80.0 ± 0.7*	1,170,000 ± 6,000*	5,880,000 ± 50,000*

- 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



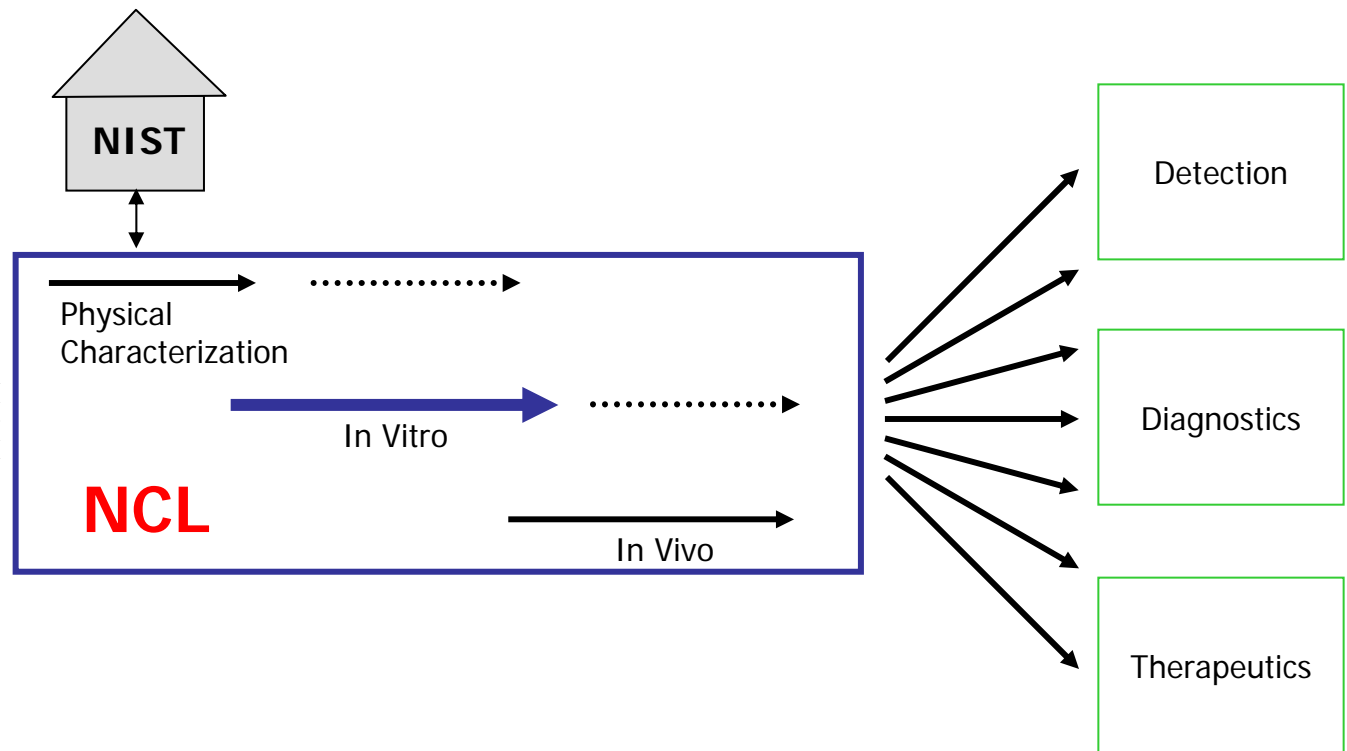
NCL Objectives

- 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.



