# Preclinical
# Characterization of Nanomaterial

Anil K. Patri, Ph.D.
Nanotechnology Characterization Lab
(SAIC Frederick)
National Cancer Institute at Frederick

**Nanotechnology and OSEWER: New Opportunities and Challenges**
**July 12, 2006**

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

## Treatment Options for Cancer

- Surgery
- Radiotherapy
- Chemotherapy

Limitations and side effects

**Urgent need to utilize novel technologies and ideas**

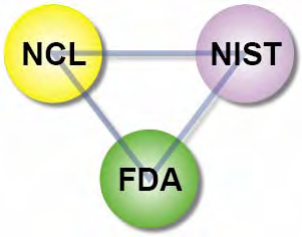
- Early diagnosis
- Targeted therapy
- Minimize side effects
- Monitor
- Provide better living standards

*Dr. Anil K. Patri*
*2 of26*

# Nanotechnology at the National Cancer Institute



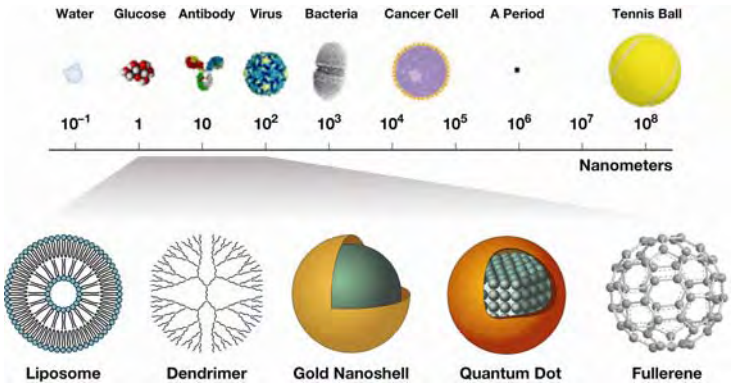
- NCI has funded exploratory work since 1999 on integrating nanotechnology into biomedical research
- Unconventional Innovations Program (UIP)
  - Diagnostics (Imaging)
  - Therapeutics
- Priority is to now transition that research into the clinical realm.




Dr. Anil K. Patri
3 of26

# Nanotechnology 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)***



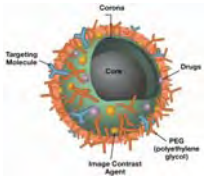

Dr. Anil K. Patri
4 of26

# Why Nano?

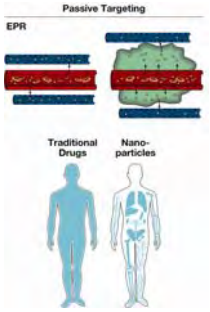
NANOTECHNOLOGY CHARACTERIZATION LABORATORY

## Therapeutic Benefits

- Solubility
  - Carrier for hydrophobic, hydrophilic therapeutics
- Multifunctional capability
  - Targeting, Imaging, Drug payloads
- Change in pharmacokinetics and pharmacodynamics
- Active and passive targeting
  - Antibody and ligand conjugates
- Reduced systemic toxicity




