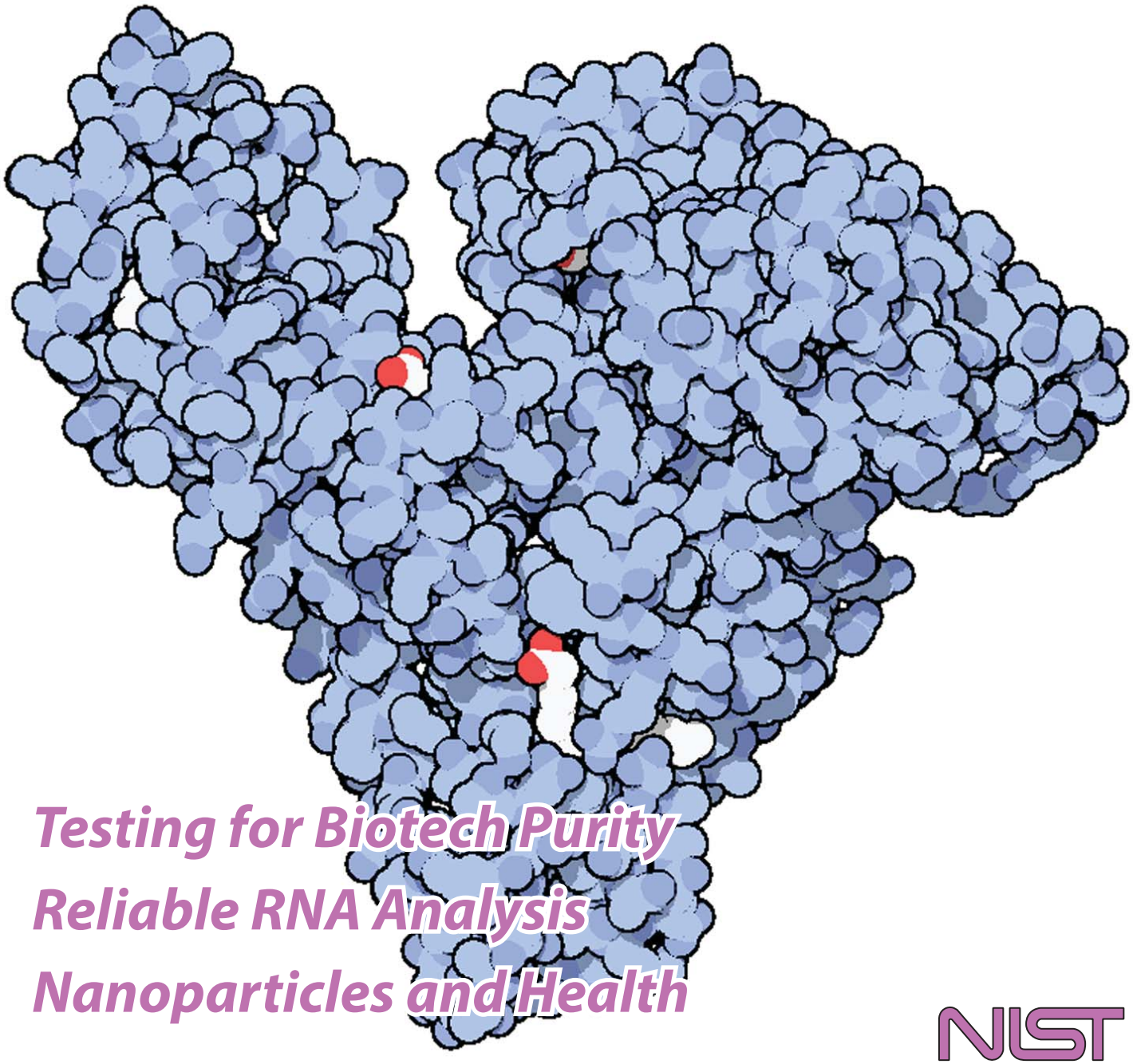
Material Matters

The Quarterly Magazine of NIST's Material Measurement Laboratory

Winter 2015



Testing for Biotech Purity

Reliable RNA Analysis

Nanoparticles and Health

National Institute of Standards and Technology
U.S. Department of Commerce

About NIST's Material Measurement Laboratory

The Material Measurement Laboratory (MML) is one of two metrology laboratories within the National Institute of Standards and Technology (NIST). The laboratory supports the NIST mission by serving as the national reference laboratory for measurements in the chemical, biological and material sciences. Our activities range from fundamental and applied research on the composition, structure and properties of industrial, biological and environmental materials and processes, to the development and dissemination of tools including reference measurement procedures, certified reference materials, critically evaluated data, and best practice guides that help assure measurement quality. Our research and measurement services support areas of national importance, such as:

- Advanced materials, from nanomaterials to structural steels to complex fluids
- Energy, from characterization and performance of fossil and alternative fuels to next-generation renewable sources of energy
- The environment, from the measurement of automotive exhaust emissions and other pollutants to assessment of climate change and the health and safety aspects of man-made nanomaterials
- Food safety and nutrition, from contaminant monitoring to ensuring the accuracy of nutrition labels
- Health care, from clinical diagnostics to tissue engineering and more efficient manufacturing of biologic drugs
- Infrastructure, from assessing the country's aging bridges and pipelines to the quality of our drinking water
- Manufacturing, from lightweight alloys for fuel-efficient automobiles to biomanufacturing, advanced electronics, and data for chemical manufacturing
- Safety, security and forensics, from gunshot and explosive residue detection, to ensuring the performance of body armor materials, to DNA-based human identity testing

The Material Measurement Laboratory also coordinates the NIST-wide Standard Reference Materials® (SRM) and Standard Reference Data programs, which include production, documentation, inventory, marketing, distribution and customer service.

The Material Measurement Laboratory is home to more than 900 staff members and visiting scientists at six locations:

- NIST main campus in Gaithersburg, MD
- NIST Boulder Laboratories in Boulder, CO
- Hollings Marine Laboratory in Charleston, SC , where NIST staff work side-by-side with scientists from NOAA, the South Carolina Department of Natural Resources, the College of Charleston, and the Medical University of South Carolina to provide the science, biotechnology and standards needed to understand links between environmental conditions and the health of marine organisms and humans
- Institute for Bioscience and Biotechnology Research (formerly CARB) in Rockville, MD, where scientists from NIST, the University of Maryland College Park, and the University of Maryland School of Medicine conduct research on measurement science and standards issues associated with advanced therapeutics
- Brookhaven National Laboratory in Upton, NY where, in partnership with the Department of Energy, the laboratory has a user facility that enables researchers from industry, academia and other government agencies to apply synchrotron-based x-ray spectroscopy techniques to the development of products like oil additives and next-generation electronics
- The Advances in Biological and Medical Measurement Science (ABMS) Program at Stanford University in Palo Alto, CA, where NIST staff are working elbow-to-elbow with Stanford faculty groups and commercial affiliates to develop standards and tools that enable translation of innovations in quantitative biology and engineered biology to clinical and commercial practice

Cover image: Human serum albumin (HSA) manufactured by genetically engineered seeds is commonly used for a variety of biomedical applications, from cell-growth media to clinical uses, but a new study suggests that plant-derived HSA may be contaminated by low levels of plant enzymes that could affect its properties.

Credit: Illustration by David S. Goodsell, RCSB PDB

A Message from the MML Director

Here at NIST's Material Measurement Laboratory (MML), the fundamental material measurements we make and the standards we help establish affect our world in many ways. When President Obama recently outlined his Administration's priorities for 2015 in his State of the Union address, I was pleased that MML is already working to solve several of the challenges he identified.

One highlight of his Address was the announcement of his intention to establish a Precision Medicine Initiative, to enhance our ability to "help deliver the right treatment to the right patient at the right time." This revolutionary approach to medicine requires that clinical measurements be accurate and reliable. In support of this effort, MML is developing improved standards and protocols for performance testing and calibration of a wide variety of clinical measurements including whole genome sequencing and assays for other biomarkers such as proteins and metabolites.

The President also emphasized our need for modern and state-of-the-art infrastructure and globally competitive manufacturing. MML supports American manufacturers with measurement solutions that help optimize manufacturing processes, assess the manufacturability of raw chemicals and materials, and assess manufacturing sustainability. Our support of manufacturing cuts across many sectors including advanced materials, automotive, biomanufacturing, chemicals, electronics, energy, and infrastructure, including materials for bridges, roadways, and pipelines. Our work contributes to an advanced manufacturing infrastructure that supports the development of new generations of technology-based products, creating jobs and a stronger economy.

