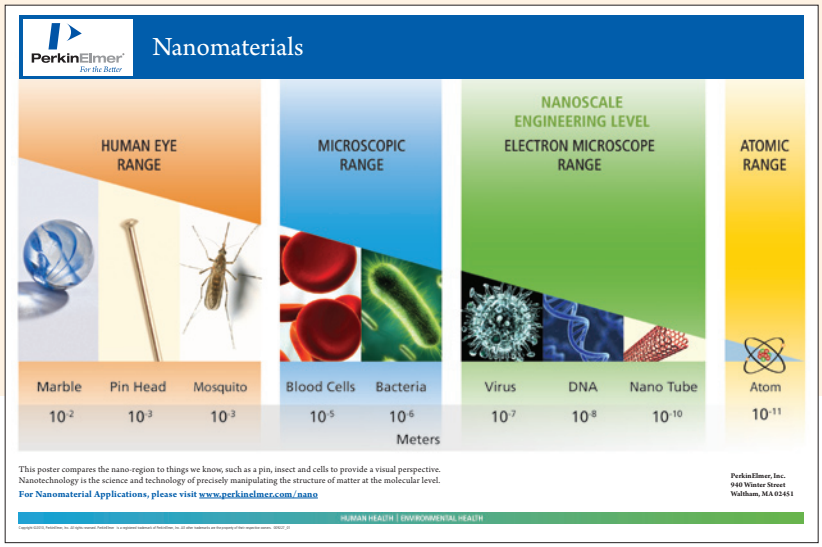
WHITE PAPER

# Nanopharmaceuticals





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# Nanopharmaceuticals and PerkinElmer

## Introduction

"Nanotechnology is widely anticipated as one of the key technologies of the 21st century."[1] PerkinElmer supplies nanomaterial characterization instruments for industrial and academic nanotechnology research. Industrial nanotechnology applications are far-reaching, spanning all science and engineering disciplines. One of the most promising nanotechnology fields is Nanopharmaceuticals. Because nanomaterials may enter the body through dermal exposure, inhalation, ingestion, or ocular contact, they lend themselves to innovative drug delivery systems. Pharmaceutical research, toxicology studies, formulation, and manufacture of pharmaceutical products require material characterization to ensure consistent drug safety and effectiveness. PerkinElmer has been providing analytical instruments to the pharmaceutical industry for more than 60 years. As such, nanopharmaceutical material applications are no exception for PerkinElmer.

## What are Nanomaterials?

Nanomaterials are materials that range in size from approximately 1 nm to 100 nm. There are more rigorous definitions that are specific to certain applications such as cosmetics. In Europe's efforts to label cosmetics that contain nanoparticles, this definition evolved: "nanomaterial means an insoluble or biopersistant and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm."[2]

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Engineered nanoparticles are of great scientific interest. They effectively bridge a gap between bulk materials and atomic and molecular structures. Nanoparticle mechanical properties are different than bulk material. Surface area is disproportionate to weight, for instance, an 8 nm gold material has a surface area of 32 square meters per gram. Materials of nanoscale proportions exhibit unique characteristics. Examples are gold nanopartilces and silver nanoparticles smaller than 12 nm that exhibit an affinity for magnetism. In bulk form gold and silver are non-magnetic.

There is a diverse field of applications over a broad range of industries:

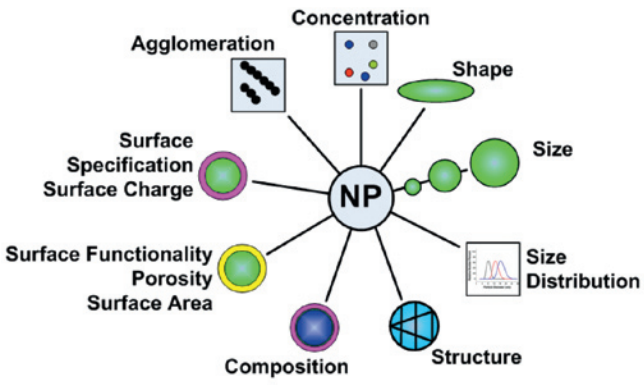
- Energy, energy-conservation, pharmaceuticals, chemicals, catalysts
- High performance-composite engineered materials – military to leisure time applications
- Coatings, electronics, sensors and displays
- And more

## What materials are used to make Engineered Nanomaterials?

There are several categories of nanomaterials, naturally occurring nanomaterials are found in nature, engineered nanomaterials are synthesized for a specific purpose or function, manufactured nanomaterials are produced for commercial purposes, and incidental nanomaterials are generated as an unintentional by-product of a process.[3]

Some engineered nanomaterials are:

- Gold, Silver, Copper, Selenium, Iron, Titanium, Zinc, and Aluminum
- Zinc oxide, Titanium oxide
- Carbon – Carbon Nanotubes, Buckyballs, and Graphene.
- Clay
- Organic materials/biodegradable




Key parameters to characterize nanomaterials.