Sources of Nanomaterials

- Cancer Centers of Nanotech Excellence (CCNEs)
- Academia
- Big Pharm
- Small Business
- NCI, NIH, NSF Grants
- DoD, DoE
- Unconventional Innovative Program (UIP)



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

NANOTECHNOLOGY CHARACTERIZATION LABORATORY

NCL Facilities

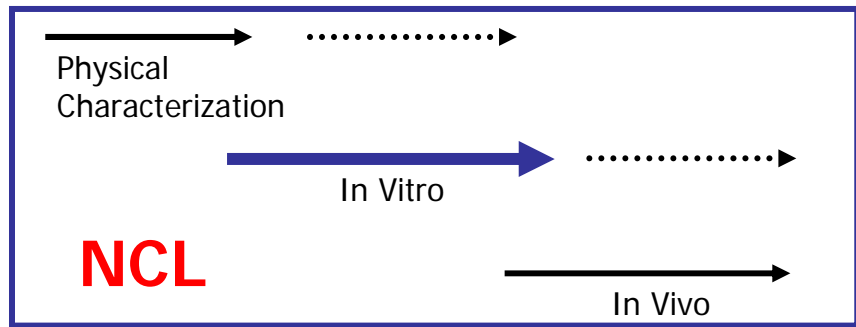
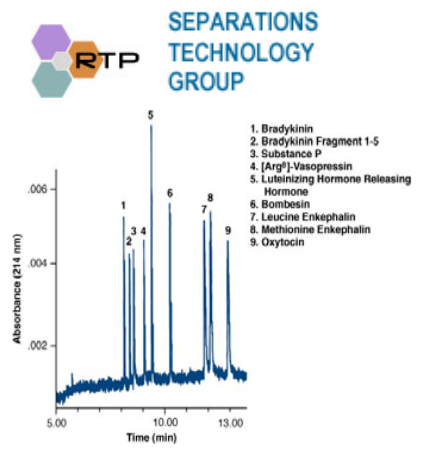
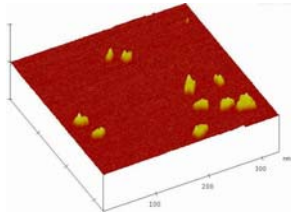


IMAGE ANALYSIS LABORATORY

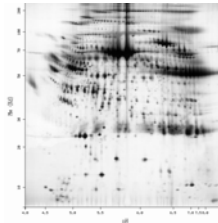


NCL Assay Cascade



Physical Characterization:

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



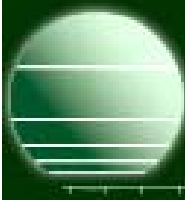
In Vitro:

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

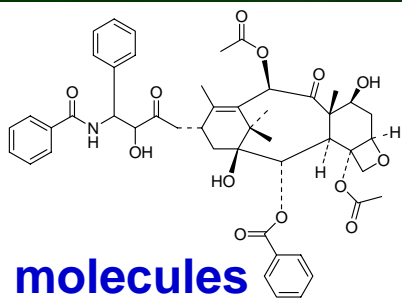


In Vivo:

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



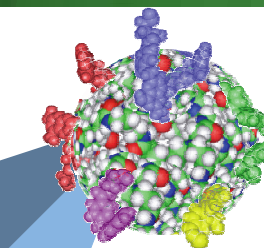
Core parameters to define physicochemical property of material



