NCL
Nanotechnology
Characterization
Laboratory

Annual Report 2025

Nanotechnology Characterization Laboratory
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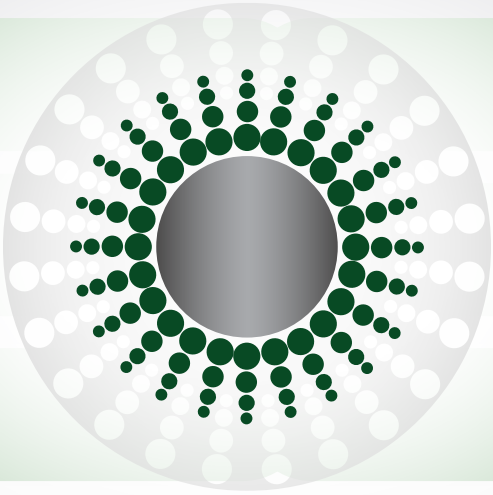
CONTENTS

The Nanotechnology Characterization Laboratory (NCL), located in Frederick, MD, is a National Cancer Institute-funded laboratory dedicated to advancing the science of cancer nanotechnology. The NCL has 20+ years of expertise in characterizing the physicochemical, immunology, pharmacology, and toxicology properties of nanomaterials. Developers of novel cancer nanomedicine technologies (chemotherapy, immunotherapy, vaccine, imaging agent, etc.) can apply to have their formulations characterized in the NCL's Assay Cascade characterization program—a multidisciplinary battery of tests that have been developed and tested to work with the multitude of nanotech platforms being explored in the biomedical research space. To date, the NCL has characterized more than 600 novel nanotechnology strategies, many of which have advanced to first-in-human clinical trials, with several now on the commercial market. Mechanisms for collaborating with the NCL are outlined on the next page, and highlights from various projects are shared throughout.

Assay Cascade for cancer nanomedicines

CRADA individualized formulation and research projects

Technical Service drug release and pharmacokinetic studies

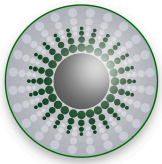


Free, downloadable protocols

Free consultations on characterization, study design, and more

Free training opportunities on all NCL capabilities

COLLABORATION MECHANISMS



While the NCL's free Assay Cascade characterization program for cancer nanomedicines is the most utilized collaboration mechanism, there are several opportunities for collaboration with the NCL. Individualized research projects can be conducted under a contractor Cooperative Research and Development Agreement (cCRADA). Fee-for-service assays, specifically in vitro drug release studies or in vivo pharmacokinetic studies analyzed using NCL's stable isotope tracer ultrafiltration assay (SITUA), can be purchased using a Technical Service. Key differences between the three collaboration mechanisms are highlighted below.

Assay Cascade Characterization Program

Application: ✓ **Cost:** ✗

What is it?

Free physicochemical and biological (immunology, pharmacology, and toxicology) characterization service for nanomaterials with an oncology indication

When is it?

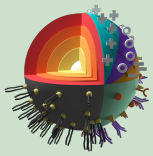
Applications deadlines in March and September every year

What to expect?

Thorough interdisciplinary testing of nanoformulation; regular updates and high-quality deliverables; assistance with method transfer to CRO/CDMO; attend meetings with regulatory agencies

Requirements?

Prior efficacy; stability; adequate physicochemical characterization data; sufficient quantities



Physicochemical Characterization

In Vitro and In Vivo Immunology

In Vitro and In Vivo Pharm. and Tox

Individualized Research Projects (cCRADA)

Application: ✓ **Cost:** ✓

What is it?

Individualized statement of work, beyond NCL's standardized Assay Cascade, tailored to your specific needs

When is it?

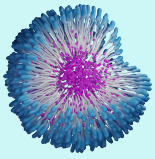
Proposals accepted year-round

What to expect?

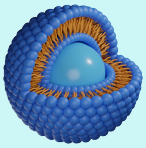
Low-cost, customized research—formulation development, method development, mechanistic studies, and more

Requirements?

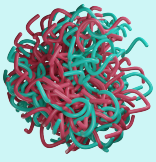
Acceptances are based on current workload demands and will require NCI/FNLCR approval and payment in advance



Polymeric Nanoparticles



Liposomes and Lipid-based



Polyelectrolyte Complexes

Technical Services

Application: ✗ **Cost:** ✓

What is it?

Specialized drug fractionation methods for in vitro and in vivo pharmacokinetic analyses

When is it?

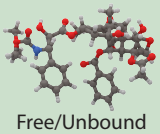
Available year-round; no application necessary

What to expect?