The President made clear that we must act forcefully to combat climate change. Our ability to do so is dependent on how well we are able to measure the many chemicals associated with it. MML scientists respond to the measurement and standards needs associated with global climate change, as determined by the Intergovernmental Panel on Climate Science (IPCC), and continue to assess new needs as the world moves from assessment to mitigation.

As you will read in this issue of Material Matters, MML scientists are working at the forefront of their fields to advance understanding across a wide range of priority areas. Our work helps make our lives better, and will help improve the lives of future generations with scientific solutions to the challenges we face.

Laurie Locascio, Ph.D.
Director, Material Measurement Laboratory
NIST

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MedImmune and NIST Partner to Advance Development of Biological Therapies

Note: A version of this story previously appeared on www.nist.gov on February 20, 2015

MedImmune, the global biologics research and development arm of AstraZeneca, and NIST recently announced the signing of a five-year agreement to jointly support research that will help advance drug discovery and manufacturing. The effort will focus on tools and measurements that will be used in the development and production of biopharmaceuticals, which are drugs and treatments derived from biological, rather than chemical, sources.

Under the new agreement, MedImmune is providing first-year funding for seven NIST postdoctoral scientists, each working on a joint MedImmune/NIST research project. These projects will seek to better understand mechanisms of action, structures and other biological and chemical principles useful in drug development, engineering and formulation, and help create measurement tools to facilitate that knowledge.

"MedImmune is committed to partnering with premier institutions that can translate strong science into patient benefit," said Bahija Jallal, executive vice president, MedImmune. "NIST offers a unique expertise that can facilitate more precise, timely and innovative approaches to drug discovery, and being conveniently located with us in Gaithersburg, further supports the burgeoning life sciences community in Maryland."

"This partnership brings together MedImmune's deep industry understanding and NIST's measurement expertise to expand our knowledge of biopharmaceuticals—a growing field with huge economic and health impacts," said Willie May, acting under secretary of commerce for standards and technology and acting NIST director.

Among the initial MedImmune/NIST collaborations are:

Developing a new, sensitive form of Raman spectroscopy (a technique that provides information about molecular vibrations that can be used to identify and quantify samples) to rapidly determine that proteins used in biopharmaceuticals are properly folded and able to interact with other molecules as intended;

Helping researchers identify potential targets for therapeutic agents by establishing a library of the mass spectra, the "fingerprints" of molecules, for proteins on the surface of cells that have roles in specific diseases;

Developing methods to produce three-dimensional structural maps with resolution at the atomic level for the largest class of proteins used for medical therapies, called monoclonal antibodies, and

Using neutron beams to understand at the molecular level why some proteins used in biopharmaceuticals unfold during their manufacture.

Along with funding for the seven postdoctoral associates, MedImmune will supply NIST with monoclonal antibodies and other proprietary materials needed by the researchers. Work will be conducted at both the MedImmune and NIST campuses, which are located just over a kilometer from each other in Gaithersburg, Md. The effort will be supported by two MedImmune departments—Biopharmaceutical Development and Antibody Discovery and Protein Engineering—and NIST's Materials Measurement Laboratory. Access also will be provided to two NIST national user facilities, the NIST Center for Neutron Research and the Center for Nanoscale Science and Technology.

The research will be conducted under a Cooperative Research and Development Agreement (CRADA), the principal mechanism used by federal laboratories to engage in collaborative efforts with nonfederal partners to achieve the goals of technology transfer. CRADAs allow the exchange of resources with private industry to advance technologies that can then be commercialized for the benefit of the public and the U.S. economy. Both parties plan to publish the results of their research under the CRADA.

MedImmune is the global biologics research and development arm of AstraZeneca, a global, innovation-driven biopharmaceutical business that focuses on the discovery, development and commercialization of small molecule and biologic prescription medicines. MedImmune is pioneering innovative research and exploring novel pathways across key therapeutic areas, including respiratory, inflammation and autoimmunity; cardiovascular and metabolic disease; oncology; neuroscience; and infection and vaccines. The MedImmune headquarters is located in Gaithersburg, Md., one of AstraZeneca's three global R&D centers. For more information, visit www.medimmune.com.

- Michael Newman, NIST

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Intercomparison of Particle Size and Count for the Biopharmaceutical Industry

Protein particles are composed of aggregated proteins and can vary in size from 10 nm to 100 µm. Such particles can form at interfaces or when the protein solution is subjected to thermal or chemical stress. Because protein particles may have greater immunogenicity than the component un-aggregated protein, industry and regulatory bodies seek methods and standards to accurately count and size these particles. Unfortunately, different counting methods give results that may differ by as much as a factor of ten or more. To understand the bias and repeatability of techniques in common use in the biopharmaceutical community, MML's Biomolecular Measurements Division has conducted an interlaboratory comparison for sizing and counting subvisible particles from 1 µm to 25 µm. Twenty-three laboratories from industry, government, and academic institutions participated. The circulated samples consisted of a polydisperse suspension of abraded ethylene tetrafluoroethylene (ETFE) particles, which closely mimic the optical contrast and morphology of protein particles. The results of the comparison provide a baseline data set on the repeatability of particle measurements within a single laboratory, the reproducibility of measurements among a set of laboratories, and expected differences between measurements with different instrument types. The comparison results will also guide the development of a NIST Standard Reference Material composed of a suspension of ETFE particles. This work was recently published* in a special February 2015 issue of the Journal of Pharmaceutical Sciences, titled "Two Decades of Publishing Excellence in Pharmaceutical Biotechnology."

* Ripple, D. C., Montgomery, C. B. and Hu, Z. (2015), An Interlaboratory Comparison of Sizing and Counting of Subvisible Particles Mimicking Protein Aggregates. J. Pharm. Sci., 104: 666–677. doi: 10.1002/jps.24287

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Ensuring Quality: NIST MML Suggests New Purity Test for Biotech Products

Note: A version of this story previously appeared in NIST's TechBeat *on December 2, 2014*

To avoid contaminating their experiments, biomedical researchers want to know that the scientific products they buy are pure. But how pure does something need to be to really be pure? Using a new test, scientists at the Institute for Bioscience and Biotechnology Research (IBBR) have found traces of plant enzymes in batches of supposedly pure, commercially available human blood protein genetically manufactured from plant seeds.* Because they are active agents that promote biochemical reactions, enzyme contamination at even low levels could have an outsized effect on measurement reproducibility, and quality control in biomanufacturing.

The IBBR is a joint institute of the University of Maryland and NIST.

The enzyme concentrations found in the study are so low that standard quality control tests cannot detect them. But even very small amounts of the catalytic proteins could cause spurious results that render experiments confusing or meaningless. And in a clinical context, if the enzymes end up in blood products given to patients, they might cause toxic or immune reactions.

The contamination discovery came about by chance, while IBBR chemists Robert Brinson and John Marino and their colleagues were studying how different molecules bind to a protein called human serum albumin (HSA). HSA makes up around three to five percent of our blood and is an important carrier for natural substances as well as drugs in the bloodstream. HSA also has proven useful as a component of media for growing cell cultures, which are used to manufacture proteins for many biotechnology and clinical purposes. Thanks to recombinant DNA technology, HSA no longer has to come from humans. To mass-produce the protein at low cost, scientists have engineered the gene that codes for it into rice seed. Rice-derived HSA is now available from a number of vendors.

In their study, the team used a technique called nuclear magnetic resonance, or NMR, to look for particular types of chemical bonds between commercially available HSA and other molecules. But the scientists saw something unexpected in their NMR results. Bonds between phosphate groups and carbon atoms were breaking in one of the tested molecules, adenosine triphosphate, or ATP. Few enzymes can break such bonds, and because the researchers were studying a plant-derived product, they suspected one culprit in particular: a group of plant enzymes called phytases.

Apparently, the phytases had eluded manufacturers' quality control tests. These tests, which can include the commonly used enzyme-linked immunosorbent assay (ELISA) as well as a combination of liquid chromatography and mass spectrometry, are targeted to specific contaminants. That is, you have to know what you're looking for to find it. And even though these tests can detect impurities down to around 1 part per billion, enzymes can "have an outsized effect" even at very low concentrations, Marino says, because, like chemical catalysts, they accelerate biochemical reactions without being consumed in the process.

Because their background effect might raise or lower concentrations of key biomolecules, phytase contamination could cause researchers to misinterpret experimental results based on cell cultures grown in HSA-based medium, Brinson and Marino say. And while they did not test any drug products, they note that HSA-based therapies often come in relatively large doses, making even slight contamination a potentially serious health risk.