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



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

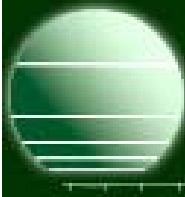


Nanomaterial

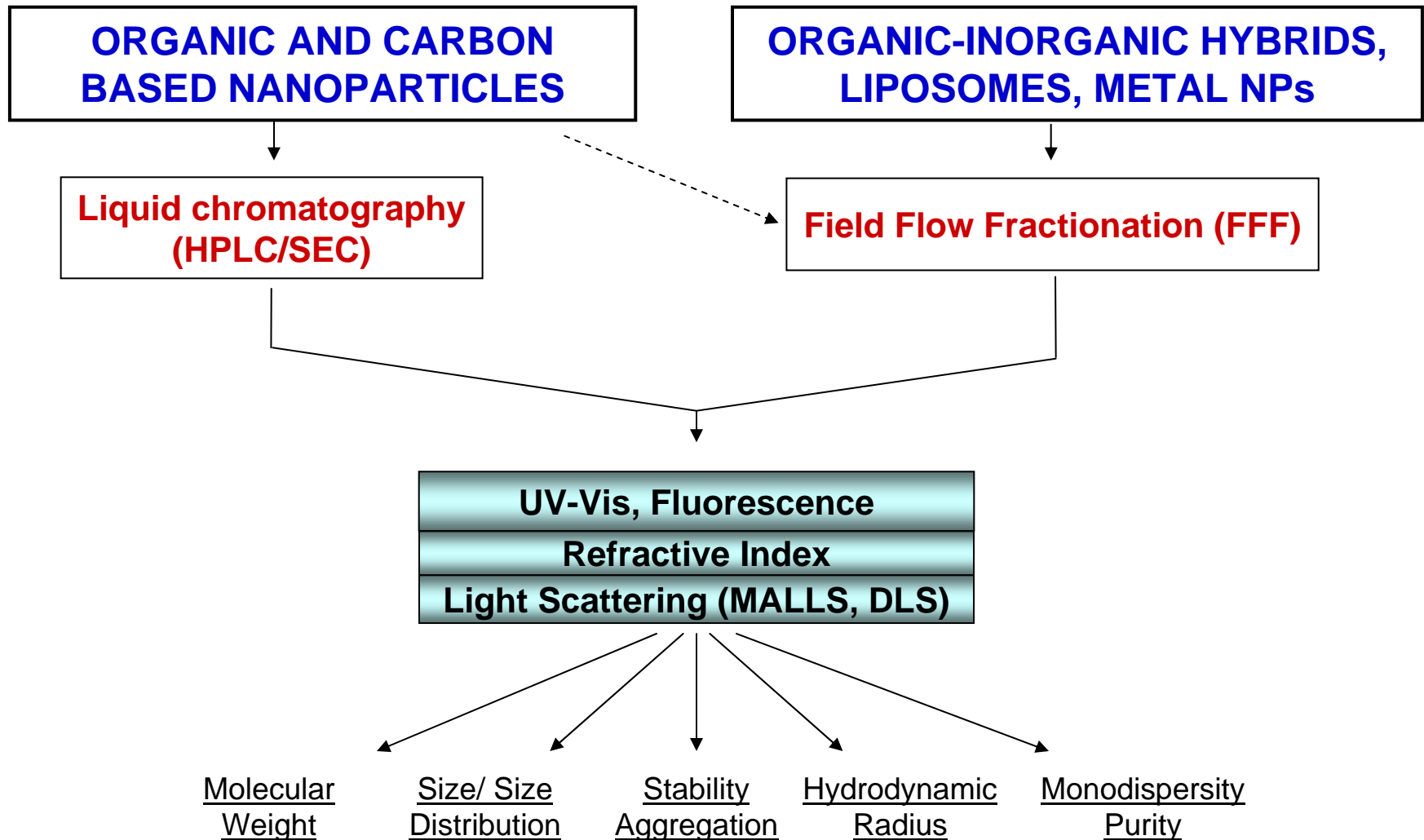
- Microscopy (AFM, TEM, SEM)
- Light scattering (Static, Dynamic)
- SEC, FFF
- Electrophoresis (CE, PAGE)
- Zeta sizer
- Fluorimetry

James Baker
University of Michigan

Same parameters – different/additional characterization methods




Flow mode analysis of Nanoparticles



In Vitro

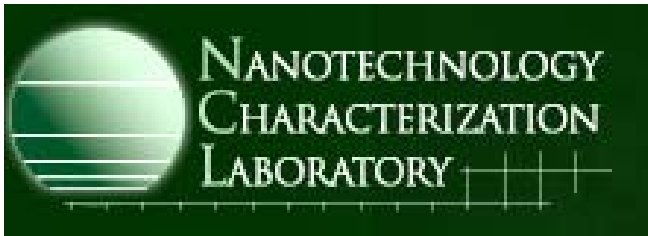
- Sterility
 - Bacterial/Viral/Mycoplasma
 - Endotoxin
- Targeting
 - Cell Binding/Internalization
- Blood Contact Properties
 - Plasma Protein Binding
 - Hemolysis →
 - Platelet Aggregation
 - Coagulation
 - Complement Activation
 - CFU-GM
 - Leukocyte Proliferation
 - Macrophage/Neutrophil Function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