Figure adopted from Hassellöv, M., and Kaegi, R., *Analysis and characterisation of manufactured nanoparticles in aquatic environments,* Chapter 6 in *Environmental & Human Health Impacts of Nanotechnology,* Eds., Lead, J.R. & Smith, E., 2009 Blackwell Publishing Ltd.

## What material parameters are important?

To completely characterize nanomaterial it is necessary to know a multitude of chemical and physical pararmeters including: the size of the particle, their shape, surface characteristics, the presence of surface coatings, and the presence of impurities.

Consequently, at the nanoscale, analytical measurement challenges are considerable and the ability to use, for example, one technique such as inductively coupled plasma and mass spectrometry (ICP-MS) to measure the elemental concentration of gold in a suspension as the only metric, does not provide enough information.

## How are engineered nanomaterials measured?

Seven of the nine nanomaterial characteristics: Particle Size, Size Distribution, Surface Charge, Surface Area, Shape, Agglomeration, and Structure, are characterized by one of the following analytical techniques:

- Scanning Electron Microscopy (SEM)
- Transmission Electron Microscopy (TEM)
- Atomic Force Microscopy (AFM)
- Confocal Microscopy (CFM)
- Dynamic Light Scattering (DLS)
- Field Flow Fractionation (FFF)
- Molecular Gas Adsorption (BET)
- Electrophoresis Particle Size

## Note

*Ultraviolet/Visible Spectroscopy* and *Fluorescence Spectroscopy* are used for particle size identification as long as the material is known and it is reflective. *Fluorescence Spectroscopy* is also used for agglomeration studies.

Nanoparticle Concentration and Composition are two nanoparticle characteristics that that are not covered by the analytical techniques described in the paragraph above. There are many analytical techniques that do cover concentration and composition. The correct analytical technique is determined by the material, coatings, and nano application.

For Nanoparticle Concentration you might choose one or several of the following analytical techniques:

- *Inductively Coupled Plasma and Mass Spectroscopy (ICP-MS)*
- *Liquid Chromatography and Mass Spectroscopy (LC-MS)*
- *Ultraviolet/Visible Spectroscopy (UV/Vis)*
- *Fluorescence Spectroscopy (FL)*

For Nanoparticle Composition you might choose one of the following analytical techniques:

- *Inductively Coupled Plasma and Mass Spectroscopy (ICP-MS)*
- *Liquid Chromatography and Mass Spectroscopy (LC-MS)*
- *Ultraviolet/Visible Spectroscopy (UV/Vis)*
- *Fluorescence Spectroscopy (FL)*
- *Thermogravimetry (TGA)*
- *Differential Scanning Calorimetry (DSC)*
- *Dynamic Mechanical Analysis (DMA)*
- *Fourier Transform Infrared Spectroscopy (FT-IR)*
- *Raman Spectroscopy*
- *Thermogravimetry, Gas Chromatography, and Mass Spectroscopy (TGA-GC/MS)*
- *Thermogravimetry and Mass Spectroscopy (TGA-MS)*

For **composition,** you may be concerned with purity or the coatings on nanomaterials besides the substrate material of the nanoparticle. All of the *italicized* analytical techniques are nano-characterization instruments that PerkinElmer offers. Please remember that there is not just one analytical technique that can characterize a nanomaterial. All analytical techniques are also listed in Table 1.

## What pharmaceutical applications are likely to utilize nanomaterials?

Nanopharmaceutical markets include **products for Humans, Pets, and Farm animals.** From the list below you can see that nanotechnology innovation will affect most people:

- Medicines for most diseases and illnesses – tablet or liquid form
- Vaccines for most diseases and illnesses
- Chemotherapeutic agents
- Anti-cancer drugs
- Personal care products: shampoos and body washes, etc.
- Medical devices and diagnostics, Molecular diagnostics, Diagnostic tests
- Dental health products
- Over-the-counter medicines
- Nutritional products
- Managing-obesity products
- Medical and Surgical devices
- Ocular health products and instruments
- Cardiology and Pulmonary medicine
- Osteoporosis
- Injury healing
- Generic pharmaceuticals
- Smoking cessation