After their initial discovery, the team tested seven HSA products from four different vendors and found varying levels of phytase activity in each batch. They also tested human serum-derived and yeast-derived HSA products but did not detect carbon-phosphate bonds breaking, providing further evidence for contamination by plant enzymes in the other products.

Brinson and Marino say that NMR screening could provide an important quality control test for the increasing number of plant-produced biotechnology products. Human proteins, vaccines and other biological substances are now produced in corn, tobacco and alfalfa, as well as rice. In all cases, manufacturers need to ensure that enzymes like phytases do not end up in the finished product, and NMR could be useful for this process, Marino says. "It's a very useful assay specifically tailored to plant production of these proteins."

But the researchers emphasize that their method is an addition to, not a replacement for, traditional screening methods. "What we're trying to propose is that our NMR methods will directly complement what's already being used," Brinson says.

- Michael Newman, NIST

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* R.G. Brinson, G.G. Giulian, Z. Kelman, J.P. Marino. Detection of contaminating enzymatic activity in plant-derived recombinant biotechnology products. Analytical Chemistry. Published online Nov. 13, 2014. DOI: 10.1021/ac503864m.

Moving Biomaterials for Oral Health into the 21st Century

The lifetime of polymeric dental composites for restoring tooth decays is limited by failures at the tooth-composite interface and subsequent decay from bacterial biofilm ingrowth. Efforts to develop the next generation of dental materials are hampered by the lack of validated methods and tools that provide quantitative information about critical composite properties and performance under complex biological environments.

MML's Biosystems and Biomaterials Division is actively addressing these needs with an interdisciplinary team focused on developing cutting edge measurements for photopolymerization properties and biofilm-material interactions. The team has recently developed an instrument that can provide simultaneous measurement of polymerization shrinkage stress evolution, polymer conversion kinetics, and exotherm development in real-time. These key polymerization properties affect clinical performance of dental restorations. This robust measurement tool along with theory and simulation are being used to explain apparent contradictory results well known to the research community. Measurement of biofilm viability is critical to evaluating antimicrobial materials. Traditional viability methods such as viable plate counting can be applied to biofilms with reasonable repeatability, but accuracy is low due to bacterial aggregation. The team concluded that measuring bacterial aggregation, an overlooked parameter, via particle sizing and counting can help to determine uncertainties and biases and improve accuracy. Taken together, measurements for polymerization properties and biofilm-material interactions increase assurance in assessment of dental materials.

These efforts have garnered significant interest in industry and academia. MML researchers Martin Chiang and Nancy Lin were recently invited to share their work with 3M ESPE and Dentsply Caulk, leading developers and manufacturers of professional dental products. Ongoing discussions are focused on technology transfer of NIST developed instruments and measurement solutions, including licensing of NIST tensometer (patent: US20120085178 A1). Chiang, Lin and NIST's Sheng Lin-Gibson also introduced NIST methods to six premier dental materials research groups in the country. These groups received funding through a large NIH/NIDCR collaborative U01 grant to accelerate development of novel dental chemistries by enabling data sharing via uniform test methods. Further, photopolymerization and antibiofilm technologies are of great commercial interest; NIST efforts are applicable beyond dental materials to many other areas including coatings, biofouling, and medical device infections.

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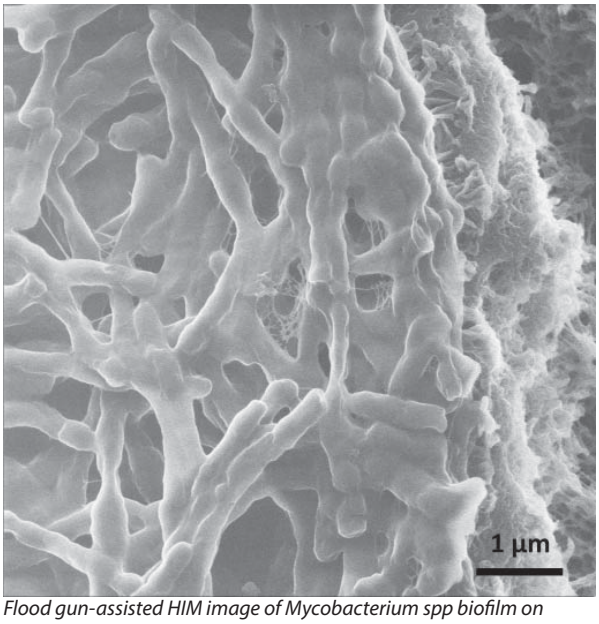
Mycobacterium Biofilms Get Their Close-up: He-ion Microscopy Fills the Gap in High-resolution Imaging of Insulating Materials

Equipped with an electron flood gun, and able to image insulating materials down to the nanometer, the He-ion microscope (HIM) at the Boulder Laboratories sits perfectly positioned to serve the needs of MML's Applied Chemicals and Materials Division (ACMD). In the fields of fuels and biocorrosion, water treatment, cell growth and catalysis, the need arises for microscopy of insulating samples. In the past, the standard method for micro- and nano-scale imaging was to coat such samples with a metal thin film for scanning electron microscopy (SEM). The HIM eliminates the need for this coating by providing unparalleled charge mitigation via a highly tunable electron flood gun. Imaging without a coating allows for the characterization of surfaces without pollution from the texture of the thin film itself. By optimizing the ion and electron scattering at the sample surface, the performance of the HIM may be preserved.

NIST researchers have already exploited flood gun imaging in their collaboration with the National Renewable Energy Laboratory (NREL) to study the effects of genetic mutation and surface treatment on the digestibility of Arabidopsis thaliana (Green Chemistry 16(5), 2014). HIM imaging of freeze-dried biomass samples showed clear differences in nanofibril exposure that correlated with data from contract resonance force microscopy, transmission electron microscopy (TEM) and enzymatic digestibility assays.

In studies on the incorporation of gold nanoparticles into rat neural progenitor cells, the HIM was able to image cross sections of cell bodies to count individual 30 nm particles (see accompanying article). While this technique does not provide the same information as a TEM tomograph, excluding specifics about the nanoparticle location within organelles, the lack of time-consuming sample preparation and capability for whole-cell imaging fills a gap not met by other techniques. The HIM is easily able to answer questions about the distribution and incorporation of particles into the cell body without staining or embedding in epoxy resin.

Most recently, flood gun-assisted HIM of polymeric reverse osmosis membranes (from a wastewater reuse pilot plant) provided a unique view of the biofilms inhabiting the membrane surface. During reverse osmosis, microbes colonize the surface of polyamide membranes leading to fouling and decreased performance. Ozone pretreatment of the feed water selects for a dominant microorganism, while the population of the untreated membrane was more diverse. Flood gun imaging of the untreated filters showed a diversity of bacterium shapes and sizes, while images of the ozone-treated sample showed a biofilm made up of a single cell morphology. Next generation DNA sequencing corroborates these observations, and identifies the dominant organism in the ozonated biofilms as Mycobacterium spp. Also observed were thin polymer "webs" between bacteria, with nanometer-wide strands. In a traditional FESEM coating process, these features would not have been observable.



Credit: Alexandra Curtin, NIST

Flood gun-assisted HIM image of Mycobacterium spp biofilm on ozonated polymeric reverse osmosis membrane used in waste water treatment.

As MML moves into new frontiers in energy, biology and nanotechnology, the HIM and its flood gun sit ready and waiting to characterize the diversity of materials knocking on the laboratory door. The simplicity of preparation combined with the power of imaging bare surfaces with the world's most surface-sensitive ion microscope opens the door to nanoscale imaging of insulating samples in a single afternoon.

