



NCI **Alliance** for
Nanotechnology
in Cancer

Overview of the Nanotechnology Characterization Lab (NCL)

<http://ncl.cancer.gov>



NATIONAL[®]
CANCER
INSTITUTE

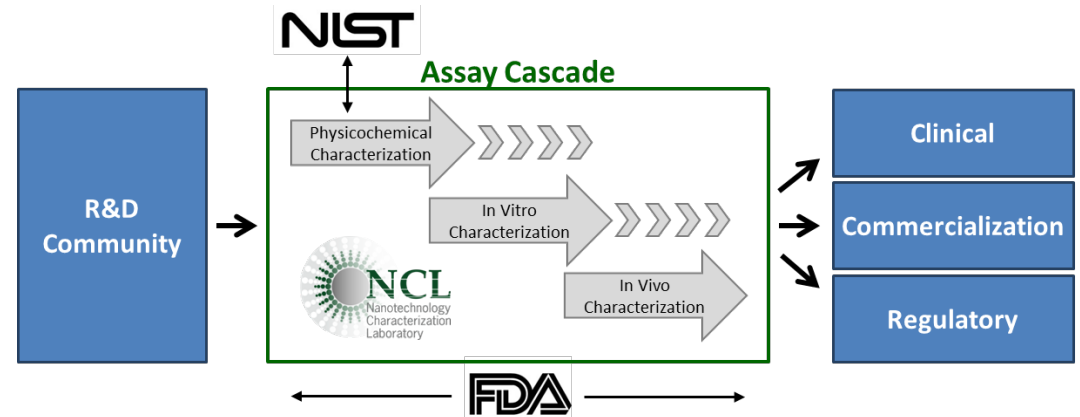
Frederick
National
Laboratory
for Cancer Research



Historical NCL - NCI, NIST & FDA Collaboration

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

NCI realized nanomedicine was a fundamentally new approach to treating cancer and NCL required enlisting new partners.



NIST

Provides the NCL with access to state-of-the art metrology

FDA

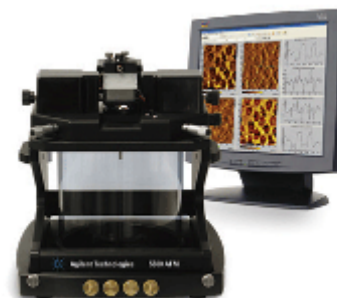
Provides NCL with guidance on risk management and regulatory concerns.

Physicochemical Characterization

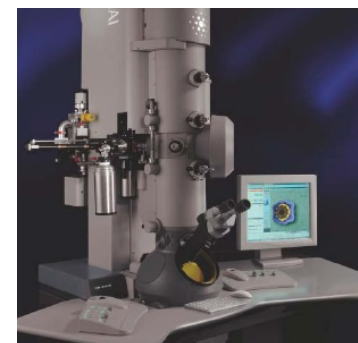
Size/Size Distribution

- 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

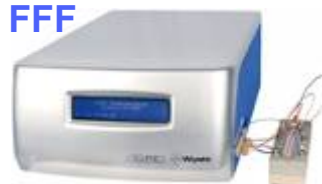
Surface Chemistry

- Biacore
- Zeta Potential

Stability

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

FFF



ICP-MS



In Vitro Characterization

Sterility

- Bacterial/Viral/Mycoplasma
- Endotoxin

Cell Uptake/Distribution

- Cell Binding/Internalization
- Targeting

Hematology

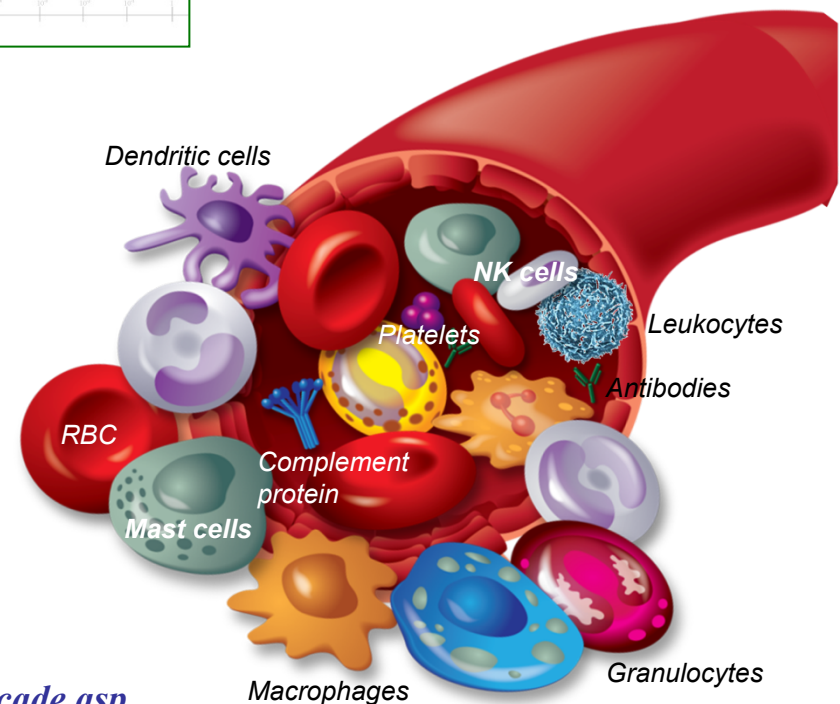
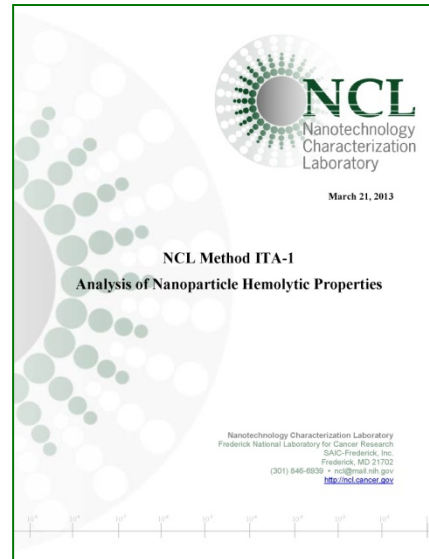
- Hemolysis
- Platelet Aggregation
- Coagulation
- Complement Activation
- Plasma Protein Binding

