



# cancer NANOTECHNOLOGY

## **Going Small for Big Advances**

Using Nanotechnology to Advance  
Cancer Diagnosis, Prevention  
and Treatment

U.S. DEPARTMENT OF  
HEALTH AND HUMAN SERVICES  
National Institutes of Health  
National Cancer Institute

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# NANOTECHNOLOGY AND CANCER

To help meet the goal of eliminating death and suffering from cancer by 2015, the National Cancer Institute is engaged in efforts to harness the power of nanotechnology to radically change the way we diagnose, image, and treat cancer. Already, NCI programs have supported research

**Nanotechnology will change the very foundations of cancer diagnosis, treatment, and prevention.**

on novel nanodevices capable of one or more clinically important functions, including detecting cancer at its earliest stages, pinpointing its location within the body, delivering anticancer drugs specifically to malignant cells, and determining if these drugs are killing malignant cells. As these nanodevices are evaluated in clinical trials, researchers envision that nanotechnology will serve as multifunctional tools that will not only be used with any number of diagnostic and therapeutic agents, but will change the very foundations of cancer diagnosis, treatment, and prevention.

The advent of nanotechnology in cancer research couldn't have come at a more opportune time. The vast knowledge of cancer genomics and proteomics emerging as a result of the Human Genome Project is providing critically important details of how cancer develops, which, in turn, creates new opportunities to attack the molecular underpinnings



of cancer. However, scientists lack the technological innovations to turn promising molecular discoveries into benefits for cancer patients. It is here that nanotechnology can play a pivotal role, providing the technological power and tools that will enable those developing new diagnostics, therapeutics, and preventives to keep pace with today's explosion in knowledge.

To harness the potential of nanotechnology in cancer, NCI is seeking broad scientific input to provide direction to research and engineering applications. In doing so, NCI will develop a Cancer Nanotechnology Plan. Drafted with input from experts in both cancer research and nanotechnology, the Plan (see pages 4 and 5) will guide

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NCI in supporting the interdisciplinary efforts needed to turn the promise of nanotechnology and the postgenomics revolution in knowledge into dramatic gains in our ability to diagnose, treat, and prevent cancer. Though this quest is near its beginning, the following pages highlight some of the significant advances that have already occurred from bridging the interface between modern molecular biology and nanotechnology.

# DEVELOPING A CANCER NANOTECHNOLOGY PLAN

NCI's Cancer Nanotechnology Plan will provide critical support for the field through extramural projects, intramural programs, and a new Nanotechnology Standardization Laboratory. This latter facility will develop important standards for nanotechnological constructs and devices that will enable researchers to develop cross-functional platforms that will serve multiple purposes. The laboratory will be a centralized characterization laboratory capable of generating technical data that will assist researchers in choosing which of the many promising nanoscale devices they might want to use for a particular clinical or research application. In addition, this new laboratory will facilitate the development of data to support regulatory sciences for the translation of nanotechnology into clinical applications.

The six major challenge areas of emphasis include:

## Prevention and Control of Cancer

- ▶ Developing nanoscale devices that can deliver cancer prevention agents
- ▶ Designing multicomponent anticancer vaccines using nanoscale delivery vehicles

## Early Detection and Proteomics

- ▶ Creating implantable, biofouling-indifferent molecular sensors that can detect cancer-associated biomarkers that can be collected for *ex vivo* analysis or analyzed *in situ*, with the results being transmitted via wireless technology to the physician

- ▶ Developing “smart” collection platforms for simultaneous mass spectroscopic analysis of multiple cancer-associated markers

## Imaging Diagnostics

- ▶ Designing “smart” injectable, targeted contrast agents that improve the resolution of cancer to the single cell level
- ▶ Engineering nanoscale devices capable of addressing the biological and evolutionary diversity of the multiple cancer cells that make up a tumor within an individual

## Multifunctional Therapeutics

- ▶ Developing nanoscale devices that integrate diagnostic and therapeutic functions
- ▶ Creating “smart” therapeutic devices that can control the spatial and temporal release of therapeutic agents while monitoring the effectiveness of these agents

## Quality of Life Enhancement in Cancer Care

- ▶ Designing nanoscale devices that can optimally deliver medications for treating conditions that may arise over time with chronic anticancer therapy, including pain, nausea, loss of appetite, depression, and difficulty breathing

## Interdisciplinary Training

- ▶ Coordinating efforts to provide cross-training in molecular and systems biology to nanotechnology engineers and in nanotechnology to cancer researchers
- ▶ Creating new interdisciplinary coursework/degree programs to train a new generation of researchers skilled in both cancer biology and nanotechnology