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



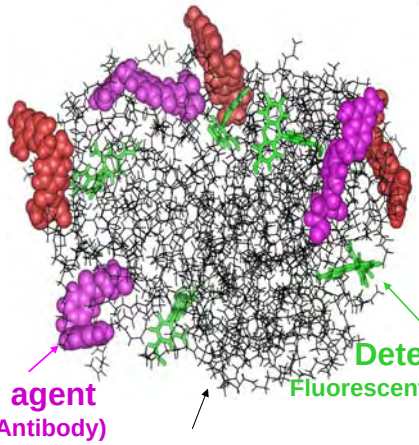

↑Solubility ↑Stability ↑Specificity = ↓Toxicity ↑Efficacy

*Dr. Anil K. Patri*
*5 of26*

# Multifunctional platform

NANOTECHNOLOGY CHARACTERIZATION LABORATORY

**Therapeutic Agent (Methotrexate)**



**Detecting agent**
**Fluorescent probe or Gd chelate**

**Targeting agent**
**(Folic acid or Antibody)**

**G5-polyamidoamine**
**(dendrimer platform)**

**Dr. James Baker, University of Michigan**

*Dr. Anil K. Patri*
*6 of26*

# Active Targeting

Dr. James Baker, University of Michigan

Free MTX
30 mg/kg total

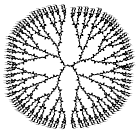
Nanodevice MTX
3 mg/kg total MTX

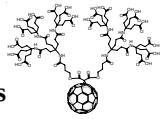
Cancer Res **2005**; 65: (12) 5317-5324

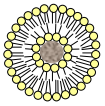


# Nanoparticles by type for medical applications

- **Organic Nanoparticles (e.g.: Polymers, Dendrimers, Functionalized fullerenes)**
- **Organic/Inorganic hybrids (e.g.: Quantum dots, Nanocomposites, Gd-chelates)**
- **Liposomes (e.g.: Functionalized, inclusion complexes**
- **Nanoemulsions (e.g.: Oil-water-surfactant mixtures)**
- **Biological nanoparticles (e.g.: Protein and peptide based nanoparticles with other active components)**







# NCL Concept of Operations

Nanotechnology Characterization Laboratory

**Sources of Nanomaterials**

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

NIST

Physical Characterization

In Vitro

NCL

In Vivo

- Detection
- Diagnostics
- Therapeutics

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

*Dr. Anil K. Patri*
*9 of26*

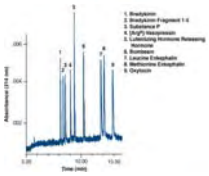
# NCI Facilities

Nanotechnology Characterization Laboratory

NCI/SAIC Research Technology Program

ABCC Advanced Biomedical Computing Center
A Center Devoted To Biocomputing

RTP SEPARATIONS TECHNOLOGY GROUP



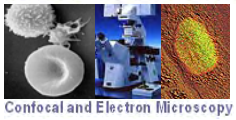
Physical Characterization

In Vitro

NCL

In Vivo

IMAGE ANALYSIS LABORATORY



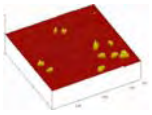
Confocal and Electron Microscopy

LASP
Laboratory Animal Sciences Program

RTP MASS SPECTROMETRY CENTER

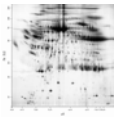
*Dr. Anil K. Patri*
*10 of26*

# NCL Assay Cascade



**Physico-chemical Characterization:**

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



**In Vitro:**

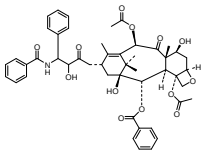
- Pharmacology
- Blood contact properties
- Effects on immune cell function
- Cytotoxicity
- Mechanistic toxicology
- Sterility

**In Vivo:**

- ADME
- Safety
- Efficacy

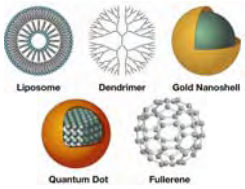
*Dr. Anil K. Patri*
*11 of26*

# Instrumentation for Physicochemical Characterization



**Small molecules**

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




**Nanomaterial**

- Microscopy (AFM, TEM, SEM)
- Scattering techniques (PCS, MALS, SAXS, SANS)
- SEC, AFFF
- Electrophoresis (CE, PAGE)
- Zeta potential
- Fluorimetry

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

**Same parameters – different/additional characterization methods**

*Dr. Anil K. Patri*
*12 of26*

# Physico-chemical Characterization

- Size, Size distribution
- Shape
- Molecular weight
- Surface characteristics
  - Net charge
  - Zeta potential
- Functionality

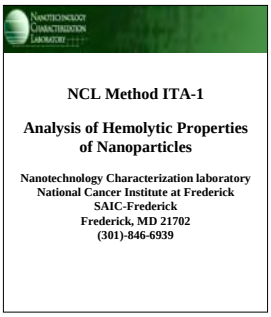
  Functional component
  - Identification
  - Quantitation
  - Functional and stability assessment
- Composition
  - Elemental
  - Core-shell
- Purity
  - Homogeneity/Inhomogeneity in Ligand distribution
  - Free components
- Stability
  - Thermal
  - pH
  - Photo
  - Aggregation
  - Freeze-thaw
  - Lyophilization
  - Centrifugation
  - Short-term storage
  - Long-term storage
  - Release kinetics
  - Stability of the 'coating'