NCL Method ITA-1

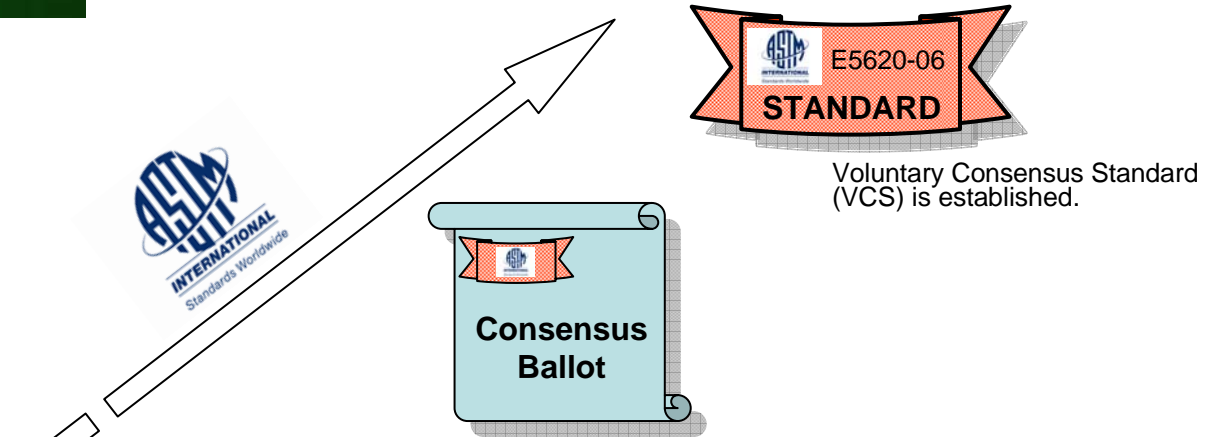
**Analysis of Hemolytic Properties
of Nanoparticles**

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(301)-846-6939

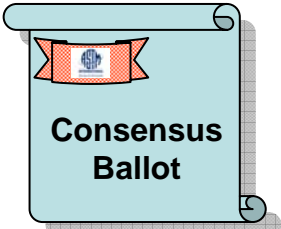


VCS informs regulatory agencies and promotes commercialization of nanotechnology for medical applications

Industry, Academia, Government



Voluntary Consensus Standard (VCS) is established.



Ballot of Standard is submitted to ASTM members for consensus.

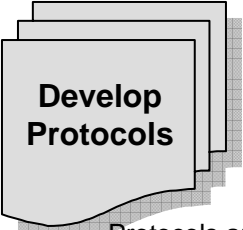
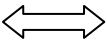


Draft of Standard is submitted to ASTM E-56.20 Subcommittee for comment and revision



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

NCI
FDA
NIST



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



NCL Method ITA-1

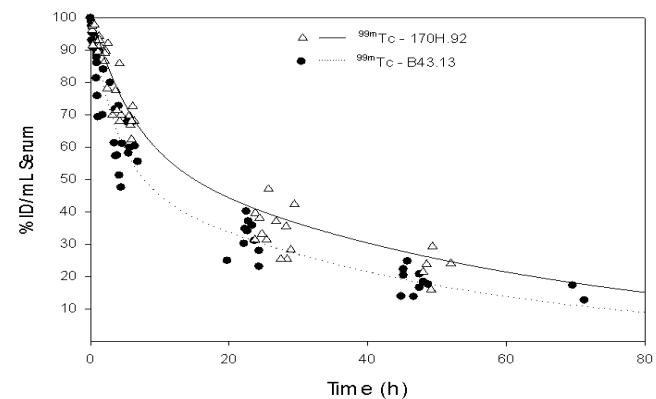
Analysis of Hemolytic Properties of Nanoparticles