## Who is involved in Nanopharmaceuticals?

**All major pharmaceutical companies** are involved in Nanopharmaceuticals.

- GlaxoSmithKline (GSK)
- Merck
- Johnson & Johnson
- Novartis
- Pfizer

**Small "start-up" nanopharmaceutical companies** play an important role in research and development. Some not-so-well-known, small, new, nanopharmaceutical-focused companies are:

- Cerulean Pharma Inc.
- Bind Biosciences
- Selecta Biosciences

**All U.S. universities** that conduct pharmaceutical or medical research are involved in nanopharmaceuticals. Some of the most well known academic nano-research institutions are:

- UCLA
- Rice University
- Georgia Tech
- MIT
- Yale University
- Many more…

**Academic authors** of nanopharmaceutical scientific research papers span the globe. They originate from:

- Iran
- Israel
- Poland
- Italy
- Germany
- Russia
- China
- Australia
- Japan
- UK
- Many more…

***Table 1.*** **Nanomaterial characteristics and applicable analytical technologies.**

| Analytical Technique | | Concentration | Particle Size | Particle S Distribut |
|---|---|---|---|---|
| Inductively Coupled Plasma – Mass Spectrometry | ICP-MS | ● | | |
| Field-flow Fractionation + ICP-MS | FFF-ICP-MS | ● | ● | |
| Liquid Chromatography – Mass Spectrometry | LC-MS | ● | | |
| Optical Spectroscopy – UV/Vis | UV/Vis | ● | ● | |
| Fluorescence Spectroscopy | FL | ● | ● | |
| Turbidity | | | ● | ● |
| Scanning Electron Microscopy | SEM | | ● | ● |
| Transmission Electron Microscopy (+EDX) | TEM | | ● | ● |
| Atomic Force Microscopy | AFM | | ● | ● |
| Confocal Microscopy | | | ● | ● |
| Field Flow Fractionation | FFF | | ● | ● |
| Dynamic Light Scattering | DLS | | ● | ● |
| Static Light Scattering | SLS | | ● | |
| Molecular Gas Adsorption (BET) | BET | | | |
| Dialysis | | | ● | |
| Electrophoresis and Capillary Electrophoresis | | | ● | ● |
| Ultrafiltration | | | ● | ● |
| Centrifugation | | | ● | ● |
| Filtration | | | ● | ● |
| Nanoparticle Tracking Analysis | NTA | | ● | ● |
| Size Exclusion Chromatography | SEC | | ● | ● |
| Selected Area Electron Diffraction | SAED | | ● | ● |
| Zeta Potential by DLS | | | | |
| X-ray Diffraction | XRD | | | |
| Thermogravimetric Analysis | TGA | | ● | |
| Quartz Microbalances | | | ● | |
| Differential Scanning Calorimetry | DSC | | | |
| Dynamic Mechanical Analysis | DMA | | | |
| Fourier Transform Infrared Spectroscopy | FT-IR | | | |
| FT-IR Imaging | | | | |
| Raman Spectroscopy | | | ● | |
| TGA coupled with Gas Chromatography – Mass Spectrometry | TGA-GC/MS | | | |
| Laser Induced Plasma Spectroscopy | LIPS | | ● | |
| Hydrodynamic Chromatography | HDC | | ● | ● |
| Laser Induced Breakdown Detection | LIBD | | ● | ● |
| X-ray Photoelectron Spectroscopy | XPS | | | |
| Electron Energy Loss Spectroscopy | EELS (+EDX) | | | |

● Commonly used in the characterization of nanomaterials

● Microscopy techniques

| Nanomaterial Characteristics | | | | | | |
|---|---|---|---|---|---|---|
| Size ion | Surface Charge | Surface Area | Shape | Agglomeration | Structure | Composition |
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● Not widely applicable

● Available from PerkinElmer

## What are nanomaterials used for in pharmaceuticals?

Nanomaterials are used primarily for drug delivery systems, but also are used for product packaging, colorants; bone, skin, and muscular growth; and medical imaging.

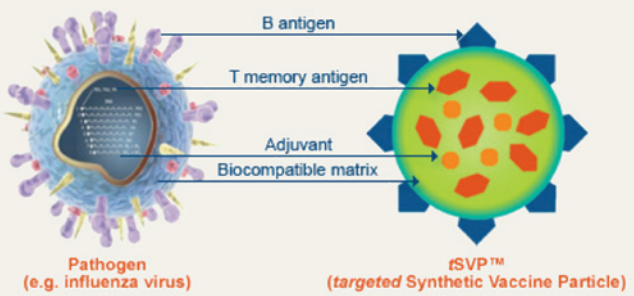
Drug delivery systems can be simple such as gold nanoparticles coated with a vitamin or nutrient. Or it could be as complex as a nanoparticle that is coated with functional groups that target specific tumor cells or organs and then are able to release the drug in some manner; time-released, released by heat, released by light, or released by magnetism. There are even nano-delivery systems that seek and destroy cells by entering the targeted cells and explode. Thus exploding within the cell and completely destroying the cell. Some time-released nanopharmaceuticals are encapsulated in lipids for use in salves and ointments. Titanium Oxide nanoparticles are used for white colorant in some salves and ointments and also in Dental Health products.