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

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, $t_{1/2}$, t_{max} , V_{ss}

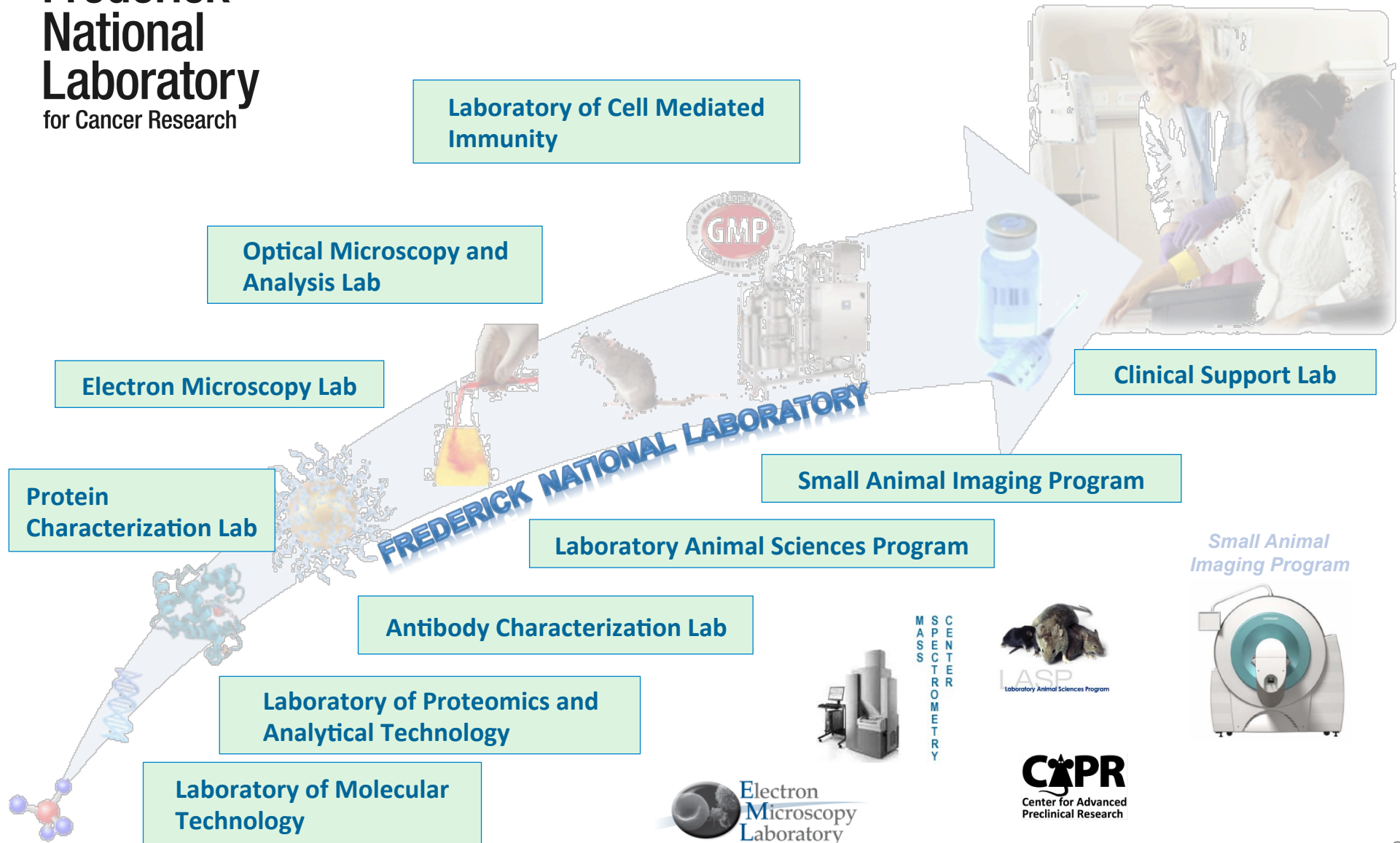


*S*mall
*A*nimal
*I*maging
*P*rogram

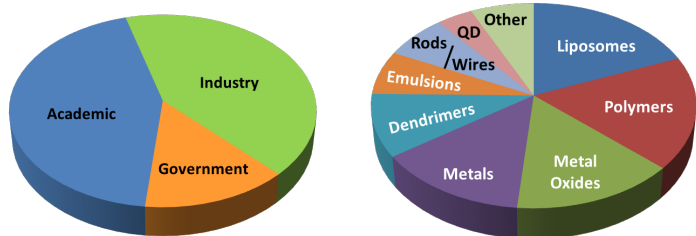
FNL Capabilities

**Frederick
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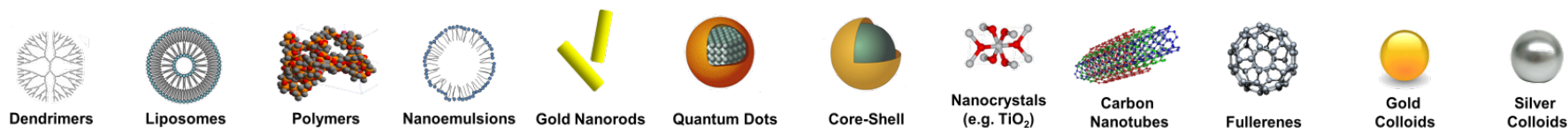
From the molecular level into clinical trials!



Source and Types of Samples



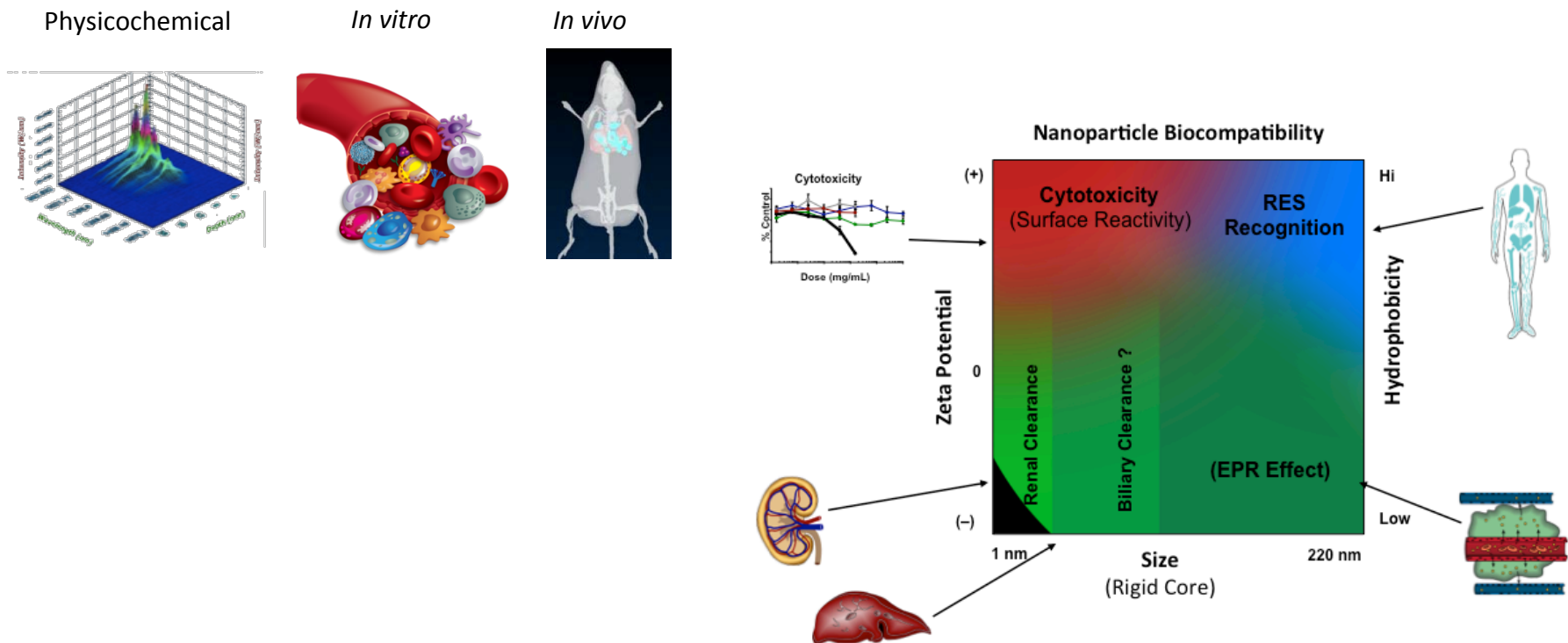
- NCL has characterized over 300 different nanomaterials and a wide range of platforms. Ten collaborators with products in clinical trials.
- NCL has an average of 15 active collaborations at any given time and characterizes an average of 75 samples each year.



NCL is one of only a few labs evaluating the wide variety of platforms used in nanomedicine.

NCL's Unique Breadth of Expertise

NCL testing is tailored to the platform properties, API, route of administration, and intended therapeutic outcome of the individual nanomedicine.



NCL testing links physicochemical properties to biological outcomes.