# WHAT IS NANOTECHNOLOGY?

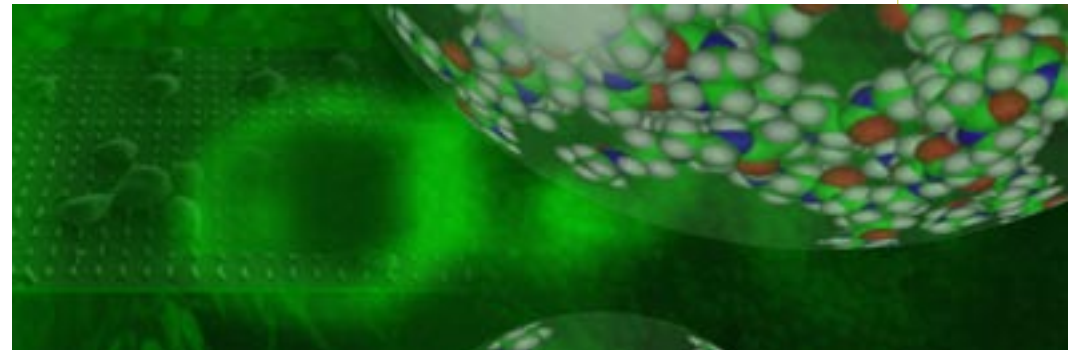
Nanotechnology refers to the interactions of cellular and molecular components and engineered materials—typically clusters of atoms, molecules, and molecular fragments—at the most elemental level of biology. Such nanoscale objects—typically, though not exclusively, with dimensions smaller than 100 nanometers—can be useful by themselves or as part of larger devices containing multiple nanoscale objects. At the nanoscale, the physical, chemical, and biological properties of materials differ fundamentally and often

**Noninvasive access to the interior of a living cell affords the opportunity for unprecedented gains on both clinical and basic research frontiers.**

unexpectedly from those of the corresponding bulk material because the quantum mechanical properties of atomic interactions are influenced by material variations on the nanometer scale. In fact, by creating nanometer-scale structures, it is possible to control fundamental characteristics of a material, including its melting point, magnetic properties, and even color, without changing the material's chemical composition.

Nanoscale devices and nanoscale components of larger devices are of the same size as biological entities. They are smaller than human cells (10,000 to 20,000 nanometers in diameter) and organelles and similar in size to large

biological macromolecules such as enzymes and receptors—hemoglobin, for example, is approximately 5 nm in diameter, while the lipid bilayer surrounding cells is on the order of 6 nm thick. Nanoscale devices smaller than 50 nanometers can easily enter most cells, while those smaller than 20 nanometers can transit out of blood vessels. As a result, nanoscale devices can readily interact with biomolecules on both the cell surface and within the cell, often in ways that do not alter the behavior and biochemical properties



of those molecules. From a scientific viewpoint, the actual construction and characterization of nanoscale devices may contribute to understanding carcinogenesis.

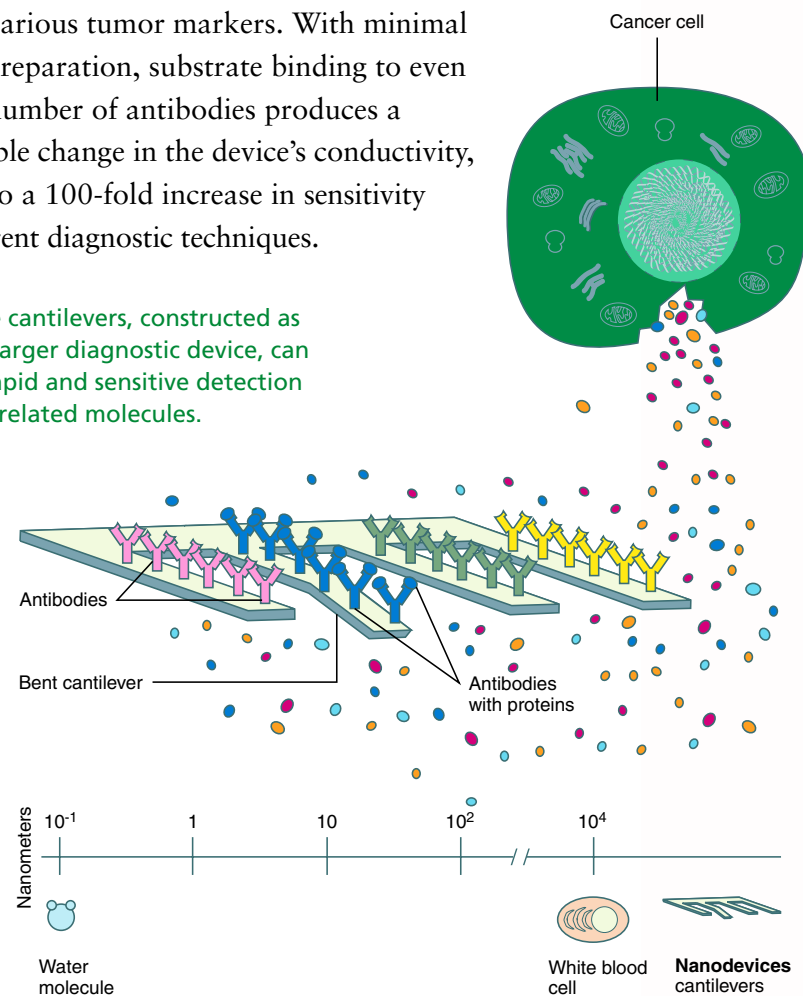
Noninvasive access to the interior of a living cell affords the opportunity for unprecedented gains on both clinical and basic research frontiers. The ability to simultaneously interact with multiple critical proteins and nucleic acids at the molecular scale should provide better understanding of the complex regulatory and signaling networks that govern the behavior of cells in their normal state and as they undergo malignant transformation. Nanotechnology