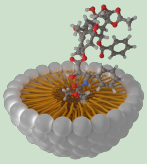
Rapid turn-around of in vitro drug release properties—ideal for formulation optimization and lot release—and in vivo noncompartmental pharmacokinetic analysis—ideal for preclinical bioequivalence evaluation

Requirements?

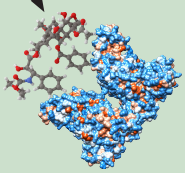
Stable isotope(s) of the API; payment in full prior to study initiation



Free/Unbound Unencapsulated



Nanomedicine Encapsulated



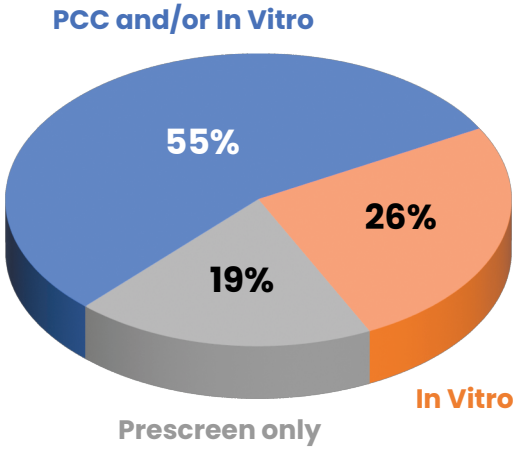
Protein Bound

NCL'S ASSAY CASCADE CHARACTERIZATION PROGRAM

Concepts accepted into the NCL's Assay Cascade program are subjected to an in-depth characterization. This includes an assessment of sterility and contamination with immune-response modulating impurities (e.g., endotoxin and beta-glucans), a comprehensive physicochemical characterization (e.g., compositional, purity, stability, and lot-to-lot analyses), in vitro safety studies (e.g., hemocompatibility, general toxicity, and immunotoxicity), and in vivo analyses (e.g., pharmacokinetics, general toxicity, immunotoxicity, and efficacy). The results from these collaborations can be used for regulatory-related purposes, publications, to attract venture capital, or to secure grant funding.

NCL supported 30 Assay Cascade projects in 2025. Research plans are individually tailored for each concept, considering the platform, API, indication, and route of administration.

Average Progression of Projects thru the Assay Cascade



The majority of Assay Cascade projects receive support with sterility, physicochemical, and in vitro safety studies.

About one-fourth of Assay Cascade projects receive support with in vivo studies.

Nearly one-fifth of projects do not advance beyond the NCL's prescreen, struggling with sterility, endotoxin contamination, or reproducibility of basic physicochemical properties. Although, researchers are consulted on corrective actions and encouraged to resubmit a new batch.

Multiple factors influence project length, including the scope of work and number of batches required, with the average length ranging from 0.8 to 2.5 years.

NCL's Assay Cascade Program for Cancer Nanomedicines

Two Application Types

Lead Candidate Characterization

- Therapeutic, diagnostic, imaging, or vaccine nanoformulations
- Novel, early-stage nanoplatforms
- Nanostrategies to alleviate adverse side effects of cancer therapy

Technology Development Characterization

- Structure–activity relationship (SAR) studies
- Method development
- Access to novel capabilities

Two Annual Deadlines

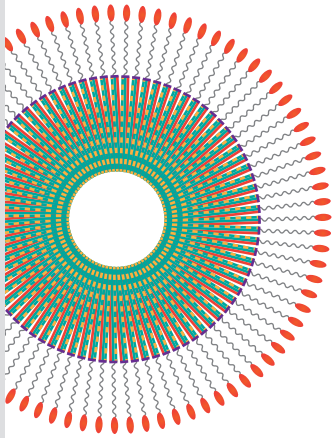
ASSAY CASCADE PROJECTS

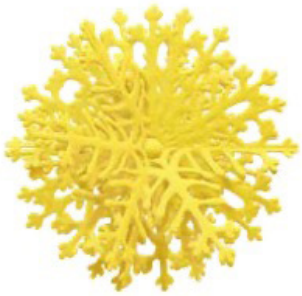
Investigators apply to the NCL's Assay Cascade with a variety of platforms, treatment strategies, and characterization needs. With the multitude of options available under the lead candidate and technology development characterization applications, the opportunities for collaboration are vast. Here, we highlight a few examples of projects accepted into the Assay Cascade characterization program, highlighting the diversity of concepts and the degree of support requested to aid researchers' developmental and translational efforts.