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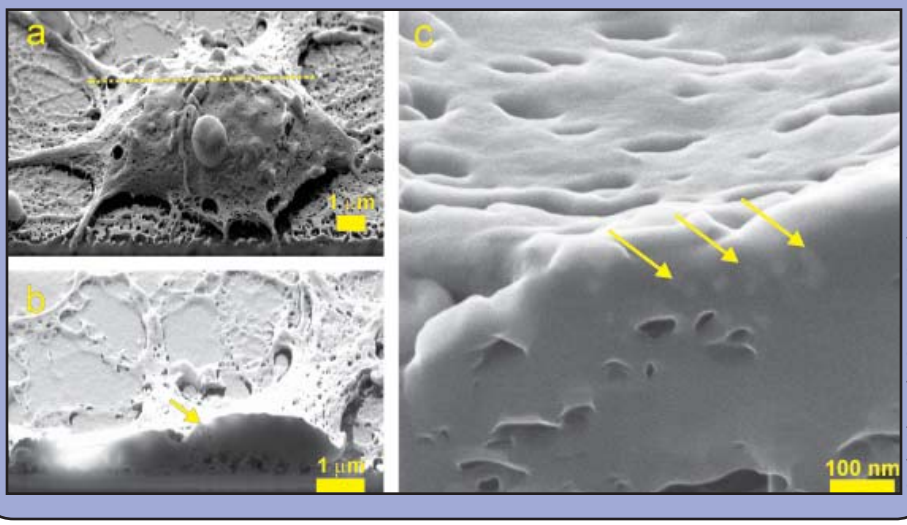
Research in Images: Cutaway View of Nanoparticles in a Neural Cell

Note: A version of this story previously appeared in NIST's TechBeat *on November 26, 2014*

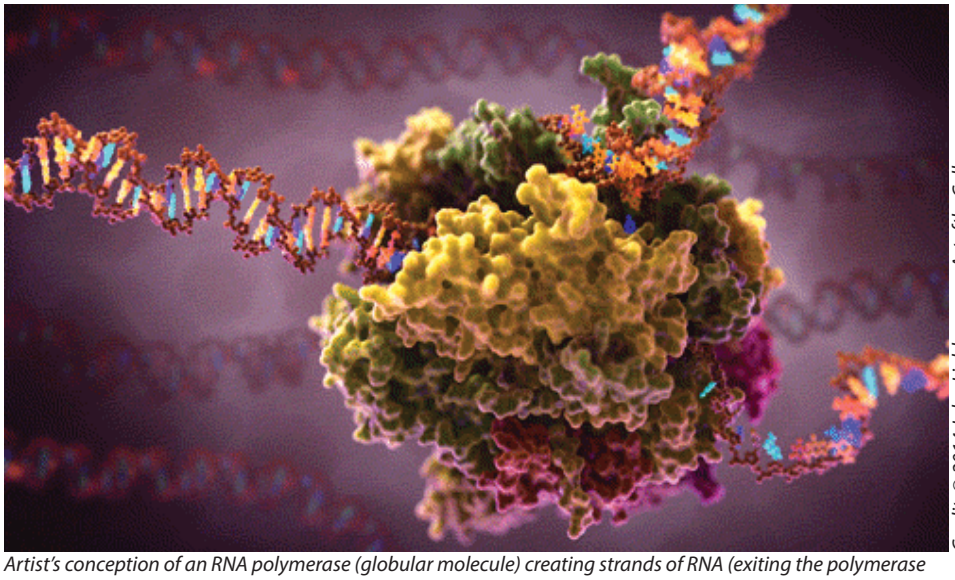
NIST's Precision Imaging Facility (PIF) in Boulder, Colo., provides a variety of advanced tools for precisely measuring the structure and chemical composition of materials at sub-nanometer scales. These images were prepared for a recent NIST study* that found that gold nanoparticles could be used as controls for in vitro neurotoxicology studies because they don't disrupt the cytoskeleton of developing neurons.

Two PIF imaging tools enabled researchers to prove that the 30-nanometer gold particles were actually inside neural progenitor cells—a new capability. The micrograph at (a) shows the cell after exposure to the nanoparticles. A focused ion beam was used to mill away portions of the cell to reveal the interior. Researchers can mill an entire cell to reveal multiple cross-sections obtaining information about nanoparticle uptake. Image (b) shows a cutaway view of the cell corresponding to the dotted line in (a). The arrow points to a cluster of nanoparticles, which were then imaged with high resolution by a helium ion microscope (c), revealing nanoparticles (light-colored dots) near the cell membrane.

* K. M. Jeerage, T. L. Oreskovic, A. E. Curtin, A. W. Sanders, R. K. Schwindt, A. C. Chiaramonti Debay, "Citrate-stabilized gold nanoparticles do not impact neural progenitor cell development" Toxicology in Vitro, Vol. 29, pp. 187-194, (05-Jan-2015) (PubID: 914824)



Credit: Alexandra Curtin, Aric Sanders, Kavita Jeerage, NIST



Credit: © 2014 John Liebler, www.ArtoftheCell.com

Artist's conception of an RNA polymerase (globular molecule) creating strands of RNA (exiting the polymerase on the right) from a DNA template (entering the polymerase on the left). Known as RNA transcription, the process is the first step in gene expression, the carrying out of biological functions as directed by DNA. Researchers studying gene expression can use a new NIST software tool to evaluate the performance of their experimental methods and be confident that the results are valid.

Reliable RNA Analysis Now Easier with NIST 'Dashboard' Tool

Note: A version of this story previously appeared in NIST's TechBeat *on December 2, 2014*

A new, innovative "dashboard" from NIST won't help you drive your car, but it will help enable reproducible research in biology.

In a recent paper in the journal Nature Communications,* an international multi-laboratory team demonstrates a new software tool, the "erccdashboard," to evaluate the performance of experimental methods used to study gene expression. The analysis tool is designed for use with RNA spike-in controls developed by the NIST-hosted External RNA Controls Consortium (ERCC**). These ERCC controls are produced from the DNA Sequence Library for External RNA Controls (Standard Reference Material 2374) that was issued by the agency in 2013.

"In gene expression experiments, scientists try to understand how a cell's biological activities arise from the genetic information contained in its genome by simultaneously quantifying the thousands of RNA molecules expressed by that genome," says Sarah Munro, lead author on the Nature Communications paper.

Munro says that the validation provided by the erccdashboard is essential to ensure that these complex experiments are reproducible. "The results of gene expression experiments are often used in making medical decisions such as identifying which drug is best for a particular patient," she explains. "Our new software tool gives researchers the ability to gauge the performance of their methods for any experiment, evaluate repeatability and reproducibility of experiments over time and between laboratories, and provide confidence that the results can be trusted."

The erccdashboard provides the first standardized approach for any lab to evaluate the quality of its gene expression analyses. The ERCC spike-in control materials are derived from NIST SRM 2374, which consists of 96 different DNA molecules, each with a specific certified gene sequence. The distinct RNA molecules produced from this DNA can be mixed together in a "cocktail" of defined proportions and then used to "spike" biological samples of RNA molecules. The RNA "spike-in" molecules act as controls to check the technical performance of the experiment. To avoid interfering with the measurements made of the sample RNA molecules, the ERCC control RNA sequences are designed to be different from the RNA sequences found in the types of mammalian cells that many researchers study, such as human or mouse cells.

Previously, Munro says, there was no standard, technology-independent approach for analyzing the data obtained from gene expression experiments. "The ERCC control materials made the development of our new method validation tool, the erccdashboard, possible," she explains.

The new NIST software, Munro says, provides a simple 'turnkey' mechanism for biologists to assess any gene expression experiment. "Its performance metrics are designed to be independent of the type of measurement technology used for an experiment, so results can be compared as technologies improve over time," she says. "Using the dashboard will enable reproducible research and prevent researchers from drawing erroneous conclusions from low-quality experimental data."

Munro says that the next goal for the NIST team that developed the dashboard is to apply the software to analyze a new suite of RNA control molecules that the ERCC is currently developing. Eventually, the team plans to extend the tool for use with protein measurements.

Free of charge to users, the erccdashboard software is an open source code written in the R statistical language. It can be downloaded from the Bioconductor repository (http://bioconductor.org/packages/release/bioc/html/erccdashboard.html) and is easily incorporated into other analysis software packages.

- Michael Newman, NIST
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* S.A. Munro, S.P. Lund, P.S. Pine, H. Binder, D-A. Clevert, A. Conesa, J. Dopazo, M. Fasold, S. Hochreiter, H. Hong, N. Jafari, D.P. Kreil, P.P. Labaj, S. Li, Y. Liao, S.M. Lin, J. Meehan, C.E. Mason, J. Santoyo-Lopez, R.A. Setterquist, L. Shi, W. Shi, G.K. Smyth, N. Stralis-Pavese, Z. Su, W. Tong, C. Wang, J. Wang, J. Xu, Z. Ye, Y. Yang, Y. Yu and M. Salit. Assessing Technical Performance in Differential Gene Expression Experiments with External Spike-in RNA Control Ratio Mixtures. Nature Communications 5, 2014, DOI: 10.1038/ncomms6125.

**ERCC, which stands for the External RNA Controls Consortium, is an industry-initiated, NIST-hosted consortium with members from more than 90 international pharmaceutical, diagnostic, biotechnology, academic, clinical and government organizations. The ERCC is charged with developing commonly agreed-upon and tested RNA controls for use in gene expression assays.