Dr. Anil K. Patri
13 of26

# *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



**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

Dr. Anil K. Patri
14 of26

# Protocols at NCL website

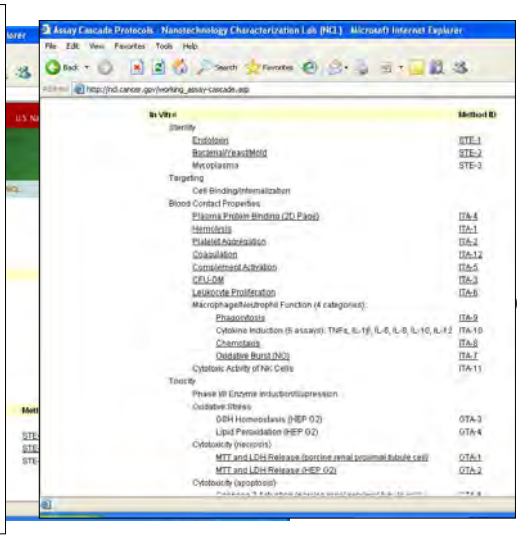

NCL Method STE-1
Version 1.0

Detection of Endotoxin Contamination by
End Point Chromogenic LAL Assay

Nanotechnology Characterization Laboratory
National Cancer Institute at Frederick
SAIC-Frederick, Inc.
Frederick, MD 21702
(301)-846-6939
ncl.mail@ctf.nci.gov

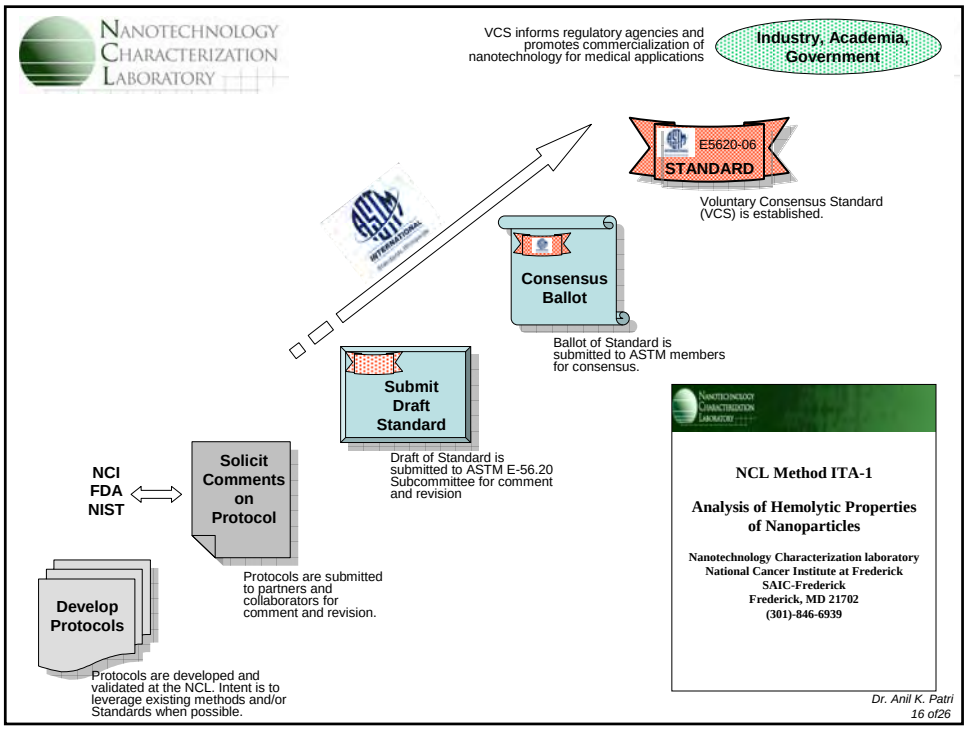
October 2005

This protocol assumes an intermediate level of scientific competency with regard to techniques, instrumentation, and safety procedures. Rudimentary assay details have been omitted for the sake of brevity.