Application to NCL's Assay Cascade is open to all US researchers, including academia, industry, non-profit, and Government organizations. Download an application from the NCL's website: https://dctd.cancer.gov/research/research-areas/nanotech/ncl

Comprehensive Characterization of Lead Clinical Candidate

Physician-scientist Dr. Jill Smith, Georgetown University, applied to the NCL's Assay Cascade program after having co-developed a CCK-B receptor-targeted PEG-poly(lysine) polyplex formulation with gastrin siRNA with the NCL's formulation team. Dr. Smith is developing the formulation for the treatment of pancreatic cancer. As part of the project, the NCL provided Dr. Smith with an analysis of sterility properties, including quantitation of endotoxin and beta-glucan contaminants, physicochemical characterization, which included an assessment of stability after complexation as well as temperature storage stability, in vitro hemocompatibility studies, in vitro cytotoxicity studies in a variety of pancreatic cancer cell lines, in vitro immunotoxicity evaluation, and a comprehensive toxicology study in rats.

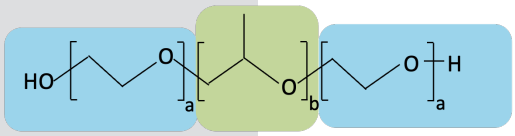




Limited Characterization of a Novel Platform

Professor Dong-Hyun Kim, Northwestern University, applied to the NCL for characterization support of gold supra-structured nanoparticles. The NCL provided physicochemical characterization to support refinement of the production process, including size distribution using orthogonal techniques, zeta potential, gold and silver metal quantitation, and detection and quantitation of residual solvents. With this data, the next step in his developmental efforts will be to incorporate a checkpoint inhibitor onto the particle surface for use as a combination radiosensitization agent and immunotherapy. The formulation is ultimately intended for treatment of cancers where local administration is possible, such as prostate, colorectal, etc.

Structure-Activity Relationship Studies



BASF, Corp., manufacturer of Kolliphor—a polymer excipient widely used in numerous FDA-approved injectable, oral, and ophthalmic drug products—applied to the NCL's program to evaluate hemocompatibility effects (e.g., platelet aggregation, complement activation, and plasma coagulation) and cytokine responses with respect to variations in polymer molecular weight and hydrophobic block lengths. Kolliphor is used in a variety of biomedical applications, including some cancer formulations. The data from the SAR study will be used to guide appropriate selection of suitable polymer properties dependent upon the intended use, thereby bettering the clinical translation potential of these polymer-based formulations.

NEW PROTOCOLS IN THE ASSAY CASCADE

The NCL has developed a multidisciplinary Assay Cascade of protocols, tests, and characterization guides to help researchers expand the knowledge-base of their technologies, filling gaps in their critical path toward translation. These protocols cover the areas of sterility and endotoxin, physicochemical characterization, immunology, pharmacology, and toxicology. The portfolio is continuously reviewed and updated with feedback and guidance from the user-base. In 2025, four new protocols were added to the Assay Cascade to help meet such demands.

91 Protocols and guides are available in the NCL Assay Cascade. All are available for download on the NCL website (https://dctd.cancer.gov/research/research-areas/nanotech/ncl/protocols-capabilities) and the National Library of Medicine's Bookshelf collection (https://www.ncbi.nlm.nih.gov/books/NBK604273/).

PCC-24: Determination of Water Content using the Karl Fischer Coulometric Method

Certain nanoformulations can be extremely sensitive to water content, and water absorption over time can negatively impact their stability and performance. Therefore, a quick and reliable means to monitor water absorption is a critical part of the Chemistry, Manufacturing, and Controls (CMC) portfolio for these formulations, helping to ensure their reliability. The NCL recently added this capability through the purchase of a Karl Fischer titration apparatus. The Karl Fischer coulometric method works by generating iodine from an applied electric current, which then reacts with water in the sample, affording quick calculation of the sample's water content.

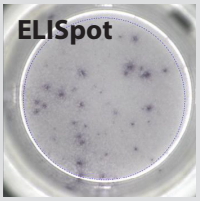
ITA-38: Analysis of Nanoparticle Effects of IgE-Dependent Mast Cell Degranulation

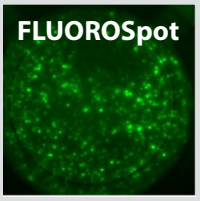
Nanoparticles may exaggerate preexisting hypersensitivity reactions and contribute to inflammation. Mast cells are an important component of true allergy. Mast cells bind antigen-specific IgEs on their surface via Fcε receptors; antigen binding to these receptors triggers mast cell degranulation, releasing secondary messengers, including cytokines, histamine, growth factors, and β-hexosaminidase, thereby driving the overall inflammatory response and the development of allergy symptoms. This protocol evaluates a nanoparticle's effect on this IgE-dependent degranulation process.