Workshop to Identify Standards Needed to Support Pathogen Identification via Next-Generation Sequencing

Recent and continuing advancements in DNA sequencing technologies have resulted in the movement of this technology from the basic research setting to the applied setting. Most notable are the areas of molecular epidemiology, culture-independent diagnostics, clinical diagnostics, and microbial forensics. On October 20-21, 2014, NIST hosted a two-day workshop to aid in identifying priority areas for standards development for next-generation sequencing (NGS), composed of stakeholders representing Federal agencies, academia, and industry, all of whom are utilizing NGS for pathogen identification and characterization. The adoption of NGS presents a number of challenges, including evaluating and certifying proper training and proficiency for end-users, establishing appropriate data storage/analysis/interpretation procedures, and implementing quality control measures throughout the entire process. The development and use of a measurement infrastructure, including documentary standards, reference materials, and reference data, will help to address these challenges, increase confidence in NGS results, and improve decision making related to pathogen detection, identification, attribution, and diagnoses. The workshop will result in a report outlining current efforts in the stakeholder community and Federal government regarding the use of NGS for pathogen identification and characterization in areas of molecular epidemiology, culture-independent diagnostics, clinical diagnostics, and microbial forensics.

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NIST at the Hollings Marine Laboratory

Objective

The unifying goal of the Hollings Marine Laboratory (HML) is, *from discovery to development and application of innovative technologies, we assess environmental impacts on marine ecosystems and potential linkages to human health through collaborative research efforts by our multi-institutional, interdisciplinary science partners.* Within this goal, NIST staff at the HML work to: (1) improve the quality of marine chemical environmental measurements through analytical methods development and application and quality assurance activities, (2) provide environmental specimen banking infrastructure, and (3) promote collaborative interdisciplinary research.

National Oceanic and Atmospheric Administration (NOAA)
National Ocean Service
National Centers for Coastal Ocean Science

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South Carolina Department of Natural Resources (SCDNR)
Marine Resources Division
Marine Resources Research Institute

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College of Charleston (CofC)
Grice Marine Laboratory

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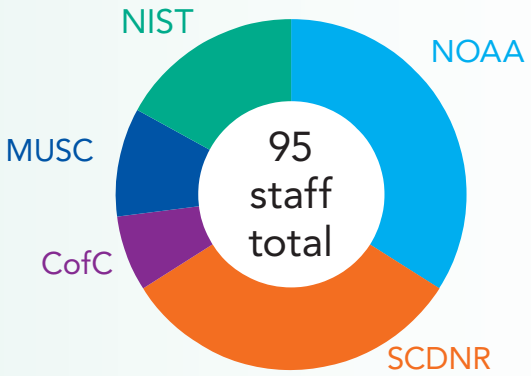
Medical University of South Carolina (MUSC)
Marine Biomedicine and Environmental Sciences



Sectors and Partnership

NIST researchers develop the advanced analytical measurement tools needed to reliably address marine environmental issues. Research themes at the HML that benefit from strong interactions among the HML partners include:

- pollution
- hazardous algal blooms
- marine animal health & human health
- sentinel species & sentinel habitats
- coastal development
- aquaculture

Capabilities

The HML campus is a unique cooperation in which land owned by South Carolina was used for the construction of a NOAA owned facility (117,000 sq.ft.) which hosts researchers from five academic, state and federal entities, all contributing research dedicated to marine science. NIST facilites at the site include:

- NIST Organic Analytical Laboratory (2,260 ft^2)
- NIST Inorganic Analytical Laboratory (1,670 ft^2)
- NIST Nuclear Magnetic Resonance (NMR) Facility (5,600 ft^2) in partnership with MUSC and NOAA
- NIST Biological & Environmental Specimen Bank (2,600 ft^2)
- NIST Reference Material Production Facility (904 ft^2)

NIST Research and Services at the HML:

Marine Environmental Specimen Bank

The NIST Environmental Specimen Bank (ESB) provides long-term cryogenic banking of biological and environmental specimens for future research, including retrospective measurements for newly recognized contaminants to determine time trends, verify previous chemical measurements (QA/QC), and provide for re-analysis of samples using new analytical techniques. The ESB also provides its services for other agencies and acts as the Chain-of-Custody Repository on the Deepwater Horizon Oil Spill for all marine mammal samples collected and used in natural resource damage assessment.

Reference Material Production

Facilities in HML are used to prepare the base material for developing fresh-frozen and freeze-dried analytical reference materials. Recent materials produced include: mussel tissue for Organics in Mussel Tissue (SRM 1974c) and Organics in Freeze-Dried Mussel Tissue (SRM 2974a).

Figure 1. Cryogenic preparation of mussel tissue for production of SRM 1974c.

NMR-based Metabolomics

NIST Scientists at the HML facility are developing advanced Nuclear Magnetic Resonance (NMR)-based metabolomics methods for applications in environmental research, animal health monitoring, and aquaculture. Recent work included using NMR: to understand how metabolic changes due to small temperature differences can turn non-virulent *Vibrio coralliilyticus* into a coral bleaching menace, to identify and quantify metabolites that could be used as biomarkers in identifying the specific sources of environmental stresses for blue crabs, and to use metabolomic analyses in better understanding the effects of experimental diets on aquaculture fish for developing economic, high-quality production procedures.

Measurement Methods and Reference Materials for Emerging Contaminants

Regulatory and health agencies within the US, Canada, and countries within Europe are continuously identifying compounds of suspected carcinogenic or toxicological risk. These compounds are often described as emerging contaminants. Once health risks are identified, accurate analytical methods must be developed and verified for quantifying these emerging contaminants in relevant samples. Recent work by NIST at HML has included measuremnts of phthalates and plasticizers. Also, work has begun in partnership with MUSC to identify and characterize new endocrine disrupting contaminants displaying receptor agonistic or antagonistic activity.

Measurements for Evaluating Exposure to Anthropogenic Toxicants

At the HML facility, NIST develops standard sample collection protocols, banks tissue and fluid samples for retrospective measurements, develops non-invasive sampling techniques to assess exposure, develops new methods to assess pollutants in wildlife blood and fluid samples, and develops analytical methods to measure "new" pollutants. Such work is being conducted for several species of marine mammals and sea turtles in partnership with NOAA.

Figure 2. Dolphin sampling to assess contaminant exposure.

Element Speciation and Metallomics

NIST staff are developing versatile measurement tools allowing for trace detection, identification, and quantification of metal species, including those associated with proteins. Speciation tools contribute to the emerging science of "metallomics," involving the detection, mapping, and/or quantification of trace elements in biological systems at organ, cell, and sub-cell levels.

Stable Isotopes of Elements as Environmental Tracers

NIST scientists at the HML facility are advancing the application of stable elemental isotopes for use in environmental research and monitoring. NIST is developing the use of mercury isotopes for identifying mercury source patterns and in understanding biogeochemical cycling through improving our understanding of fractionation of mercury in the environment. NIST is also evaluating methods for accurately reconstructing historical ocean pH using boron isotopes in coral skeletons. Such methodology would have applications to climate change and ocean acidification studies.

Figure 3. Banked seabird eggs were used to study environmental fractionation of mercury in the Arctic.

Learn More

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Making Data Stick: A New Database of Adsorption Properties from NIST MML

Adsorbent materials have long-standing application in areas such as gas purification and storage, catalysis, and environmental remediation and range from simple activated carbon to complex crystalline materials such as zeolites and metal-organic frameworks. Despite the long history of adsorption science (documented applications date to 1550 B.C. and rigorous study began in the late 1800s), ever-evolving measurement challenges exist to understand the chemical and physical behavior of the many newly emerging materials that are being used in adsorption applications. To address such challenges, NIST MML and the Advanced Research Projects Agency - Energy (ARPA-E) of the Department of Energy recently established the Facility for Adsorbent Characterization and Testing (FACT) (www.nist.gov/mml/fact), a state-of-the-art laboratory for characterizing adsorbent materials and accurately measuring gas adsorption properties of these industrially-relevant materials.

A crucial component of the FACT lab involves the dissemination of measured adsorption data and how it compares with available measurements in the literature. The FACT project thus funded the development of a new Standard Reference Data product, the NIST/ARPA-E Database of Novel and Emerging Adsorbent Materials (NIST SRD-205). This database is a catalog of adsorbent materials and measured adsorption properties of numerous materials obtained from article entries from the scientific literature and is freely accessible through a web search application at http://adsorbents.nist.gov. Search fields for the database include adsorbent material, adsorbate gas, experimental conditions (pressure, temperature), and bibliographic information (author, title, journal), and results from queries are provided as a list of articles matching the search parameters. Most important is the direct access to the adsorption isotherm data, for which multiple datasets can either be compared visually online in the web application or exported for offline analysis. Additionally, newly measured adsorption properties obtained from FACT experimental reports will also be included in the database after the laboratory is fully operating. Tools for online analysis of the isotherm data are currently in development and will be included in any future release of the database.