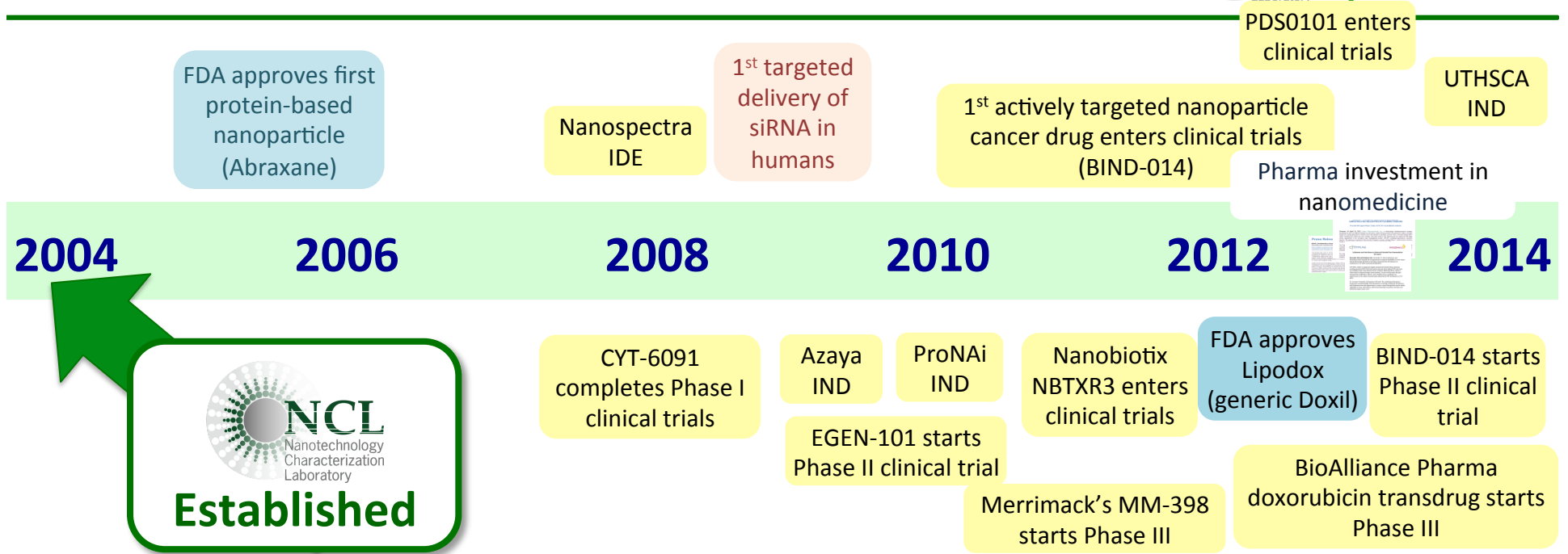
Ten years of providing NCL Assay Cascade testing has given NCL unique expertise.