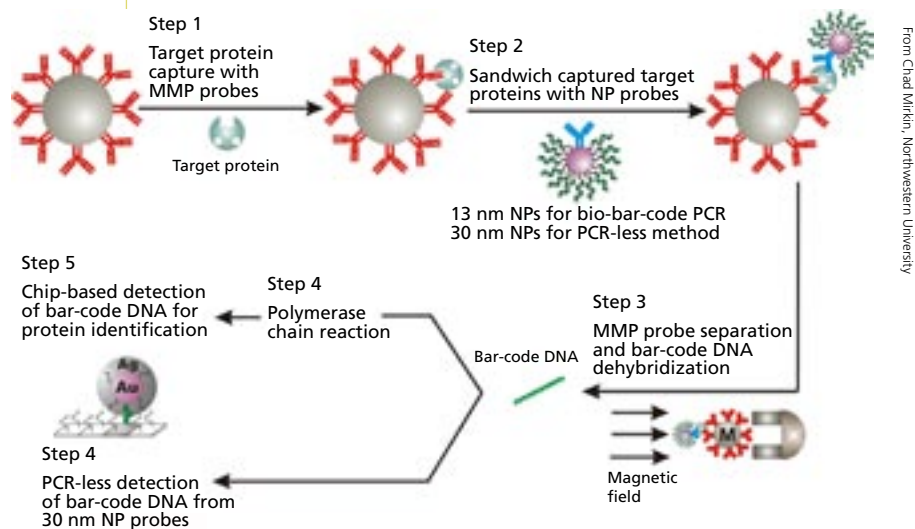
provides a platform for integrating efforts in proteomics with other scientific investigations into the molecular nature of cancer by giving researchers the opportunity to simultaneously measure gene and protein expression, recognize specific protein structures and structural domains, and follow protein transport among different cellular compartments. Similarly, nanoscale devices are already proving that they can deliver therapeutic agents that can act where they are likely to be most effective, that is, within the cell or even within specific organelles. Yet despite their small size, nanoscale devices can also hold tens of thousands of small molecules, such as a contrast agent or a multicomponent diagnostic system capable of assaying a cell's metabolic state, creating the opportunity for unmatched sensitivity in detecting cancer in its earliest stages. For example, current approaches may link a monoclonal antibody to a single molecule of an MRI contrast agent, requiring that many hundreds or thousands of this construct reach and bind to a targeted cancer cell in order to create a strong enough signal to be detected via MRI. Now imagine the same cancer-homing monoclonal antibody attached to a nanoparticle that contains tens of thousands of the same contrast agent—if even one such construct reaches and binds to a cancer cell, it would be detectable.

## NANOTECHNOLOGY AND DIAGNOSTICS

Today, cancer-related nanotechnology research is proceeding on two main fronts: laboratory-based diagnostics and *in vivo* diagnostics and therapeutics. Nanoscale devices designed for laboratory use rely on many of the methods developed to construct computer chips. For example, 1–2 nanometer-wide wires built on a micron-scale silicon grid can be coated with monoclonal antibodies directed against various tumor markers. With minimal sample preparation, substrate binding to even a small number of antibodies produces a measurable change in the device's conductivity, leading to a 100-fold increase in sensitivity over current diagnostic techniques.

Nanoscale cantilevers, constructed as part of a larger diagnostic device, can provide rapid and sensitive detection of cancer-related molecules.





DNA-coated gold nanoparticles (NPs) form the basis of a system that also uses larger magnetic microparticles (MMPs) to detect attomolar ( $10^{-18}$ ) concentrations of serum proteins. In this case, a monoclonal antibody to prostate specific antigen (PSA) is attached to the MMP, creating a reagent to capture free PSA. A second antibody to PSA, attached to the NPs, is then added, creating a “sandwich” of the captured protein and two particles that is easily separated using a magnetic field.

Nanoscale cantilevers, microscopic, flexible beams resembling a row of diving boards, are built using semiconductor lithographic techniques. These can be coated with molecules capable of binding specific substrates—DNA complementary to a specific gene sequence, for example. Such micron-sized devices, comprising many nanometer-sized cantilevers, can detect single molecules of DNA or protein.