Throughout the project, many individuals have assisted in development of the database, including an advisory committee of academic and industrial experts on adsorption science and a diverse group of students and guest researchers that have done the hard work of compiling the data in the database.

The new SRD product was formally released at the "Measurement Needs in the Adsorption Sciences" workshop hosted by NIST in November 2014, which also gave NIST staff an opportunity for first-hand feedback. Attendees were most enthusiastic about its extensive catalog of adsorption isotherms, which can be used for rapid interlaboratory comparison studies via meta-analysis of the database contents. Overall, the new database has been well received by the adsorption science community, which is eager for data resources for more easily comparing the results of adsorption experiments worldwide and will be used to more efficiently disseminate reference adsorption data and speed the development and analysis of novel materials for adsorption applications.

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NIST MML Researchers Launch Updated NIST Mass and Fragment Calculator Software

Eric Kilpatrick, a researcher from MML's Bioanalytical Science Group, has developed two software programs to aid mass spectrometry researchers.

An updated version of the NIST Mass and Fragment Calculator web tool was recently launched. The resolution of mass spectrometers has improved tremendously in recent years enabling proteomic researchers to screen for important clinical biomarkers, prepare accurate and precise Standard Reference Materials and to assimilate this method in routine clinical analysis. As this capability has increased, the predicted mass of proteins must be calculated with greater precision and allowing for the mass changes due to expected modifications including glycosylations, disulfide bonds, processing derivitizations and stable isotope labeling. Software to predict these masses must be flexible, dependable and have transparency in the calculations. Multiple programs currently exist both as stand-alone or web-based utilities however the results have been shown to differ between them. Additionally, the web-based programs may be removed from service or changed in an unknown manner affecting the ability to replicate the calculation in the future. The NIST Mass Calculator was developed to provide a dependable program with a fully described algorithm encompassing a variety of common post translational or processing modifications allowing the user to independently verify the results. The program allows flexibility to the user to select results using average or monoisotopic elemental values reported in a NIST publicly available database (http://physics.nist.gov/cgi-bin/Compositions/stand_alone.pl). Modifications may be selected including two methods for isotopic labeling, glycosylations, cysteine alkylations, two common N-terminal modifications and disulfide bonds, all based on the user input amino acid sequence of the protein or peptide. A unique feature of the program is to allow the user to assign a specific mass to a particular amino acid residue which is useful in manual interpretation of some mass spectrometry spectra. Predictions can also be requested for "fragmentation ions" which are produced in tandem mass spectrometry. The program is written in Windows Visual Basic and available as a free download from the NIST website (http://www.nist.gov/mml/bmd/bioanalytical/massfragcalc.cfm).

Kilpatrick has also developed a second software program, the NIST Isotope Enrichment Calculator. The use of isotope dilution mass spectrometry is widely used to assign concentration levels for Standard Reference Materials for small molecules of common clinical analyses including cholesterol, glucose, creatinine, etc. This technique is dependent on having a version of the molecule synthesized using heavy stable isotope elements for which the extent of isotopic incorporation is reported. Application of this technique to proteins or peptides also requires a heavy version of the analyte but due to the larger size, and subsequently complex isotopic profile, judging the degree of incorporation is a greater computational challenge. The NIST Isotope Enrichment Calculator was developed to estimate the degree of isotopic incorporation of the heavy nitrogen isotope, nitrogen-15, into a peptide. A series of theoretical computations of varying nitrogen-15 percentage are generated using accumulated multinomial probabilities and then compared against the observed masses and intensities from an experiment. The comparison which maximizes the Pearson correlation coefficient is reported as the incorporation percentage.

The program is written in Windows Visual Basic and available as a free download from the NIST website (http://www.nist.gov/mml/bmd/bioanalytical/isoenrichcalc.cfm).

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Strengthening Thin-Film Bonds with Ultrafast Data Collection

Note: A version of this story previously appeared in NIST's TechBeat *on October 17, 2014*

When studying extremely fast reactions in ultrathin materials, two measurements are better than one. A new research tool invented by researchers at Lawrence Livermore National Laboratory (LLNL), Johns Hopkins University and NIST captures information about both temperature and crystal structure during extremely fast reactions in thin-film materials.*

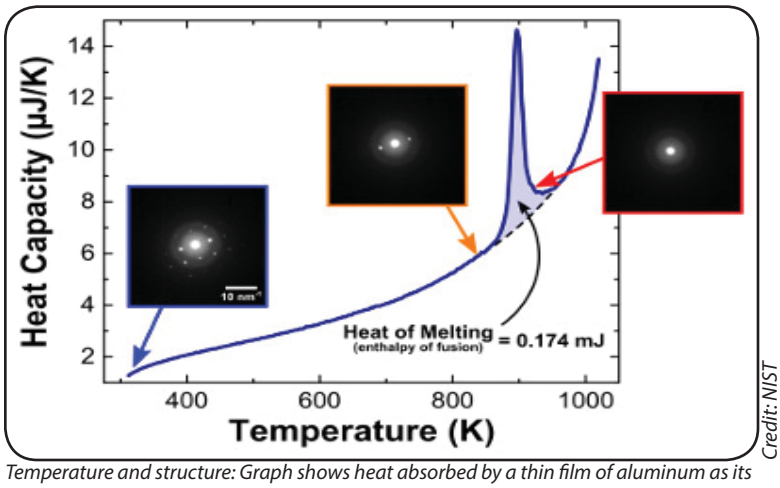
The combined device will help scientists study new materials and processes used to make advanced technologies, including state-of-the-art semiconductors and flat-screen display devices, says David LaVan, a NIST MML materials scientist who co-led the study.

Modern electronics manufacturing often pushes the limits of current measurement technology. Making a flat-screen display requires bonding a large sheet of a pure, rare material to an underlying metal substrate with as few defects as possible. To do so, manufacturers typically sandwich a thin film between the two materials and heat it rapidly to high temperatures, causing it to react and bond the metals.

This method usually works, but industry researchers would like to optimize the process. And existing tools to describe what's happening in the reactive thin film provide only incomplete information. One such technique, nanocalorimetry, can track very precisely large temperature changes—at rates up to ,1000 degrees Celsius per millisecond—that occur at a very small scale. Such a measurement can alert researchers to a material's phase transitions, for example, when a metal melts. But nanocalorimetry tells researchers little about the actual chemical processes or microstructural changes they are measuring as a material heats up or cools down.

To study these changes, LaVan's LLNL collaborators Geoffrey Campbell, Thomas LaGrange and Bryan Reed developed a different device, the dynamic transmission electron microscope (DTEM). In traditional transmission electron microscopy, diffraction and transmission patterns made by electrons passing through a thin sample provide information about how the sample's atoms are arranged. But TEM typically requires that the sample maintain one crystal structure for an extended period, as the microscope's detector captures enough electrons to generate an image.

DTEM, by contrast, captures structural information very rapidly. It relies on a pulsed laser to send short, bright blasts of electrons through a sample. LaVan and his colleagues at NIST and Johns Hopkins realized that if the LLNL group's DTEM laser pulses were synched with a rapid temperature rise, the researchers could simultaneously track phase transitions and structural changes in materials they were studying. "It's like peanut butter and chocolate," LaVan says. "If we can somehow get these two instruments working simultaneously, we'll have the whole story."



Credit: NIST

Temperature and structure: Graph shows heat absorbed by a thin film of aluminum as its temperature increased. Inset boxes show electron diffraction patterns captured by DTEM as temperature changes. The patterns reveal the crystal structure and orientation of the aluminum. At low temperatures, pattern is characteristic of a face-centered-cubic crystal structure. When the sample is heated past the large melting peak, the spots disappear indicating that the aluminum has lost its crystal structure due to melting.

But first the researchers needed to shrink the circuitry for their nanocalorimeter to a tenth of its original size, so that it could fit inside the microscope. The researchers also needed to write new software to synchronize the microscope's electron pulses with the nanocalorimeter's rapid heating pulses. "To get [the devices] to work together was really a substantial effort from three different research groups," LaVan says.

Finally, LaVan and team member Michael Grapes, a research associate at NIST, and graduate student in materials science Timothy Weihs' group at Johns Hopkins, flew the redesigned nanocalorimeter to Livermore, synchronized it with the DTEM, and ran tests on thin films of materials such as aluminum, whose microstructural and thermal properties are well understood. The scientists found that, as expected, the nanocalorimeter recorded phase transitions at the same time the DTEM recorded structural changes, and both sets of measurements were consistent with their study materials' known properties.