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

- **Single/repeat-dose PK/TK/tissue distribution**
- **Clinical Tx cycle**
 - Schedule
 - Duration
 - Route
 - Formulation
- **Quantitation method**
 - radiolabeled nanoparticle (Scintillation)
 - Imaging
 - ELISA
- **PK Parameters**
 - AUC, C_{max}, CL, t^{1/2}, t_{max}

Based on FDA Pre-clinical Guidance

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)

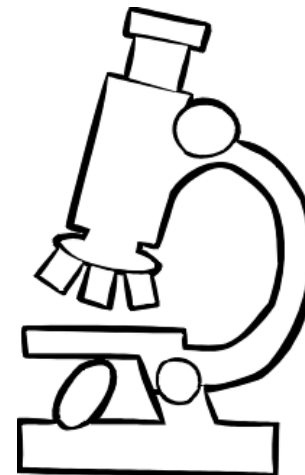


- **Single/Repeat-Dose Acute/Subacute Toxicity**
 - Rats (determine STD10/NOAEL/Lethal dose)
- **Clinical Tx Cycle**
 - Schedule
 - Duration
 - Route
 - Formulation
- **Endpoints monitored**
 - Hematology
 - Clinical chemistry
 - Gross pathology
 - Histopathology
 - Clinical signs

Based on NCI DTP toxicology protocols

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 \geq 20%)



Histopathology

Brain
Lymph node
Thyroid
Pituitary
Thymus
Spleen
Ileum
Cecum
Lymph node
Prostate
Urinary bladder
Hardian gland
Femur
Mammary gland

Pancreas
Esophagus
Trachea
Heart
Gall Bladder
Lung
Rectum
Colon
Epididymis
Seminal vesicle
Uterus
Nasal Sections
Vertebra
Skin/Subcutis

Salivary gland
Parathyroid
Adrenal
Kidney
Liver
Duodenum
Stomach
Jejunum
Ovary
Testis
Eye
Femur
Spinal cord
Tongue

Hematology

Erythrocyte count (RBC)

Hemoglobin (HGB)

Hematocrit (HCT)

Mean corpuscular volume (MCV)

Mean corpuscular hemoglobin (MCH)

Mean corpuscular hemoglobin concentration (MCHC)

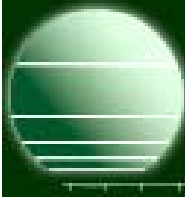
Platelet count (Plate)

Reticulocyte count (RETIC)

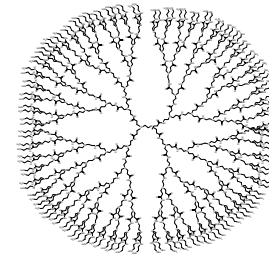
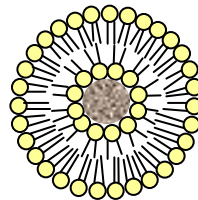
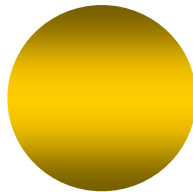
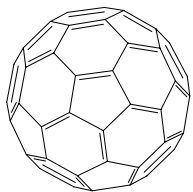
Total leukocyte count (WBC)