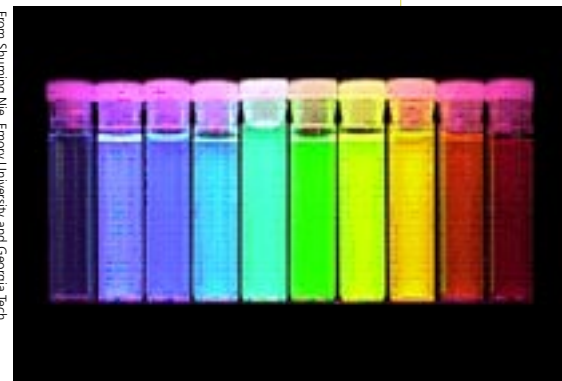
Researchers have also been developing a wide variety of nanoscale particles to serve as diagnostic platform devices. For example, DNA-labeled magnetic nanobeads have the potential to serve as a versatile foundation for detecting

virtually any protein or nucleic acid with far more sensitivity than is possible with conventional methods now in use. If this proves to be a general property of such systems, nanoparticle-based diagnostics could provide the means of turning even the rarest biomarkers into useful diagnostic or prognostic indicators.

Quantum dots, nanoscale crystals of a semiconductor material such as cadmium selenide, are another promising nanoscale tool for laboratory diagnostics. A product of the quest to develop new methods for harvesting solar energy, these coated nanoscale semiconductor crystals act as molecular light sources whose color depends solely on particle size. When linked to an antibody or other molecule capable of binding to a substance of interest, quantum dots act like a beacon that lights up when binding occurs.

Because of the multitude of colors with which they can emit light, quantum dots can be combined to create assays capable of detecting multiple substances simultaneously. In one demonstration, researchers were able to simultaneously measure levels of the breast cancer marker Her-2, actin, microfibril proteins, and nuclear antigens.

From Shuming Nie, Emory University and Georgia Tech

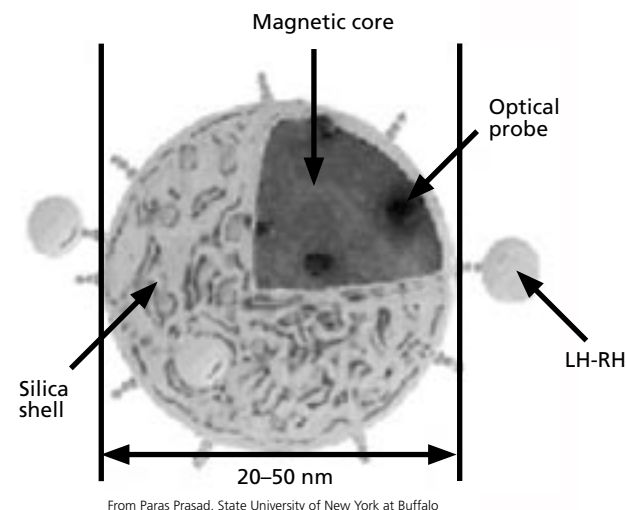


The color of a quantum dot depends on its size. These quantum dots emit across the entire visible spectrum even though all are irradiated with white light.

# NANOTECHNOLOGY AND CANCER THERAPY

Nanoscale devices have the potential to radically change cancer therapy for the better and to dramatically increase the number of highly effective therapeutic agents. Nanoscale constructs, for example, should serve as customizable, targeted drug delivery vehicles capable of ferrying large doses of chemotherapeutic agents or therapeutic genes into malignant cells while sparing healthy cells, which would greatly reduce or eliminate the often unpalatable side effects that accompany many current cancer therapies. Already, research has shown that nanoscale delivery devices, such as dendrimers (spherical, branched polymers), silica-coated micelles, ceramic nanoparticles, and cross-linked liposomes, can be targeted to cancer cells. This is done by attaching monoclonal antibodies or cell-surface receptor ligands that bind specifically to molecules found on the surfaces of cancer cells, such as the high-affinity folate receptor and luteinizing hormone releasing hormone (LH-RH), or molecules unique to endothelial cells that become co-opted by malignant cells, such as the integrin  $\alpha_v\beta_3$ . Once they reach their target, the nanoparticles are rapidly taken into cells. As efforts in proteomics and genomics uncover other molecules unique to cancer cells, targeted nanoparticles could become the method of choice for delivering anticancer drugs directly to tumor cells and their supporting endothelial cells. Eventually, it should be possible to mix and match anticancer drugs with any one of a number of nanotechnology-based delivery vehicles and targeting agents, giving researchers the opportunity to fine-tune therapeutic properties without needing to discover new bioactive molecules.

Multifunctional nanoparticles can be targeted to cancer cells using receptor ligands.