The research team is already moving on to study other, less well-understood materials. Recently, the scientists have used their combined nanocalorimeter-DTEM to measure what happens when aluminum and nickel combine to form thin-film alloys. The team's study provides, for the first time, simultaneous structural and thermal data on this reaction at high heating rates, LaVan says.

- Michael Baum, NIST
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*M.D. Grapes, T. LaGrange, L.H. Friedman, B.W. Reed, G.H. Campbell ,T.P. Weihs and D.A. LaVan. Combining nanocalorimetry and dynamic transmission electron microscopy for in situ characterization of materials processes under rapid heating and cooling. Review of Scientific Instruments 85, 084902. Published online Aug. 18, 2014.

NIST MML Scientists Demonstrate the Use of ATMP to Control Particle Size in Synthesis of Iron Nanoparticles

NIST MML scientists have recently shown* the merits of a phosphonate chelator, aminotris (methylene phosphonate) (ATMP) as a stabilizer during iron nanoparticle synthesis in aqueous solution. By varying the molar ratio of ATMP to iron, particle size can be varied from 155 nm ± 66 nm to 3.0 nm ± 0.4 nm, and lower mass concentrations of the ATMP stabilizer are needed due to its strong association with the iron in solution, as compared to typical polymer-type stabilizers. Iron nanoparticles have tremendous potential for use across a variety of diverse fields, including environmental remediation of recalcitrant organic and inorganic chemicals, and magnetic resonance imaging (MRI) with increased bio-imaging sensitivity. The ability to control the size of the particles during synthesis improves the ability to tune their performance in these applications. Water based synthesis techniques of these nanoparticles avoids the environmental disadvantages of using hazardous solvents, but requires the use of a chelator to keep the nanoparticles from agglomerating. Synthesized particles display broad peaks in x-ray diffraction analysis, suggesting a high level of disorder, and possibly nanocrystalline or amorphous character. Future work includes the use of ATMP-stabilized iron nanoparticles as core materials for bimetallic core-shell nanoparticles, as well as experimental studies of how chelator-type stabilizers affect nanoparticle chemical stability and reactivity.

* "ATMP-stabilized iron nanoparticles: Chelator-controlled nanoparticle synthesis," Lauren F. Greenlee and Nikki S. Rentz, in the Journal of Nanoparticle Research (DOI: 10.1007/s11051-014-2712-8), 2014

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NIST MML Study Suggests Light May Be Skewing Lab Tests on Nanoparticles' Health Effects

Credit: © GoodMood,Photo-Gabriele,Maltini-renamarie-Fotolia.comrenamarie - Fotolia

Titanium dioxide nanoparticles are widely used not only in paints but in sunscreen and even salad dressing.

Note: A version of this story previously appeared in NIST's TechBeat *on November 18, 2014*

Truth shines a light into dark places. But sometimes to find that truth in the first place, it's better to stay in the dark. That's what recent NIST MML findings* show about methods for testing the safety of nanoparticles. It turns out that previous tests indicating that some nanoparticles can damage our DNA may have been skewed by inadvertent light exposure in the lab.

Nanoparticles made of titanium dioxide are a common ingredient in paint, and they also are considered safe for use both on the body (in sunscreen, where they help block ultraviolet light) and even within it (in foodstuffs such as salad dressings to make them appear whiter). It is well known that in the presence of light and water, these particles can form dangerous, highly reactive chemicals called free radicals that can damage DNA. Because light does not reach the human body's interior, scientists have long accepted that these nanoparticles would not damage cells by forming free radicals from light activation.

However, some recent studies using cells suggest that titanium dioxide can damage DNA even in darkness—a disturbing possibility. Because such findings could have major health implications, the NIST team set out to determine whether light was indeed required for the nanoparticles to cause DNA damage.

"We didn't set out to test the safety of the particles themselves—that's for someone else to determine," says NIST's Elijah Petersen. "Our main concern is to ensure that scientists have enough knowledge to make accurate measurements. That way, tests will give accurate representations of reality."

The NIST team exposed samples of DNA to titanium dioxide nanoparticles under three different conditions: Some samples were exposed in the presence of visible or ultraviolet light while others were kept carefully and intentionally in complete darkness from the moment of exposure to the time the DNA damage was measured. The team found that only when exposed to laboratory or ultraviolet light did the DNA form base lesions, a form of DNA damage associated with attack by radicals. Their conclusion? The culprit in earlier studies may be ambient light from the laboratory that inadvertently caused DNA damage.

"The results suggest that titanium dioxide nanoparticles do not damage DNA when kept in the dark," Petersen says. "These findings show that experimental conditions, such as lighting, must be carefully controlled before drawing conclusions about nanoparticle effects on DNA."

- Chad Boutin, NIST

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*E.J. Petersen, V. Reipa, S.S. Watson, D.L. Stanley, S.A Rabb and B.C. Nelson. The DNA damaging potential of photoactivated P25 titanium dioxide nanoparticles. Chemical Research in Toxicology, October 2014 issue, DOI: 10.1021/tx500340v.

Nanomaterial Standards: No Small Task

In November 2014, MML researchers Vytas Reipa and John Elliott travelled to New Delhi for the ISO TC 229 Nanotechnologies 17th Plenary Meeting. Reipa and Elliott hold both project leader and expert positions in Working Group 3 (WG3): Health, Safety and Environmental Aspects of Nanotechnologies. WG3 is composed of 40 members from many countries. The meeting was dedicated to furthering several current WG3 projects as well as proposing new ones.

Metal oxide nanoparticles are used in household products such as cosmetics, food, paint, and polymers. However, there is concern over their phototoxicity once they are released to the environment. Reipa and colleagues from KRISS (Korean Research Institute of Standards and Science) are teaming up to develop a method to measure the photocatalytic activity of metal oxides using the biocompatible reagent - NaDH. Previous methods that measure photocatalytic activity of powders utilized toxic dyes and required large quantities of test material. This high throughput, fluorescence based method can be used with biomolecules, cells or small organisms.

Elliott presented the latest updates on two standards that were developed from NIST research in collaboration with the International Alliance for NanoEHS Harmonizations (IANH). The first work item he presented is a standard protocol for evaluating cytotoxicity by measuring the effect of nanoparticles on the viability of a macrophage cell line. The second work item is also a standard protocol for characterizing nanoparticle-induced reactive oxygen species, an indicator of environmental stress, in a macrophage cell line. The collective decision by the international members of the working group was to move both documents to balloting. The positive outcomes of this meeting illustrate NIST leadership in and reputation for high quality measurements in nanotechnology.

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Liquids and Glasses Relax, Too. But Not Like You Thought

Note: A version of this story previously appeared in NIST's TechBeat *on November 18, 2014*

A new insight into the fundamental mechanics of the movement of molecules recently published* by NIST MML researchers offers a surprising view of what happens when you pour a liquid out of a cup. More important, it provides a theoretical foundation for a molecular-level process that must be controlled to ensure the stability of important protein-based drugs at room temperature.

Proteins depend critically on their three-dimensional structure, the shape the long and complex molecules tend to fold into. Modern protein-based drugs—for example, vaccines or antibodies created to fight cancers—generally are not stable at room temperature or in the liquid formulations most convenient for clinical use. To preserve them for use in parts of the world without reliable refrigeration, manufacturers freeze-dry the proteins and coat the complex molecules with glassy sugars to keep their structure intact. "It's like a lollypop," observes NIST biochemist Marcus Cicerone, "but these lollypops are only 10 microns or smaller."

The challenge is to design the sugar coating to get the maximum shelf life for a given pharmaceutical protein, which ideally would be measured in years. The issue revolves around what chemists refer to as "relaxation"—broadly, any molecular motion that leads to transport of the molecule. About 10 years ago, NIST researchers discovered a testing shortcut.** Using neutron radiation, they discovered that measuring tiny molecular movements in the proteins at very short timescales—picoseconds***—could reliably predict the long-term stability of a formulation. The sugars that worked the best were the ones that suppressed the tiny, rapid motions. Exactly why this was so was not particularly clear, but it worked.

This new paper finally explains the underlying principles. The neutron experiments, says Cicerone, measure mean square displacement. "Imagine a jarful of molecules. It's how far the average molecule jiggles around for a given timescale," he says. "In condensed matter like a liquid or glass, we usually think that all the molecules are identical, and on the average they all have the same environment with a little bit of space for them to jiggle, but not very much."