**Below is a diagram of a product from Selecta Biosciences Company. This complex nanopharmaceutical delivery system is designed to combat an influenza virus.[5]**

### *t*SVP™ – A new class of synthetic vaccines for Optimal Immune Response

Selecta's *targeted* Synthetic Vaccine Particle (*t*SVP™) product platform enables, for the first time, the highly-precise and modular development of therapeutic and prophylactic vaccines with optimal efficacy, duration of coverage and safety, to greatly improve the lives of patients.

Selecta's *t*SVP™ platform creates fully-integrated synthetic nanoparticle vaccines engineered to mimic the properties of natural pathogens to elicit a maximal immune response. The *t*SVP™ vaccines are rationally designed to optimize the presentation of antigens to the nexus of the immune system and ensure a focused and undistracted response. Selecta's *t*SVP™ platform accomplishes this by delivering antigens and adjuvants, within the same biodegradable nanoparticle, directly to antigen-presenting cells. This approach maximizes the immune response while minimizing undesirable off-target effects.

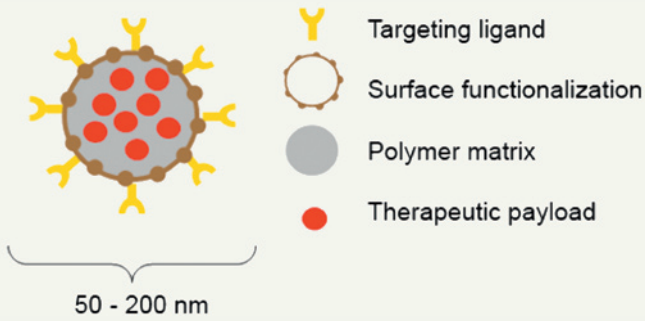



Selecta's unique *t*SVP™ platform includes a self-assembling nanoparticle platform that is synthetic, modular, and engineered for highly-effective targeting to immune cells. The *t*SVP™ vaccines incorporate only the essential elements required for a specific, robust immune response, based on precise engineering that is only possible with Selecta's proprietary, nanoparticle self-assembly process.

**Below is a diagram of a product from Bind Biosciences Inc. that describes another type of complex nanopharmaceutical delivery system.[6]**

### Targeted Nanoparticle Platform

BIND's targeted nanoparticles consist of the following components that facilitate optimization and control:




Targeting ligand provides recognition, enabling targeted nanoparticles to identify and bind to their intended target site. They are designed to recognize specific proteins or receptors that are found on the surface of cells involved in disease or the surrounding extracellular matrix.

Surface functionalization shields targeted nanoparticles from immune surveillance, while providing attachment for the targeting ligand through proprietary linkage strategies. We have developed proprietary methods for precisely controlling the surface characteristics necessary to ensure the drug is delivered efficiently and consistently.

Polymer matrix encapsulates payload molecules in a matrix of clinically validated biodegradable and biocompatible polymers that can be designed to provide the desired drug release profile.

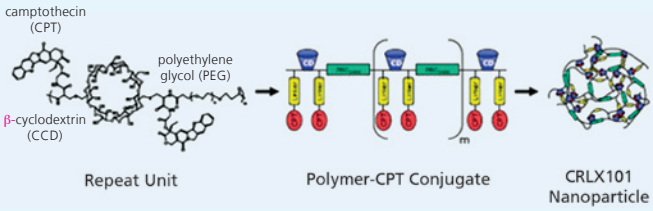
Therapeutic payloads can be incorporated into our targeted nanoparticles, including small molecules, peptides, proteins and nucleic acids, such as siRNA.

**Below is a diagram from Cerulean Pharma Inc. that describes a complex nanopharmaceutical delivery system.**

## CRLX101

CRLX101 is comprised of the high potency anti-tumor agent camptothecin coupled to a cyclodextrin based polymer that self-assembles into nanoparticles of consistent size and other physical attributes.

Below is a schematic representation of CRLX101.