ITA-41: Detection of Interferon-gamma (IFNγ) by Enzyme-Linked Immunosorbent Spot (ELISpot) Assay

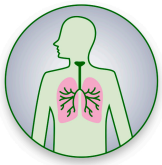
ITA-42: Detection of Interferon-gamma (IFNγ) and Interleukin-2 (IL-2) by Fluorescent Enzyme-Linked Immunosorbent Spot (FLUOROSpot) Assay

Nanotechnology-based immunotherapy and vaccine concepts have become an important part of the cancer nanotechnology landscape. IFNγ and IL-2—biomarkers of T cell activation—are often upregulated following treatment with this class of materials. To facilitate in vitro efficacy testing of these formulations, the NCL added ELISpot and FLUOROSpot assays to the Immunology Assay Cascade. These techniques are advantageous over traditional ELISA, offering higher sensitivity and the ability to assess the number of activated cells. The FLUOROSpot assay underwent interlaboratory studies and bioanalytical method validation as part of the method's development; these data are summarized in a recent publication in *The AAPS Journal* (https://doi.org/10.1208/s12248-025-01072-3).





STRUCTURE-ACTIVITY RELATIONSHIP STUDIES

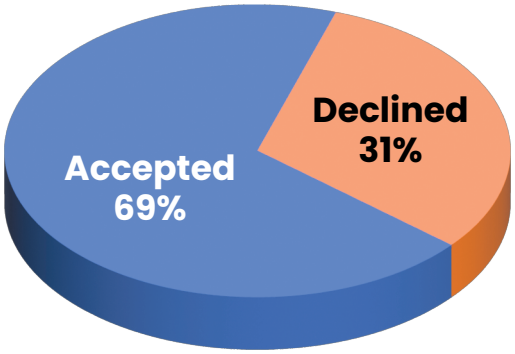


Structure-activity relationship (SAR) studies have been an important part of the advancement of the cancer nanomedicine field. Such studies have uncovered protein corona correlations to nanoparticle surface coverage, hemocompatibility connections to nanoparticle surface charge and composition, and cell uptake and biodistribution changes in response to nanoparticle size. The elucidation of these cause-and-effect relationships has afforded the strategic design and development of safer and more effective nanoparticle-based treatment strategies.

Part of the NCL's mission is to explore SARs, and the NCL has now opened its Assay Cascade program to help with this undertaking. Researchers developing cancer-related nanotechnology therapies and imaging agents can apply to have SAR studies conducted on their formulations, with the goal of assisting your formulation design and optimization.

Applications for SAR studies are now accepted as part of the Assay Cascade characterization program. These are more focused projects, with a narrower scope as compared to applications seeking in-depth characterization of lead concepts, but have a much higher average acceptance rate at 69%.

Average SAR/Technology Development Application Acceptance Rate



Example SAR Projects

Physicochemical Property
- Nanoparticle size
- Surface charge density
- PEG length
- Coating composition

Biological Response
- Hemolysis
- Plasma coagulation time
- Cytokine response
- Phagocytosis

A recent Assay Cascade collaborative project with Prof. Nicole Steinmetz (UC San Diego) explored the relationship of S protein cleavage in a cowpea mosaic virus (CPMV) investigational immunotherapy concept. CPMV is comprised of 60 copies each of large and small (S) coat proteins. This S coat protein contains a 24-amino acid peptide on its C-terminus that has been shown to be important in viral replication, RNA packaging, and assembly. Different CPMV preparation techniques, however, can affect this peptide, forcing different degrees of cleavage. This most recent study explored three CPMV preparations: one with an intact S protein, one with a cleaved S protein, and one with a mixture of the two. Studies investigated in vitro cytokine responses, in vitro immune cell uptake, and in vivo efficacy in an A20 murine diffuse large B cell lymphoma mouse model, and the results show that the S protein cleavage state does not negatively impact the immunomodulatory properties of the CPMV immunotherapy concept.

Structure-function relationship of S protein cleavage in cowpea mosaic virus intratumoral immunotherapy.
Simms, A., Affonso de Oliveira, J.F., Minasov, N., Cedrone, E., Dobrovolskaia, M.A., and Steinmetz, N.F.
Biomater Sci. **2025**, 13(19), 5422-5428. DOI: 10.1039/d5bm00969c. PMID: 40856604.