On an equally unconventional front, efforts are focused on constructing robust “smart” nanostructures that will eventually be capable of detecting malignant cells *in vivo*, pinpointing their location in the body, killing the cells, and reporting back that their payload has done its job. The operative principles driving these current efforts are modularity and multifunctionality, i.e., creating functional building blocks that can be snapped together and modified to meet the particular demands of a given clinical situation. A good example from the biological world is a virus capsule, made from a limited set of proteins, each with a specific chemical functionality, that comes together to create a multifunctional nanodelivery vehicle for genetic material.

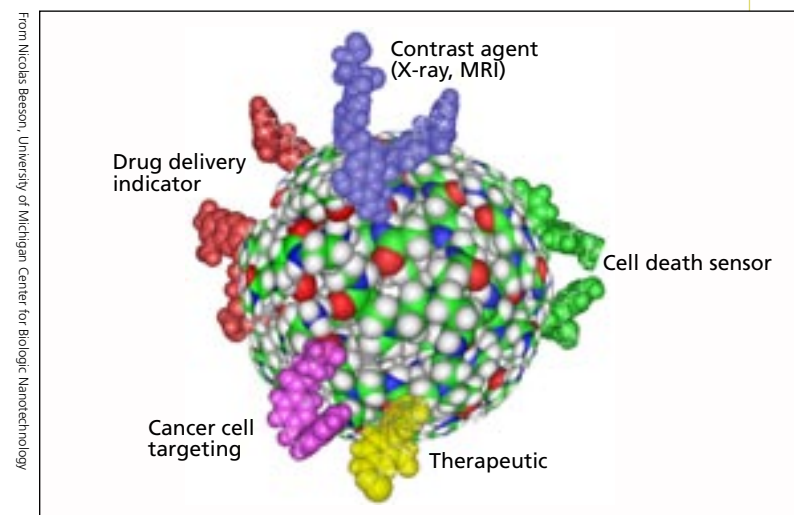
In fact, at least one research group is using the empty RNA virus capsules from cowpea mosaic virus and flockhouse virus as potential nanodevices. The premise is that 60 copies



of coat protein that assemble into a functional virus capsule offer a wide range of chemical functionality that could be put to use to attach homing molecules—such as monoclonal antibodies or cancer cell-specific receptor antagonists, and reporter molecules—such as magnetic resonance imaging (MRI) contrast agents, to the capsule surface, and to load therapeutic agents inside the capsule.

While such work with naturally existing nanostructures is promising, chemists and engineers have already made substantial progress turning synthetic materials into multifunctional nanodevices. Dendrimers, 1- to 10-nanometer spherical polymers of uniform molecular weight made from branched monomers, are proving particularly adept at providing multifunctional modularity. In one elegant demonstration, investigators attached folate—which targets the high-affinity folate receptor found on some malignant cells, the indicator fluorescein, and either of the anticancer drugs methotrexate or paclitaxel to a single dendrimer. Both *in vitro* and *in vivo* experiments showed that this nanodevice delivered its therapeutic payload specifically to folate receptor-positive cells while simultaneously labeling these cells for fluorescent detection. Subsequent work, in which a fluorescent indicator of cell death was linked to the dendrimer, provided evidence that the therapeutic compound was not only delivered to its target cell but also produced the desired effect. Already, some dendrimer-based constructs are making their way toward clinical trials for treating a variety of cancers.

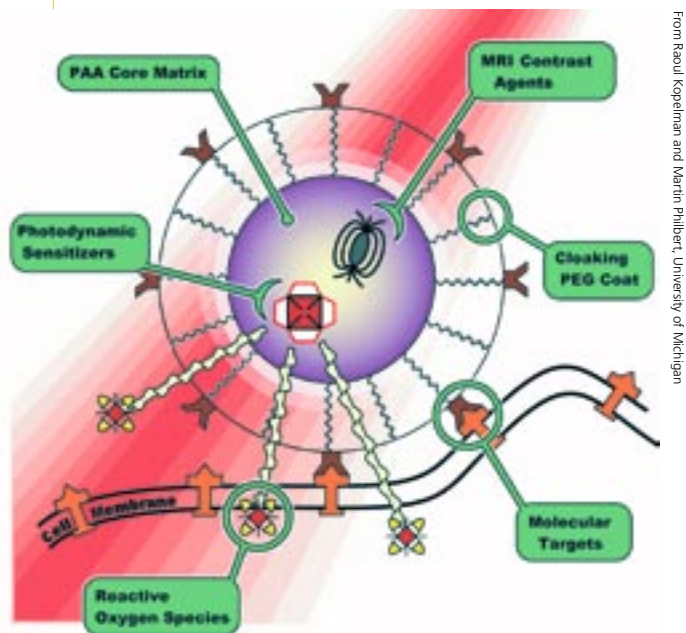
Such multifunctional nanodevices, sometimes referred to as nanoclinics, may also enable new types of therapeutic



Dendrimers can serve as versatile nanoscale platforms for creating multifunctional devices capable of detecting cancer and delivery drugs.

approaches or broader application of existing approaches to killing malignant cells. For example, silica-coated lipid micelles containing LH-RH as a targeting agent have been used to deliver iron oxide particles to LH-RH receptor-positive cancer cells.