NCL Extramural Collaborators

In clinical trials



Nanomedicine Development

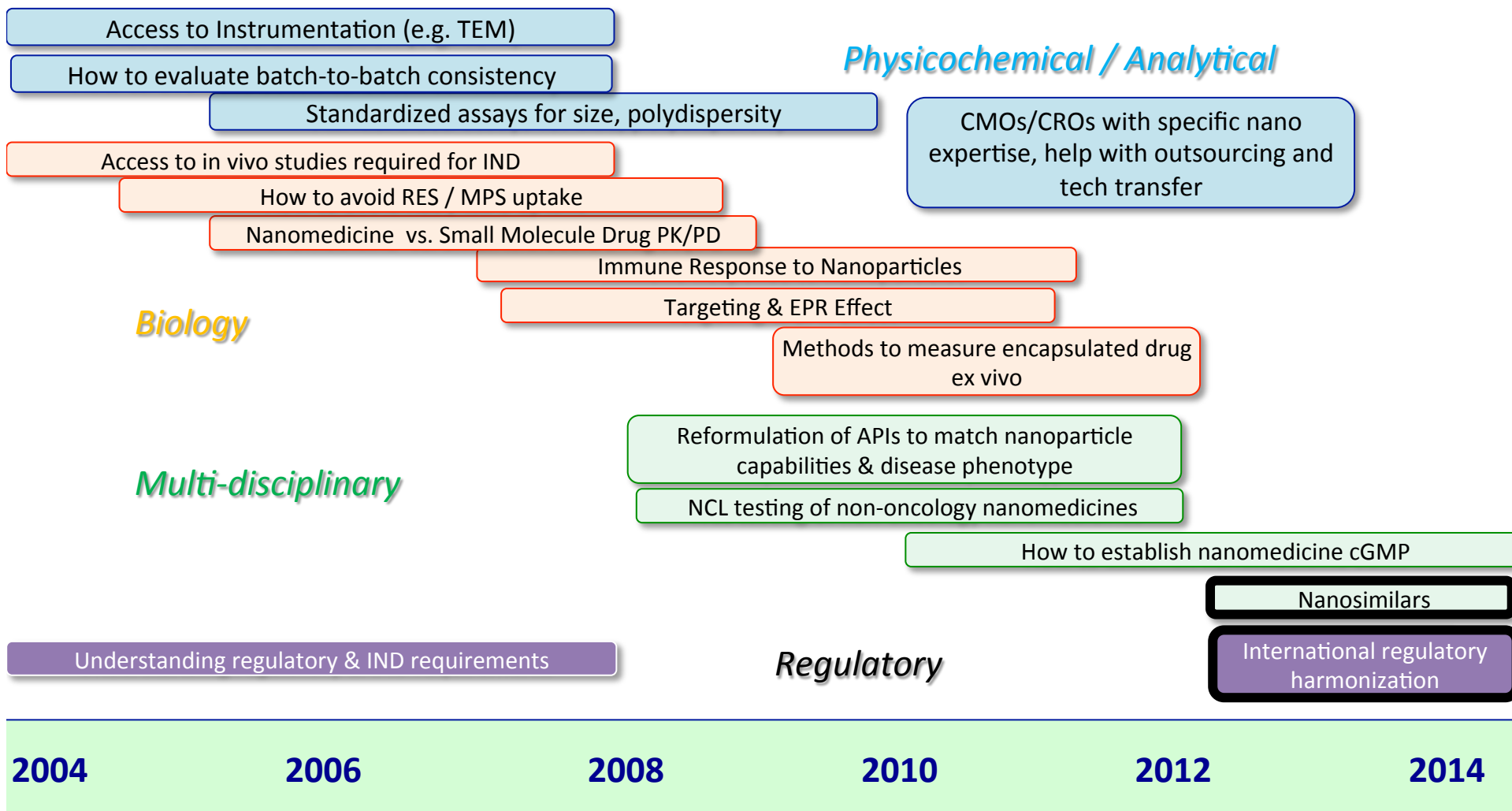


Nanomedicine is maturing. More advanced products are entering and proceeding through clinical trials.

Evolving Requests from the Nanomedicine R&D Community



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As nanomedicine matures, collaborators request NCL assistance in new and changing areas. NCL has developed new expertise to meet this demand.

Continue to Provide Assay Cascade Resource

- Provides “pharmaceutical mentorship” for materials scientists and engineers



Reformulation & cGMP

- Collaborations with Pharma, CMOs & industry consortia



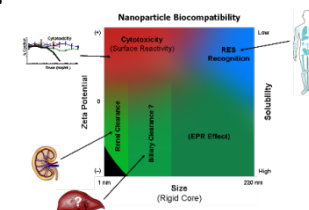
Non-oncology Nanomedicines

- Infectious disease, cardiovascular, etc.



Metrology & New Methods

- Working with instrument manufacturers



Basic Research & Grand Challenges

- Immunotox
- Active targeting

Informing Regulatory

- Equivalence testing for nanosimilars
- Addressing FDA’s scientific questions
- NBCDs



Transnational Collaboration

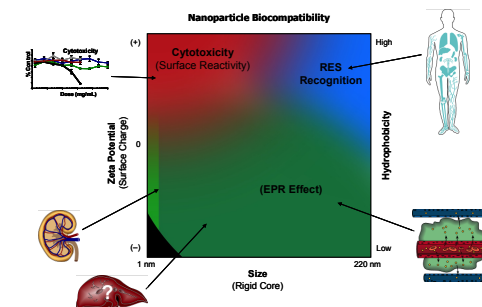
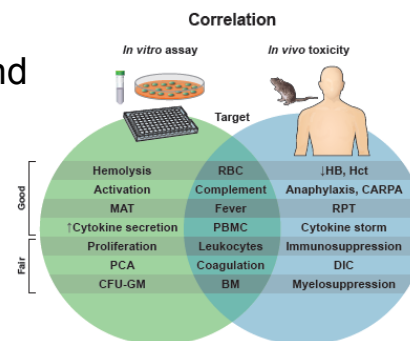
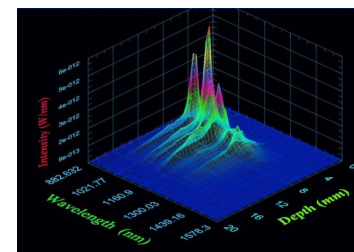
- EU-NCL



NCL is a Translational Resource for Nanomedicine Developers

Physicochemical Properties

- Answering FDA questions regarding material integrity and stability
- Characteristics of surface coatings that impact safety and efficacy: nature of attachment, surface coverage, coating stability
- Improved quantification of size polydispersity and nanomaterial impurities



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

Pharmacology and Toxicology

- Defining quality attributes that affect performance:
 - Metabolite modeling of drug release
 - Stable isotope method for drug release
- Autophagy & lysosomal dysfunction

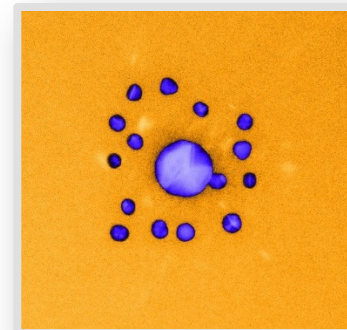
Immune Response

- Protein coronas
- Hematocompatibility
- Immunogenicity
- In vitro – In vivo correlations

Assisting Nanomedicine Developers with Answers to Regulatory Questions

- Gold nanoparticles with conjugated therapeutic protein

- FDA asked for quantification of amount of protein bound to gold
- Analysis of batch-to-batch consistency of gold shape and size