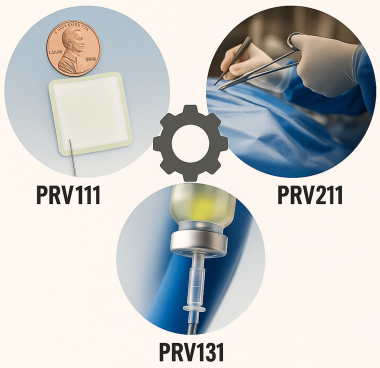
REGULATORY SUCCESSES OF ASSAY CASCADE COLLABORATORS

The NCL's interdisciplinary team works with dozens of investigators and companies every year, each developing a unique nanoparticle treatment strategy for improving cancer-related treatment outcomes. NCL's vast Assay Cascade portfolio provides these investigators with data they use to further the understanding of their formulation, optimize their final product, and address regulatory-related questions and concerns. Much of the data generated at the NCL can be used directly in Investigational New Drug (IND) and Investigational Device Exemption (IDE) filings with the Food and Drug Administration (FDA). Furthermore, NCL subject-matter experts, upon request, will attend regulatory meetings to address reviewer questions on NCL study designs and data interpretation.

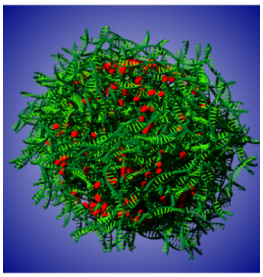
24 NCL Collaborators have advanced novel-nanotechnology concepts into human clinical trials since the program began accepting projects in 2005. Several of these are now marketed in both the US and EU.

NCL collaborator, Privo Technologies, announced the start of clinical trials in 2025 with PRV131, a cisplatin formulation designed for intratumoral administration of oral squamous cell carcinomas. PRV131 was accepted into the NCL Assay Cascade in December 2023, and NCL began characterizing the formulation in Q1 2024. The NCL provided physicochemical characterization, immunological studies in human primaries, in vitro drug release studies, and a repeat-dose rat toxicology study.

PRV131 expands on earlier Privo Technologies formulations (nanoengineered topical and intraoperative polymeric patches of cisplatin, PRV111 and PRV121, respectively) and aims to achieve tumor debulking prior to surgery, in order to preserve oral tissue and improve overall surgical outcomes. The phase 1/2 trial for PRV131, being run as arm 3 of the PRV111 and PRV121 CLN-004 clinical trial (NCT05893888), is a dose-escalation study to evaluate safety, tolerability, pharmacokinetics, and preliminary efficacy of the formulation.



You can read Privo Technologies' announcement of the clinical trial on their website: https://privotechnologies.com/privo-technologies-begins-dosing-prv131-in-arm-3-of-clinical-study-cln-004-a-first-in-class-cisplatin-intratumoral-injectable-targeting-oral-cavity-cancer/



Professor Young Jik Kwon of the University of California Irvine, together with commercial partner PharmaResearch, Co., Ltd., successfully filed an Investigational New Drug application with the FDA in 2025 and received authorization to proceed with clinical trials. Their concept, nanocomplexes of doxorubicin with the company's proprietary salmon DNA fragments (DNA Optimization Technology (DOT™), will be tested in a Phase 1 clinical trial in patients with locally advanced or metastatic solid tumors.

The NCL team are also working with several NCL Assay Cascade collaborators on pre-IND/pre-IDE phases, such as preparing for FDA INTERACT (Initial Targeted Engagement for Regulatory Advice on CBER/CDER Products) and SIR (Submission Issue Request) meetings and providing written responses to FDA queries.

- Professor Nicole Steinmetz, University of California San Diego, is preparing for an FDA INTERACT meeting for her team's cowpea mosaic virus (CPMV) immunotherapy concept, designed for intratumoral administration of solid tumors.
- Nanospectra Biosciences submitted written responses to the FDA in preparation for an SIR meeting to review their AuroShell technology, gold shell-silica core nanoparticles which produce heat upon near IR irradiation to afford tumor cell death. The technology will first be used to treat prostate cancer.

NANOTECH FORMULATION

NCL has expertise in the design and preparation of a variety of formulation platforms, with specialties in targeted lipid- and polymer-based technologies, and has formulated small molecule, nucleic acid, and peptide active pharmaceutical ingredients (API). You can request NCL support for formulation of your drug of choice to overcome solubility issues, toxicity concerns, alter biodistribution, etc. Interested researchers apply by first completing a short interest form with some background information. If the request appears to be within NCL's scope of capabilities, you will be asked to present your project to the NCL Formulation Review Committee.

Unlike the Assay Cascade program, which is a free, NCI-funded program, formulation development is performed under a cost-recovery mechanism. However, work is billed at-cost, meaning there is zero profit made on these collaborations. Formulation projects for extramural collaborators are performed under a cCRADA (see page 1 for more details on the cCRADA). Projects for intramural researchers will involve cost-sharing through appropriate mechanisms.