Once these so-called nanoclinics have been taken up by the target cell, they can not only be imaged using MRI, but can also be turned into molecular-scale thermal scalpels: applying a rapidly oscillating magnetic field causes the entrapped  $\text{Fe}_2\text{O}_3$  molecules to become hot enough to kill the cell. The critical factor operating here is that nanoparticles can entrap 10,000 or more  $\text{Fe}_2\text{O}_3$  molecules, providing both enhanced sensitivity for detection and enough thermal mass to destroy the cell.



From Raoul Kopelman and Martin Philbert, University of Michigan

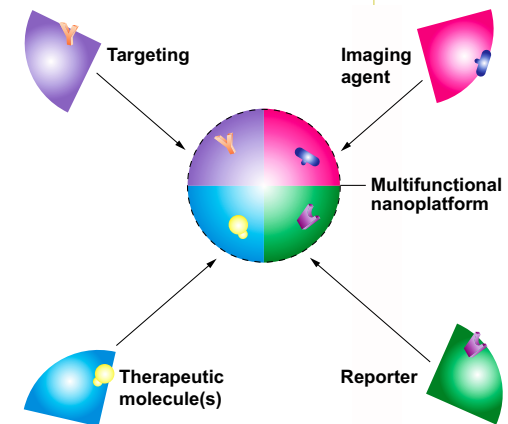
“Smart” dynamic nanoplatforms have the potential to change the way cancer is diagnosed, treated, and prevented. The outside of such “nanoclinics” could be decorated with a tumor-homing monoclonal antibody and coated with polyethylene glycol (PEG) to shield the device from immune system detection. The polymer matrix of such particles could be loaded with contrast agents, which would provide enhanced sensitivity for pinpointing tumor location within the body, and various types of therapeutic agents, such as reactive oxygen-generating photodynamic sensitizers that would be activated once the particle detected a malignant cell.

Photosensitizers used in photodynamic therapy, in which light is used to generate reactive oxygen locally within tumors, have also been entrapped in targeted nanodevices. The next step in this work is to also entrap a light-generating system, such as the luciferin-luciferase pair, in such a way as to trigger light production only after the nanoparticles have been taken

up by a targeted cell. If successful, such an approach would greatly extend the usefulness of photodynamic therapy to include treatment of tumors deep within the body.

Such multifunctional nanodevices hold out the possibility of radically changing the practice of oncology, perhaps providing the means to survey the body for the first signs of cancer and deliver effective therapeutics during the earliest stages of the disease. Certainly, researchers envision a day when a smart nanodevice will be able to fingerprint a particular cancer and dispense the correct drug at the proper time in a malignant cell’s life cycle, making individualized medicine a reality at the cellular level.

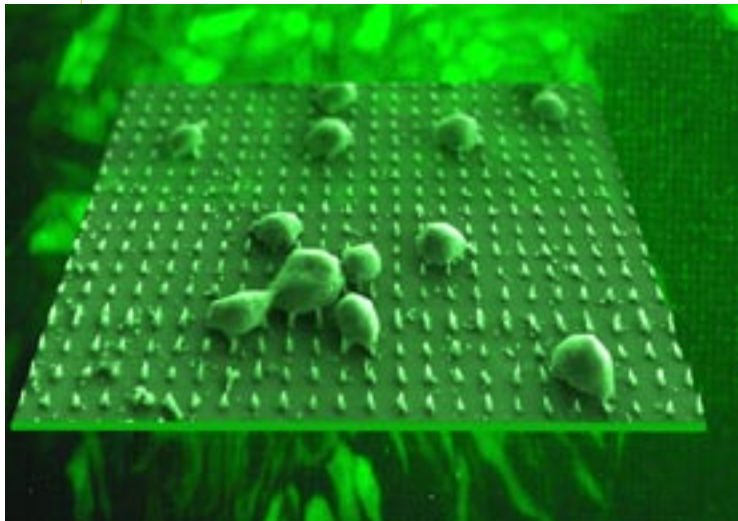
An important aspect of biomedical nanotechnology research is that most systems are being designed as general platforms that can be used to create a diverse set of multifunctional diagnostic and therapeutic devices.



With the focus on modularity and multifunctionality, one goal is to create and characterize platform technologies that can be mixed and matched with new targeting agents that will come from large-scale proteomics programs already in action and therapeutics both old and new. Accomplishing this goal, however, will require that engineers and biologists work hand in hand to combine the best of both of their worlds in the fight against cancer.