CRLX101 provides clinical validation of the CDP technology improving the tolerability of the parent drug camptothecin. Results from the Phase 1 clinical study of CRLX101 have shown that it has a favorable safety profile in patients with advanced cancer. Combining camptothecin's potency and Cerulean's nanopharmaceutical design features, we believe CRLX101 has the potential to kill tumor cells while minimizing the side effects typically associated with chemotherapy treatment.

It is easily noted that all three nanopharmaceutical delivery systems are very different and all require material characterization by some analytical technique. This ensures patient safety and product effectiveness for each.

## Amazing results

When nanopharmaceutical drug delivery systems as sophisticated as the three above are used in certain cancer cases the results have been better than traditional bulk chemotherapy with little or no side effects.

Below are excerpts from Cerulean Pharma Inc. clinical and pre-clinical Nanopharmaceutical data for their product CRLX101 and progress with pre-clinical lead CRLX288.

Cerulean Chief Medical Officer John Ryan, Ph.D., M.D., reported results from the dose-finding, safety and tolerability Phase 1 clinical study of CRLX101. Specifically, Dr. Ryan discussed data establishing the maximum tolerated dose and the recommended dose and schedule for a planned Phase 2 study. He reviewed observations of progression-free disease of greater than six months in five advanced cancer patients who had previously relapsed and progressed on multiple lines of prior therapy. Notably, these advanced cancer patients had highly aggressive tumor types, such as non-small cell lung and pancreatic cancer, with typical survival of less than six to eight months. These observations correlate with CRLX101's pharmacokinetics profile including an extended half-life of more than 30 hours and a low volume of distribution of 2.1 liters per square meter, an indication of low systemic exposure of free drug. These data are also consistent with animal pharmacokinetic data demonstrating a high and prolonged localized drug exposure in the tumor.

Cerulean Senior Director of Research Scott Eliasof, Ph.D., presented recent results on the Company's pre-clinical lead candidate, CRLX288, a docetaxel nanopharmaceutical. His presentation focused on animal studies showing a significant improvement in the therapeutic index of CRLX288 compared to the parent drug docetaxel. Specifically, Dr. Eliasof reported that CRLX288 achieved complete regression and inhibition of tumor growth in 100 percent of the animals studied for greater than 100 days post-treatment, at dose levels that were well tolerated, in both typical size xenograft tumors of 100 $mm^3$ as well as in xenograft tumors as large as 800 $mm^3$. CRLX288's superior efficacy over the parent drug docetaxel in animal studies was consistent with other pre-clinical findings showing 20 times more drug accumulating in the tumor as compared to treatment with free docetaxel [bulk material].

Together, the Phase 1 findings for CRLX101 and the pre-clinical data on CRLX288 demonstrate that Cerulean's nanopharmaceutical platform has the potential to markedly enhance efficacy and tolerability of therapeutic agents in humans. Such biological outcome is targeted to be achieved with drug-containing nanoparticles that are designed to remain intact in circulation, accumulate in tumor tissues, enter cancer cells, and provide a long and sustained drug effect with slow and controlled drug release.

Please note that CLRX101 delivers the drug, camptothecin. Camptothecin is very potent and when delivered in bulk form not only killed the tumors but in some cases killed patients. When the same dosage of camptothecin is delivered in nanoscale increments the drug is still as effective as in bulk delivery, but there are no side effects. This maybe a result of attacking the tumor on a cell by cell basis. In fact, in laboratory tests a double dose of camptothecin bulk form was delivered on the nano scale and there were still no side effects.[9]

## Conclusion

PerkinElmer diligently works at being a global leader in nanotechnology characterization. PerkinElmer participates in Nano seminars worldwide. PerkinElmer is a member of the U.S. Technical Advisory Group (TAG 229) within the International Standards Organization (ISO). This membership involves writing and reviewing ISO Nanotechnology documents for industry, including nanopharmaceuticals and toxicology applications. PerkinElmer also participates in the U.S. National Nanotechnology Initiative (NNI) which helps set U.S. strategic direction for nanotechnology. PerkinElmer is a leader in material characterization and with its strength in characterizing nanomaterials it is very well positioned as a leader in nanopharmaceuticals. These are truly exciting times in nanoscience.

## Additional Readings and websites

- *Nanotechnology and Engineering Nanoparticles – A Primer.*
- Nanopharmaceutical Applications Library

Both suggested readings **above** are found www.perkinelmer.com/nano

- U.S. National Nanomaterials Initiative (NNI), http://www.nano.gov/
- University of California Center for Environmental Implications of Nanomaterials, USA, http://cein.cnsi.ucla.edu/pages/
- International Standards Organization, http://www.iso.org/iso/home.htm

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