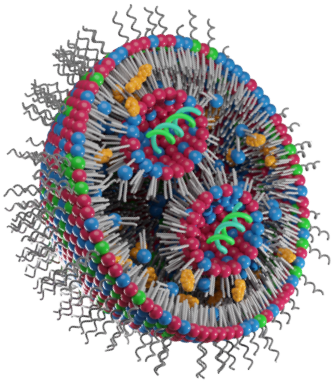
Nanoformulation design, preparation, and testing is available to both intramural and extramural researchers. Interested parties should reach out to ncl@mail.nih.gov to request a Formulation Support Request Form.

Representative Formulation Projects from 2025

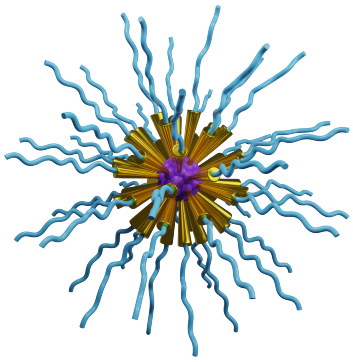
The NCL is working with the Frederick National Lab's Cancer Immunoprevention Laboratory (Dr. Jason Marshall) to develop and test prophylactic and therapeutic polyplex peptide and mRNA lipid nanoparticle vaccines for a variety of cancer indications.

In collaboration with NCI's Neuro-Oncology branch (Dr. Masaki Terabe), the NCL formulation team is helping to develop and test a tumor antigen/poly(lysine succinylated)-α-galactosylceramide vaccine for treatment of glioblastoma.

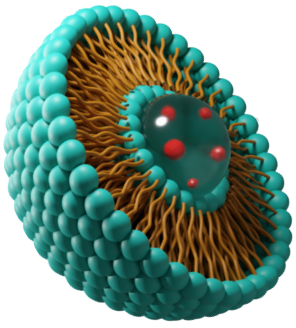
For the Cellular and Molecular Therapeutics Branch of the National Heart, Lung, and Blood Institute (Dr. John Tisdale), the NCL is helping to develop a hematopoietic stem cell-targeted CRISPR-lipid nanoparticle for treatment of sickle cell disease.