Differential leukocyte count

Nucleated red blood cell count



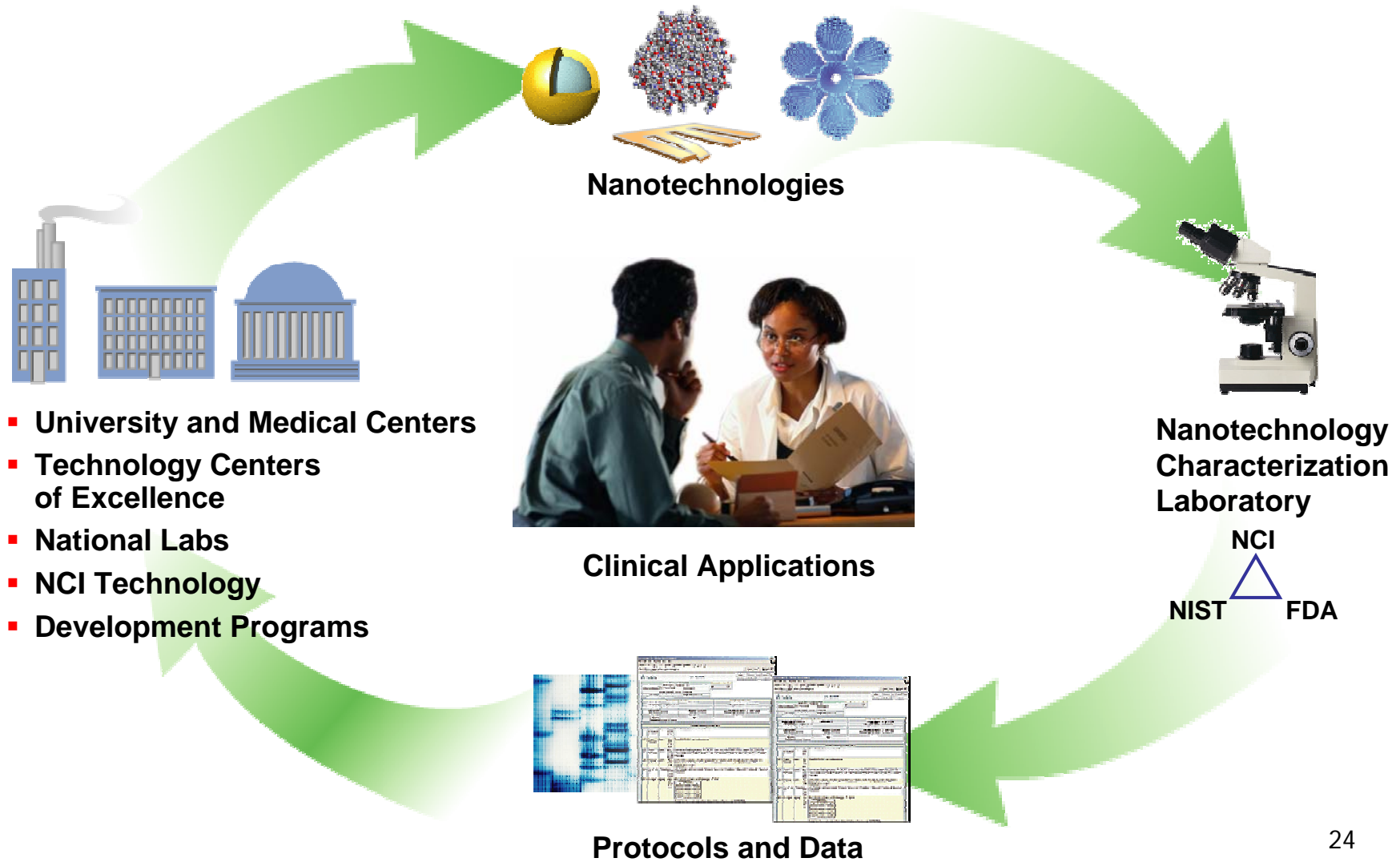
- **Organic Nanoparticles (e.g.: Polymers, Dendrimers)**
- **Inorganic Nanoparticles (e.g.: Iron oxide, gold nanoparticles)**
- **Organic/Inorganic hybrids (e.g.: Nanocomposites, core-shell type, Gd-chelates)**
- **Carbon based (e.g.: Functionalized fullerenes)**
- **Liposomes (e.g.: Functionalized,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



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 $t_{1/2}$, clearance mechanisms (i.e. metabolism, biliary excretion, renal clearance, etc.)

Summary



Questions/Comments

<http://NCL.cancer.gov>

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