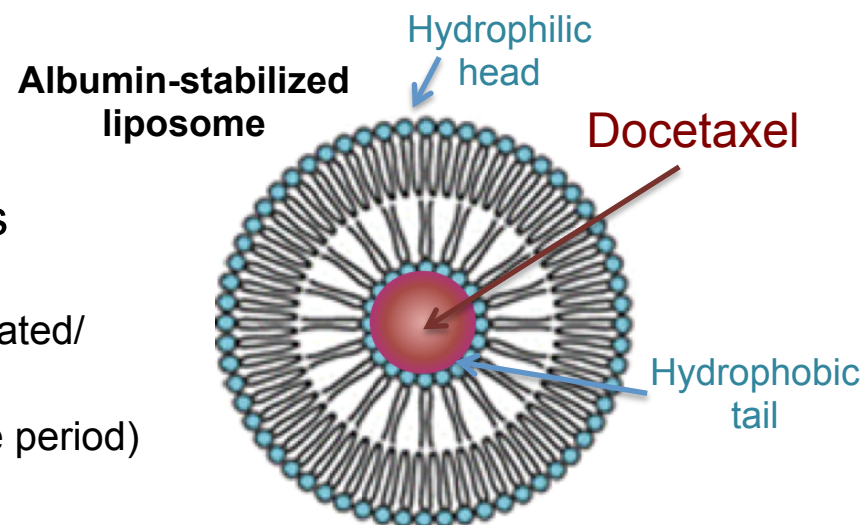


- Liposomal formulation of docetaxel

- FDA asked company to compare protein binding of liposomal docetaxel to docetaxel given as Taxotere.

- Nanoliposome radiopharmaceutical administered by catheter into GBM tumors

- FDA asked for measurement of the amount of encapsulated/unencapsulated radionuclide, chelated/unchelated radionuclide
- Changes in sample over time (In the clinical time period)
- Is the sample consistent pre- and post-catheter?
- Sterility pre- and post-catheter?



Developing Methods to Quantitate Polymer Coatings on Nanomedicines

Polyethylene glycol (PEG) is the most common type of coating polymer on nanoparticles for biomedical applications

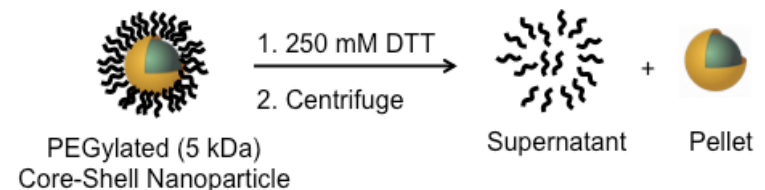
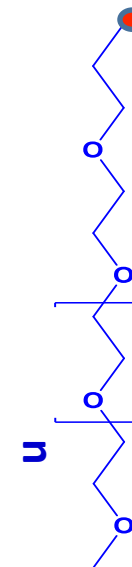
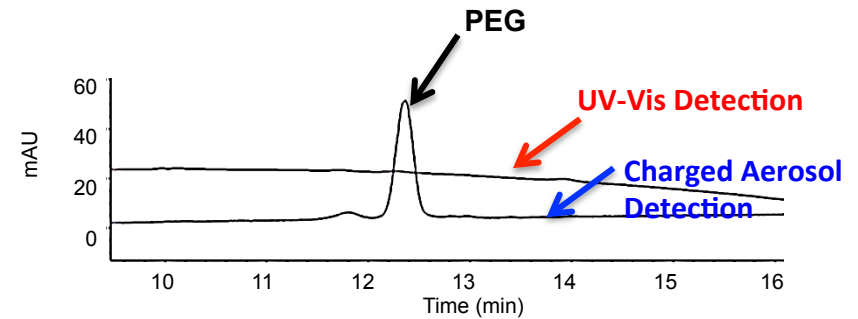
- Chemically inert backbone.
- Provides 'stealth property' to evade immune system recognition.
- End groups for covalent linkage (-SH, -NH₂, -COOH, Lipid, Silane, etc.).
- Does not have chromophore.
- Insensitive for UV-Vis detection.



Limited methods are available for accurate quantification of PEG coatings.

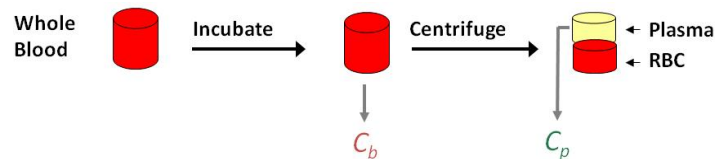
Developing Methods to Quantitate Polymer Coatings on Nanomedicines

- Charged Aerosol Detector (CAD)
 - Polyethylene Glycol (PEG)
 - Lipids
 - More sensitive than UV-Vis for most polymers.
- Thermogravimetric Analysis (TGA)
 - Monitor change in physicochemical properties as a function of increasing temperature.
 - Dehydration, decomposition pattern, organic content analysis.
- Chemical Methods
 - Example: Thiol containing species
 - Ellman's Reagent



Developing Methods for Estimating Drug Release

- Blood partitioning assay



$$F_p \% = (C_p / C_b) \times (1 - H_c) \times 100$$

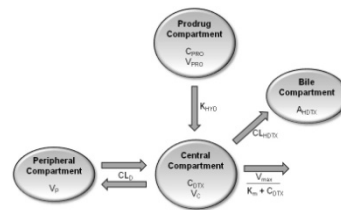
↑ Plasma Fraction ↑ Hematocrit
 Concentrations in plasma (C_p) and blood (C_b)

Zolnik et al., *Drug Metab Dispos.* 2008, 36(8):1709-15.