**http://ncl.cancer.gov/**

*Dr. Anil K. Patri*
*15 of26*




*Dr. Anil K. Patri*
*16 of26*

# Environmental Aspects

**Studies Applicable to Environmental Risk Assessment**

- **General Cytotoxicity Assays**- determining concentration-response relationships, SAR studies
- **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.)

*Dr. Anil K. Patri*
*17 of26*

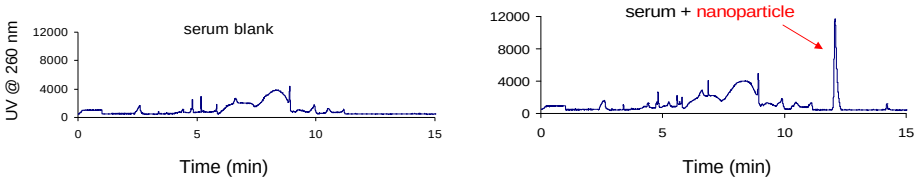
# DATA

*Dr. Anil K. Patri*
*18 of26*

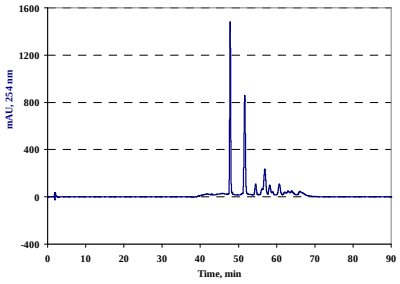
# Detection and Quantitation in Matrix

NANOTECHNOLOGY CHARACTERIZATION LABORATORY

**Capillary electrophoresis**




**HPLC (QC)**



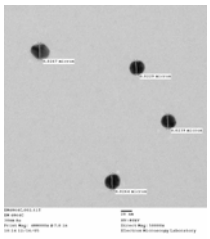

*Dr. Anil K. Patri*
*19 of26*

# Metrology: Size

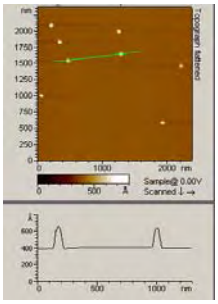
NANOTECHNOLOGY CHARACTERIZATION LABORATORY