# A POWERFUL RESEARCH ENABLER

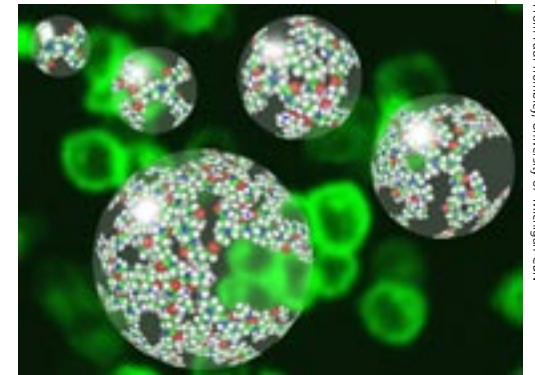
Nanotechnology is providing a critical bridge between the physical sciences and engineering, on the one hand, and modern molecular biology on the other. Materials scientists, for example, are learning the principles of the nanoscale world by studying the behavior of biomolecules and biomolecular assemblies. In return, engineers are creating a host of nanoscale tools that are required to develop the systems biology models of malignancy needed to better diagnose, treat, and ultimately prevent cancer. In particular, biomedical nanotechnology is benefiting from the combined efforts of scientists from a wide range of disciplines, in both the physical and biological sciences, who together are producing many different types and sizes of nanoscale devices, each with its own useful characteristics.



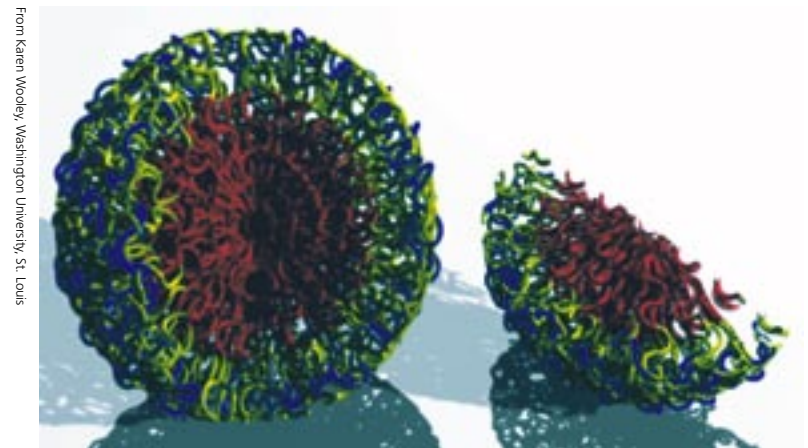
From Michael Simpson, Oak Ridge National Laboratory

An array of carbon nanotubes provides an addressable platform for probing intact, living cells.

Nanotechnology research is generating a variety of constructs, giving cancer researchers great flexibility in their efforts to change the paradigm of cancer diagnosis, treatment, and prevention. Shown here are two such structures. On the left are highly stable nanoparticles containing a cross-linked hydrophilic shell and a hydrophobic interior. On the right are spherical dendrimers, which are of rigorously defined size based on the number of monomer layers used to create a particular dendrimer. Like most of the other nanoparticles being developed, these are easily manipulated, affording researchers the opportunity to add a variety of molecules to the surfaces and interiors of the nanoparticles.



From Paul Tomblay, University of Michigan CBN



From Karen Wooley, Washington University, St. Louis

# NATIONAL NANOTECHNOLOGY CHARACTERIZATION LABORATORY FOR CANCER

As part of its cancer nanotechnology program, the National Cancer Institute is establishing a national resource laboratory at its Frederick facility that will provide critical infrastructure support to this rapidly developing field. The National Nanotechnology Characterization Laboratory (NCL) will fill a major resource gap in biomedical nanotechnology by providing nanodevice assessment and standardization capabilities that many experts have identified as a critical requirement to rapidly integrating nanotechnology into the clinical realm.



In creating the NCL, NCI will provide a stable and standardized environment for intramural and extramural researchers to bring their nanodevices and nanomaterials for assessment and development. The laboratory will also serve as an incubator in which private and public sector research efforts could be coordinated, yielding new partnerships that would accelerate the translation of basic research into clinical advances.

The basic functions carried out by the NCL will include:

- GMP synthesis of sizable quantities of a variety of nanoparticles and nanodevices;
- Characterization of nanoparticles and devices;
- Functionalization of nanoparticles;
- Development of tools and methods for characterizing both native and functionalized nanoparticles;
- Creation of reference standards and release specifications; and
- Facilitation of testing and analysis protocol development that will speed the regulatory review of novel diagnostics, therapeutics, and prevention strategies that use nanoscale devices.

In addition, the laboratory will have the capability of performing preclinical toxicology, pharmacology, and efficacy testing of nanodevices created both by NCI intramural and extramural efforts as well as by the private sector.