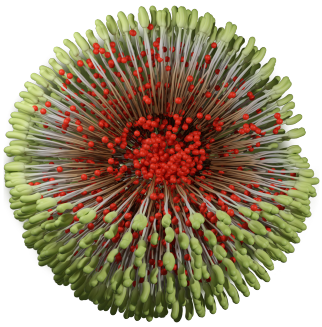
lipid nanoparticle



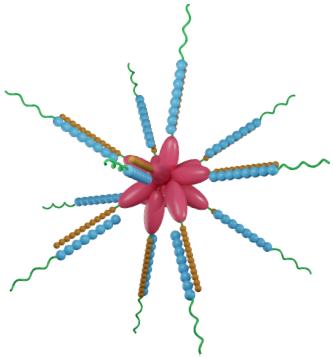
polymeric micelle



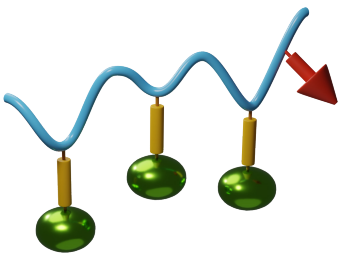
liposome



polymeric nanoparticle



polyplex

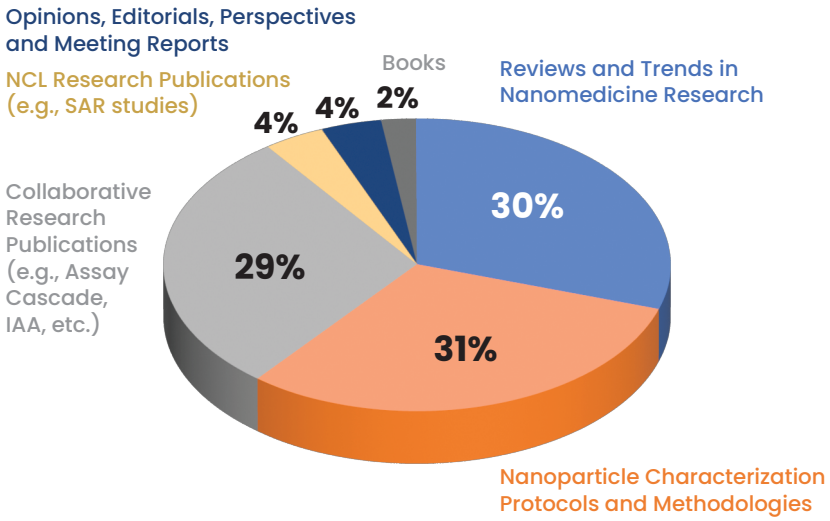


polymer-drug conjugates

NCL PUBLICATION TRENDS

In addition to using NCL characterization data for regulatory purposes, many collaborators also choose to publish these data. Nearly 30% of all NCL publications stem from collaborative research projects such as the Assay Cascade program, Inter-Agency Agreements (IAA), and other collaboration mechanisms. Another 30% of publications highlight trends observed from Assay Cascade studies and review relevant and timely topics of interest, and 31% detail characterization protocols and techniques. Research studies, such as structure-activity relationships and post-doctoral fellow research projects, perspectives, opinions and meetings reports, and books, such as the *Characterization of Nanoparticles Intended for Drug Delivery* and *Handbook of Immunological Properties of Engineered Nanomaterials* series, comprise the final 10% of NCL publications.

The NCL has over 300 publications with more than 25,000 citing articles.
The NCL's complete bibliography can be browsed on the National Library of Medicine site, https://www.ncbi.nlm.nih.gov/myncbi/1pU5B1t4kjrwtR/bibliography/public/.



Did you miss the NCL's 2024 symposium? Good news—speakers from the event worked together to provide an open-access, comprehensive meeting summary. Published in June 2025, in *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, the article summarizes all talks from the event, including key messages from keynote speakers Branden Brough (former Director of the National Nanotechnology Coordination Office), Pieter Cullis (University of British Columbia), and Mauro Ferrari (University of Washington), as well as exciting research updates from Yechezkel Barenholz (The Hebrew University of Jerusalem), Neil Desai (Aadi Bioscience), Matthieu Germain (Nanobiotix), Glen Kwon (University of Wisconsin-Madison), Len Pagliaro (Sona Nanotech), and Nicole Steinmetz (University of California-San Diego).

Advancing Medical Applications of Cancer Nanotechnology: Highlighting Two Decades of the NCI'S Nanotechnology Characterization Laboratory Service to the Research Community.
Crist, R.M., Barenholz, Y., Cern, A., Clark, K.N., Cullis, P.R., Dean, C., Desai, N., Ferrari, M., Germain, M., Giacomantonio, C.A., Grabarnik, E., Grodzinski, P., Hod, A., Kennedy, B.E., Kularatne, R.N., Kwon, G.S., Loeb, E., Noftall, E.B., Pagliaro, L., Rasoulianboroujeni, M., Roth, A., Rowles, D., Singh, K., Steinmetz, N.F., Yehtina, Z., Zhang, Y., Zilbersheid, D., Clogston, J.D., Stern, S.T., and Dobrovolskaia, M.A.
Wiley Interdiscip Rev Nanomed Nanobiotechnol. **2025**, 17(3), e70020. DOI: 10.1002/wnan.70020. PMID: 40458962.

CONSULTATIONS, TRAININGS AND PRESENTATIONS

In addition to research collaborations, the NCL also supports the nanotech community by providing free consultation and training. These services are available to all nanotech researchers, regardless of acceptance into the NCL via one of the three collaboration mechanisms described on page 1. Education and knowledge-sharing remain an important part of the NCL's overall mission. The team is dedicated to not only advancing the science of nanotechnology and the clinical translation of next-generation nanotherapies, but also to sharing trends and observations that may enable faster development and to training scientists in the most current testing strategies.

Consultation and training services are available as a free service to the nanotechnology community, regardless of application status. Please contact us to discuss your needs, ncl@mail.nih.gov.

Consultations

The NCL has gained a wealth of experience having tested more than 600 unique nanomedicines. Expertise spans characterization genres such as endotoxin contamination and testing, physicochemical properties, immunotoxicity, general toxicology, and pharmacokinetics; nanoparticles such as liposomes, micelles, lipid nanoparticles, polymeric platforms, and metallic particles; and active pharmaceutical ingredients such as small molecules, antibodies, peptides, and nucleic acids. The NCL can assist with characterization planning and study design, data interpretation, and help connecting with other experts and resources.

59 free consultations provided to extramural researchers in 2025

Training

Need more than consultation? The NCL also provides hands-on training in laboratory techniques. Common examples of training include the Limulus amoebocyte lysate (LAL) assay, including calculation of the maximum allowed dilution and preparation of inhibition-enhancement controls, preparation of peripheral blood mononuclear cells (PBMC) for cytokine ELISA screening, lipidic and polymeric nanoparticle formulation techniques, and training in the use of a variety of analytical instruments. Browse the capabilities and instrumentation on our website for a full list of available training opportunities, https://dctd.cancer.gov/drug-discovery-development/assays/nano/protocols-and-capabilities.