- Dual labeling/complementary analysis *in vivo*

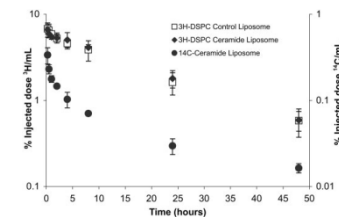
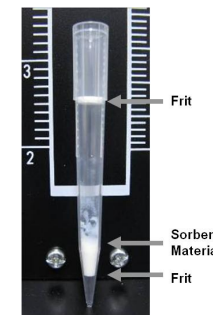
- New extraction methods to separate free and encapsulated

- Metabolite modeling to predict free drug

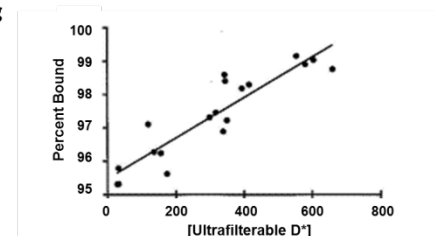
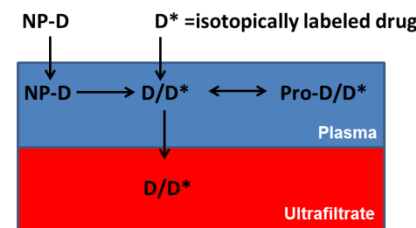


Stern ST et al., *J Control Release*, 2013, 172(2), 558-567.

- Measurement of fraction unbound to quantify drug encapsulated/released



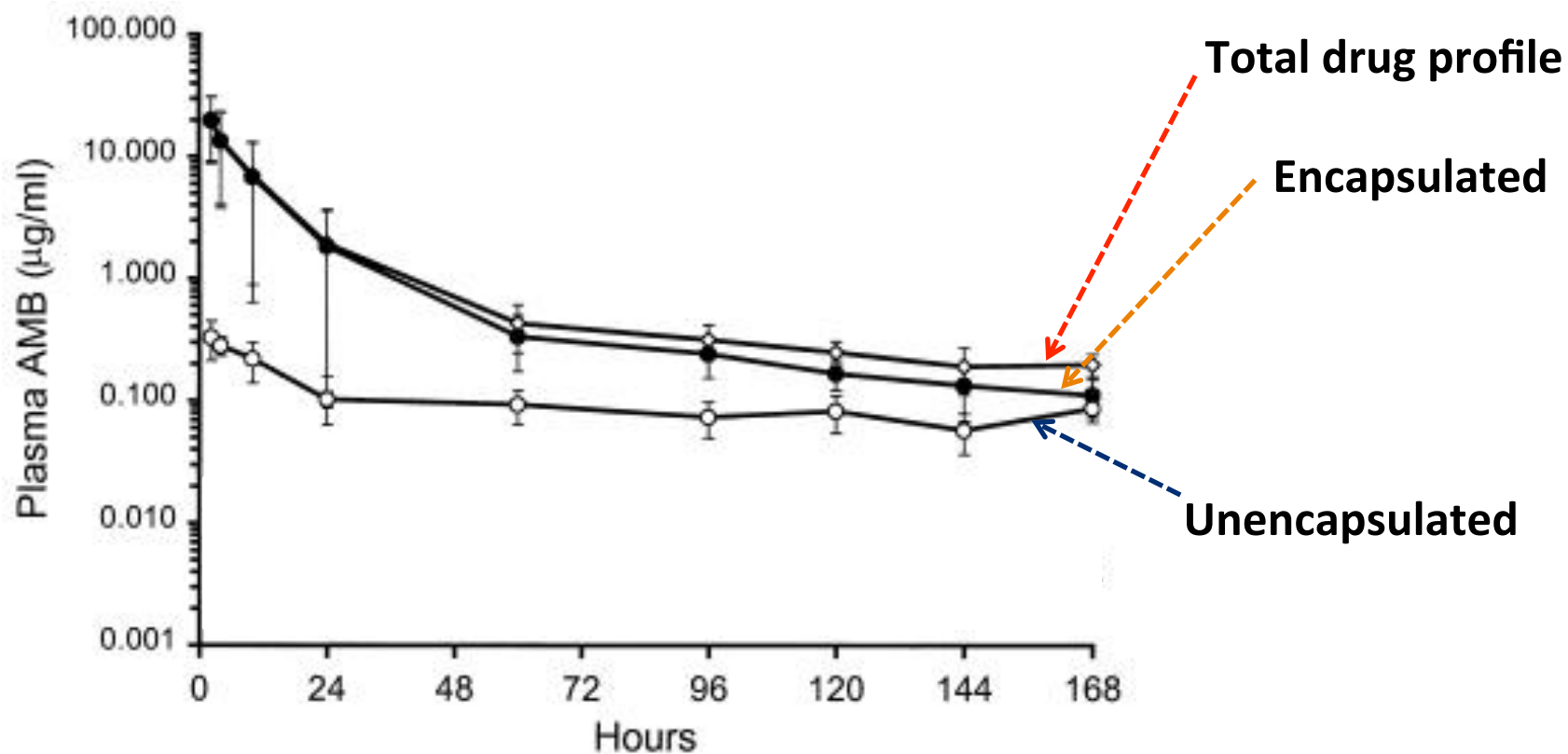
Zolnik et al., *Drug Metab Dispos.* 2008, 36(8):1709-15.



Bekersky et al., *Antimicrob Agents Chemother* 2002, 46(3):834-40.