## FOR ADDITIONAL INFORMATION ON NANOTECHNOLOGY RESOURCES

NCI Unconventional Innovations Program—  
<http://otir.nci.nih.gov/tech/uip.html>

NCI Innovative Molecular Analysis Technologies Program—  
<http://otir.nci.nih.gov/tech/imat.html>

NCI Fundamental Technologies for Biomolecular Sensors—  
<http://otir.nci.nih.gov/tech/ftbs.html>

National Nanotechnology Initiative—  
<http://www.nano.gov/>

Understanding nanodevices—  
<http://newscenter.cancer.gov/sciencebehind/nanotech/nano01.htm>

Nanotechnology and cancer—  
<http://www.cancer.gov/newscenter/nanotech>

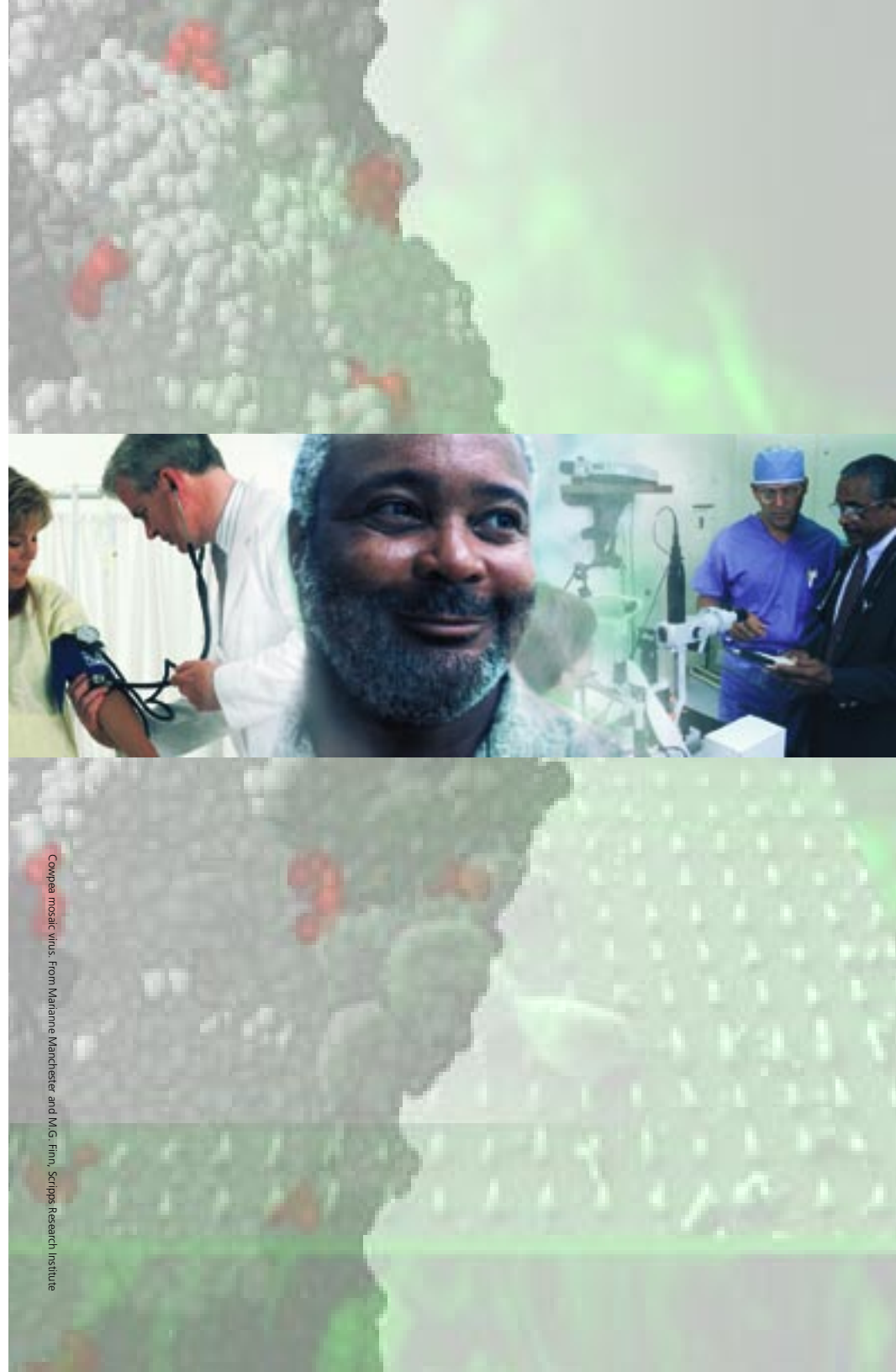
The Institute of Nanotechnology—  
<http://www.nano.org.uk/index.html>

CRADA Opportunities—  
<http://ttb.nci.nih.gov/cradaopp.html>

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Cowpea mosaic virus. From Marianne Manchester and M.G. Finn, Scripps Research Institute



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