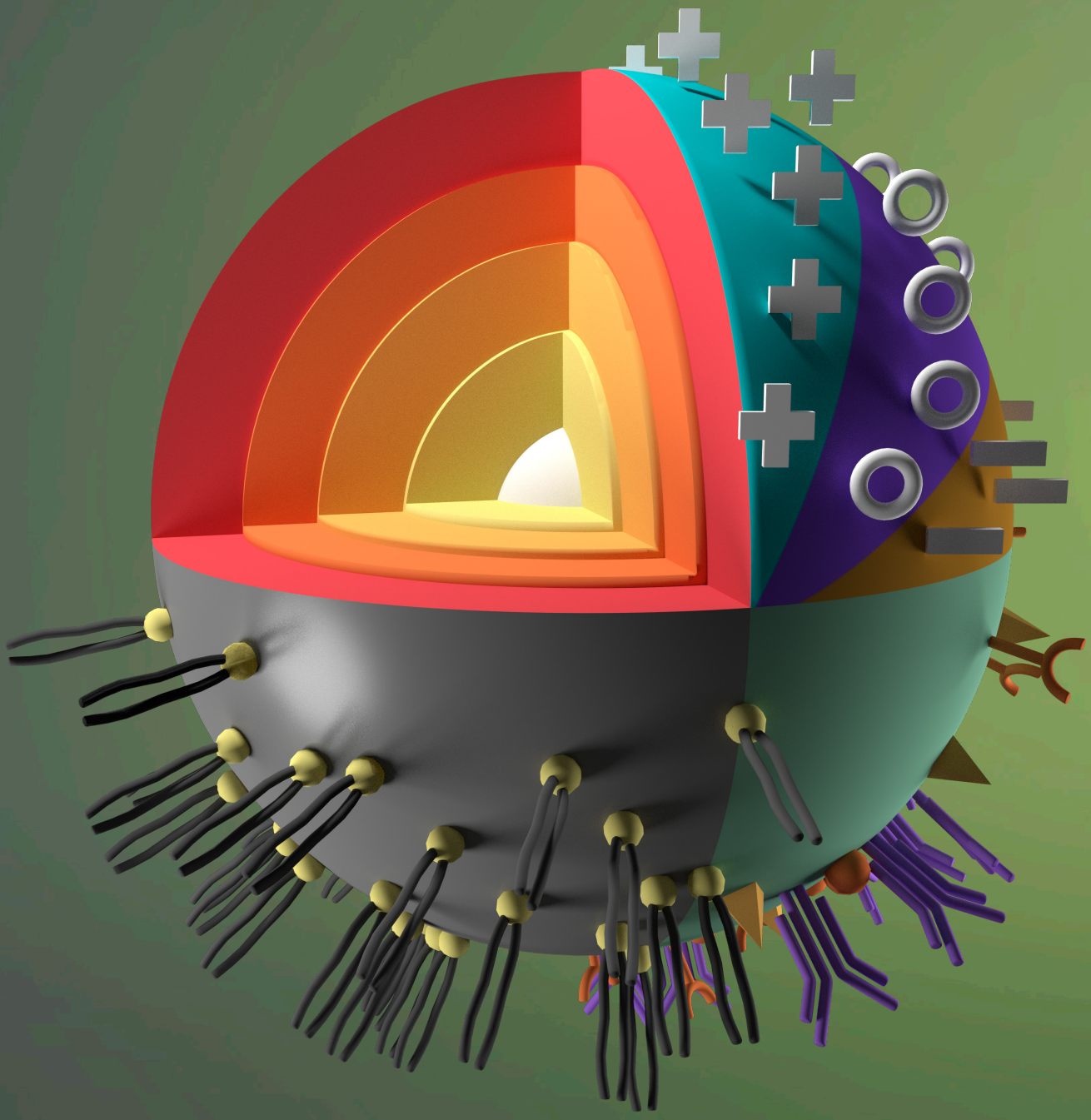
Training can be as short as **1-day** or as long as **1-year**

Presentations, Seminars, and Invited Talks

The NCL is very active in local, national, and international scientific conferences. In addition to seeing NCL staff speak at public meetings and workshops, staff are also open to invitations to speak at your institution. If interested in hosting an NCL team member, please reach out to us at ncl@mail.nih.gov and let us know your topic of interest.

29 conferences and workshops attended, including **17** invited seminars in 2025

Frederick
National Laboratory
for Cancer Research



The NCL is part of the Frederick National Laboratory for Cancer Research (FNLCR). FNLCR is operated by Leidos Biomedical Research, Inc., for the National Cancer Institute, under contract 75N91019D00024.