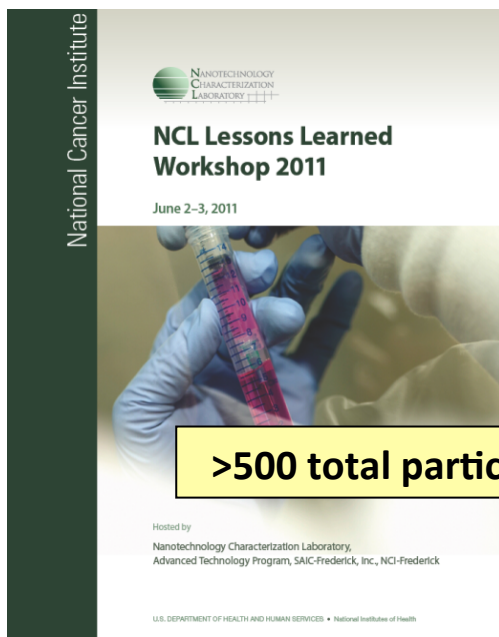
Both a nanomedicine quality and bioequivalence property.

Nanomedicine PK



Nanomedicine PK is different. Total drug profile is dominated by encapsulated drug fraction.

NCL Lessons Learned



>500 total participants

“I’ve never
been to a
meeting
that was so
relevant.”

LLW Attendee

Integrative Biology

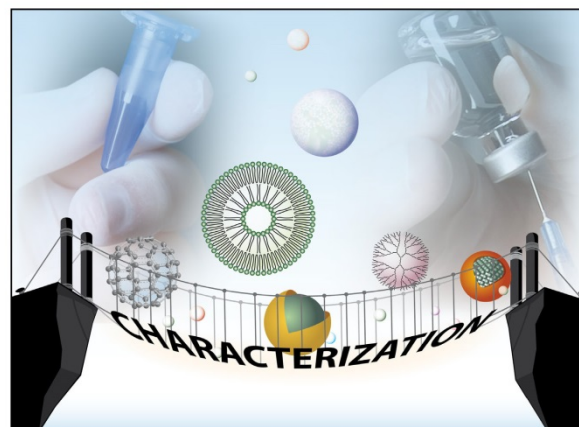
RSC Publishing

PERSPECTIVE

Cite this: *Integr. Biol.*, 2013,
5, 66

Common pitfalls in nanotechnology: lessons learned from NCI’s Nanotechnology Characterization Laboratory

Rachael M. Crist, Jennifer Hall Grossman, Anil K. Patri, Stephan T. Stern,
Marina A. Dobrovolskaia, Pavan P. Adiseshiaiah, Jeffrey D. Clogston and Scott E. McNeil*



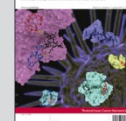
Featuring research from the Nanotechnology Characterization Laboratory (NCL) at the Frederick National Laboratory for Cancer Research, Frederick, MD, United States.

Title: Common pitfalls in nanotechnology: lessons learned from NCI’s Nanotechnology Characterization Laboratory

The NCL has characterized more than 250 different nanotechnology cancer strategies. One of the most important “Lessons Learned” from this work emphasizes the importance of characterization. From early physicochemical characterization through *in vivo* safety and toxicological profiling, exhaustive characterization is critical for successful clinical translation.

As featured in:

Integrative Biology



See Scott E. McNeil et al.,
Integr. Biol., 2013, 5, 66

RSC Publishing

www.rsc.org/ibiology

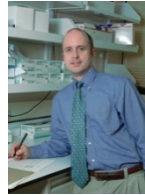
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Acknowledgements

Nanotechnology Characterization Lab



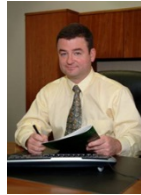
Scott E.
McNeil, Ph.D.



Stephan T.
Stern, Ph.D.,
DABT



Marina A.
Dobrovolskaia,
Ph.D.



Nick
Panaro, Ph.D.



Jeffrey D.
Clogston, Ph.D.



Pavan
Adisheshaiah,
Ph.D.



Abdullah
Mahmud, Ph.D.



Jennifer
Grossman, Ph.D.



Rachael M.
Crist, Ph.D.



Anna
Ilinskaya, Ph.D.



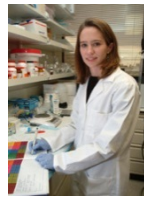
Christopher B.
McLeland, B.S., M.B.A.



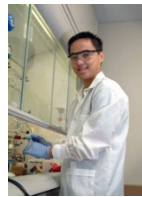
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Potter, B.S.



Barry W.
Neun, B.S.



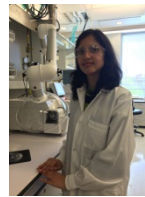
Sarah
Skoczen, M.S.



Sonny
Man, M.S.



Matthew
Hansen, M.S.



Alpana
Dongargaonkar,
M.S.



Jamie
Rodriguez, B.S.



Elizabeth
Befekadu, B.S.



Becky
Schneider, B.S.

Supporting Staff:

Electron Microscopy Lab



Ulrich
Baxa, Ph.D.



Sarah
Anderson, M.S.

Pathology/Histotechnology Lab



Diana C.
Haines, D.V.M.,
DACVP



Gloryvee
Rivera, B.S.



Wendi
Custer, B.A.



Kelly
Benauer

Contact Info:

Scott McNeil
(301) 846-6939
ncl@mail.nih.gov

<http://ncl.cancer.gov>