**30 nm Gold colloids**

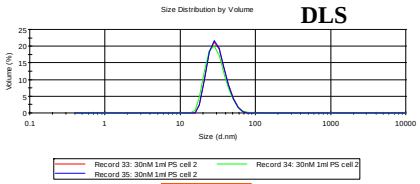
**TEM**



**AFM**



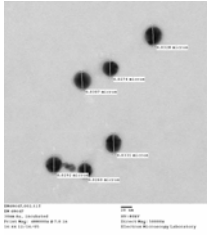
**DLS**



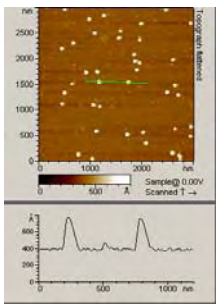

**31 nm**

**30 nm Gold colloids with protein**

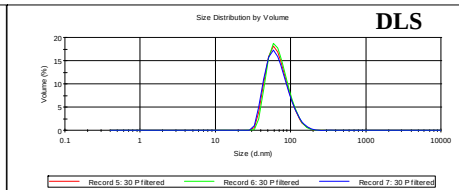
**TEM**



**AFM**



**DLS**



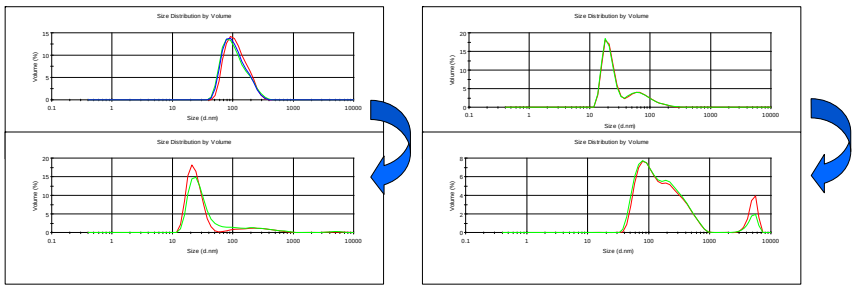

**69 nm**

*Dr. Anil K. Patri*
*20 of26*

# Stability: Liposomes


## Shelf stability

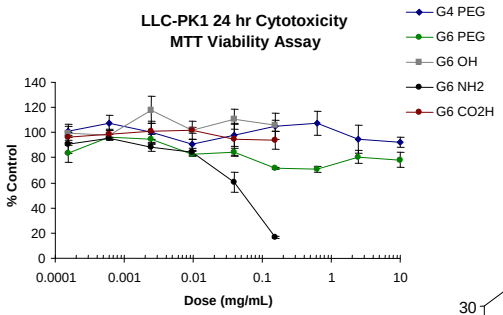
Size immediately after preparation



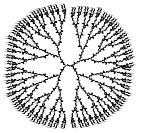
Change in size after 3 months of storage

*Dr. Anil K. Patri*
*21 of26*

# Structure Activity Relationships

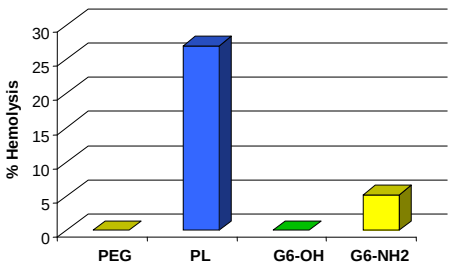



Effect of Surface Functional Group on cell viability



G6 Dendrimer

Hemolysis




*Dr. Anil K. Patri*
*22 of26*

# Acknowledgements


**NCL**

Scott McNeil, Ph.D.
Marina Dobrovolskaia, Ph.D.
Arindam Dhar, M.D., Ph.D.
Jiwen Zheng, Ph.D.
Sarah Skoczen, M.S.
Tim Potter, B.S.
Lydia Fine
Anil Patri, Ph.D.
Stephan Stern, Ph.D.
Jeff Clogston, Ph.D.
Banu Zolnik, Ph.D.
Chris McLeland, B.S.
Barry Neun, B.S.

**NCI-Frederick**

Tim Veenstra, Ph.D.
Haleem Issaq, Ph.D.
King Chan, Ph.D.
Suzanne Specht, B.S.
Anu Puri, Ph.D.
Darue Prieto, M.S.
Jack Simpson, Ph.D.
Tad Guszczynski, Ph.D.
Kunio Nagashima




# Questions/Comments

http://ncl.cancer.gov

(301)-846-6939


Contact Info:
Anil Patri
patri@ncifcrf.gov

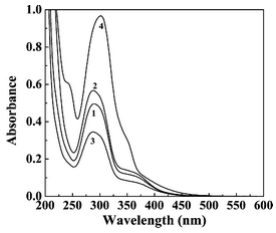
NANOTECHNOLOGY CHARACTERIZATION LABORATORY

Dr. Anil K. Patri
25 of26

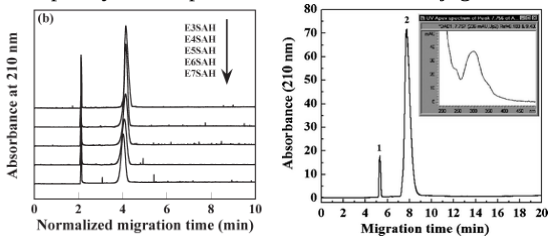
## Multifunctional Nanoparticles: Characterization of ligand distribution

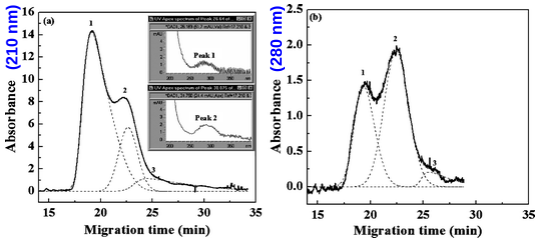
NANOTECHNOLOGY CHARACTERIZATION LABORATORY

UV-Vis spectra




Capillary Electrophoresis of dendrimer conjugates




**Multifunctional with Folic acid and Methotrexate**

Shi, X.; et al. *Electrophoresis*, **2005**, 26, 2960-2967.
Shi,X; et al. *Analyst*, **2006**, 131, 374-381.

Dr. Anil K. Patri
26 of26