Credit: Baum/NIST

At the picosecond scale, liquids move not so much in a gang rush as a follow-the-leader process akin to the space moving around in a 15 puzzle, according to a group of NIST researchers. The physical model they propose has implications for the design of protein-based drugs that must be stored for long periods at room temperature.

"What we found is that picture is not really right."

In reality, Cicerone says, there are two different environments the molecules can be in. "There is one environment like that—molecules are very well packed and on a picosecond timescale they move maybe one percent of their radius. They're hardly moving at all. But there's another environment where some molecules can move maybe 30 percent of their radius in the same time. They're really making big jumps, and in glasses, those big jumps are essentially the only way that molecules can move around. Everybody else is completely stuck. "It's kind of like a 15 puzzle.**** You can only move one at a time."

What happens is a molecule next to a region that's more loosely packed can move there, and does. Then one that was next to it suddenly has room to move, and does, and so on. On a picosecond and nanometer scale of time and space, when you pour a liquid out of a cup, it doesn't really all come out all at once. It's more follow-the-leader.

On a practical level, says Cicerone, the results explain why the short timescale mean displacement measurements can predict the results of molecular degradation measurements that would normally take months. "It gives a really good solid understanding of why these picosecond and nanosecond timescale measurements correlate with degradation processes in glass for the proteins," he says, "so it gives us confidence that the techniques we build that are based on this idea will be robust and people will be able to use them."

As a bonus, he says, the model also explains a somewhat arcane degradation process in glasses called Johari-Goldstein relaxation. "It's the timescale for the switching between the tightly packed and loosely packed regions. It's the vacancy in the game of 15 moving around," says Cicerone.

- Michael Baum, NIST
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* M.T. Cicerone, Q. Zhong and M. Tyagi. Picosecond dynamic heterogeneity, hopping and Johar-Goldstein relaxation in glass-forming liquids. Physical Review Letters 113,117801-117801 (2014).

** See the 2004 article, "Keeping Drugs Stable Without Refrigeration," at www.nist.gov/mml/msed/drugs_061604.cfm, and the 2008 article, "Candy-Coating Keeps Proteins Sweet," at www.nist.gov/mml/msed/sugar_081908.cfm.

***0.000 000 000 001 second

**** A 15 puzzle is a sliding puzzle that consists of a frame of numbered square tiles in random order with one tile missing.

NIST MML Hosts NIChE Workshop on "Measurement Needs in the Adsorption Sciences"

Adsorbent materials have many applications, including those related to gas storage, gas purification, catalytic reforming, and sustainable development. Despite major progress in adsorption technology and physical adsorption characterization during the past two decades, challenges still exist, notably those associated with accurately measuring sorption properties over a wide range of temperatures and pressures. In early November 2014, a New Industry and Chemical Engineering (NIChE) workshop (sponsored by ARPA-E and Corning Inc.) was held at NIST, through the Council for Chemical Research, on the standards and measurement needs and challenges in the adsorption sciences. The workshop focused on metrology needs in the areas of absorbent characterization, gas storage, and separations. The event brought together experts from government, industry, and academia to identify challenges and opportunities, to suggest mechanisms to address these areas, and to recommend strategies by which NIST's expertise and investments can be targeted and leveraged.

The workshop included a tour of the NIST Facility for Adsorbent Characterization and Testing (FACT), a state-of-the-art laboratory recently commissioned with support from the U.S. Department of Energy's Advanced Research Projects Agency-Energy (ARPA-E) to address the challenges inherent to measuring gas and vapor sorption properties, as means to inform the research and standards activities of the laboratory. FACT houses instruments for characterization of the pore architecture and evaluating fundamental sorption properties of singles gases and gas mixtures. At the breakout sessions, workshop participants discussed, identified, and prioritized research areas of greatest impact to the scientific community, recognized viable growth areas, and stimulated new ideas—information that will accelerate the development of new measurement technologies and methodologies. A workshop report will be released next year.

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NIST Mass Spectral Library to Play Key Role in the Search for Life on Mars

The NIST Mass Spectral Library, touted as the world's most widely used and trusted resource for identifying mass spectra*, may play a key role in extraterrestrial MS analysis as well. Results of the first Mars Organic Molecule Analyzer (MOMA) GC-MS Coupling instrument were presented at the American Astronomical Society Division for Planetary Sciences meeting in fall of 2014. The MOMA will be aboard the ExoMars rover that is planned to launch in 2018 by an international effort led by the European Space Agency. The MOMA GC-MS instrument will be a key analytical tool in providing characterization of organic content, a.k.a. evidence of life on Mars. As stated on the ESA's webpage** "MOMA's two main activities on Mars will be: 1) the detection of organic molecules, even at very low concentrations and 2) the possibility to establish their biotic or abiotic origin by molecular identification in terms of chirality. The GC-MS mode will be used to identify and analyze volatile molecules found in Martian soil samples. The powdered sample material provided by the Rover's drill will be used to fill one of twenty one-time-use, small ovens. At high temperature, all volatile materials will evaporate and will be extracted to be routed to the gas chromatograph, where they will be separated and identified. Some ovens are filled with a chemical product, the derivatization agent, used to transform the chemical compound into another similar compound, in order to make the samples suitable for chiral analysis. This type of analysis is an indicator of the biological versus abiotic origin of organic molecules. The separate volatile molecules will be ionized and then will be analyzed individually with the mass spectrometer." For unambiguous identification of detected species, the MS signals are compared with none other than the NIST Mass Spectral Library.

Credit: NASA/JPL-Caltech/MSSS

Curiosity *Self-Portrait at 'Mojave' Site on Mount Sharp*

* http://phys.org/news/2014-08-latest-nist-mass-spectral-library.html

** http://exploration.esa.int/mars/45103-rover-instruments/?fbodylongid=2132

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New Instrument Promises to Improve Understanding of Ocean Acidification as Recorded in Corals

NIST MML's Environmental Chemical Sciences Group at the Hollings Marine Laboratory in Charleston, South Carolina has recently installed a Nu Plasma II multi-collector inductively coupled mass spectrometer (MC-ICPMS) that will be the centerpiece of a new environmental isotope research program that will include new work on ocean acidification as recorded in corals. This MC-ICPMS instrument equipped with 16 faraday cups and 6 ion counting detectors will offer the ability to perform high-precision isotope ratio measurements of boron in calcium carbonate marine skeletons as a proxy for the local pH conditions of the ocean when and where these organisms grow.

Rising atmospheric CO2 concentrations from fossil fuel emissions are decreasing the pH of the oceans. This lower pH decreases calcification rates for corals and calcareous algae, thereby altering the ecology and calcium carbonate budget on coral reefs. This creates another stressor for coral reef ecosystems that are already undergoing precipitous global declines in vitality. One of the challenges identified by NOAA's Ocean Acidification (OA) Program is that the current network of OA instrumentation does not provide sufficient spatial and temporal coverage to provide robust monitoring and prediction capabilities for seawater pH. A promising proxy for indirectly determining seawater pH is the ratio of boron (B) isotopes found in biogenic calcium carbonates. In seawater, 11B is enriched in boric acid relative to borate, and the proportion of boric acid to borate in the ocean is pH dependent. By measuring the ratio of 11B to 10B (δ11B) in the carbonate skeleton, the pH of the seawater at the time of calcification can be determined. However, many challenges exist for accurately implementing this method: the differences in seawater pH to be detected are small, the matrix of seawater and carbonate skeletons are complex, and the physiological effects of the coral species may introduce confounding effects. Much of the initial research involves improving the metrology of this approach by refining analytical methods and optimizing calibration and traceability of boron and pH measurements. The true test is with the implementation and validation of the method in the field.

Credit: Stephani Gordon

NIST researcher Rusty Day deploying coral fragments on a transplant rack at the low-pH study site at the hydrothermal vent, which was dominated by boulders covered in fleshy macroalgae.

In May, 2014 NIST participated in a research cruise with NOAA's Coral Reef Ecosystem Division (CRED), Pacific Marine Environmental Lab (PMEL), Atlantic Oceanographic & Meteorological Lab (AOML), and SCRIPPS to study ocean acidification at a shallow water hydrothermal vent located at Maug Island, in the Mariana Island chain. This site presents a unique opportunity to perform a field calibration of the boron pH proxy because of the natural gradient from the highly acidified zone of the vent (6.07) where coral growth is sparse, to background pH (8.13) on a nearby vibrant coral reef. NOAA oceanography teams characterized the pH gradient by deploying SeaFET pH data-loggers, and NIST and its partners collected, stained, and transplanted corals and calcified algae concurrently with field instrumentation. The δ11B will be measured by MC-ICPMS, and compared to direct seawater pH measurements. This is a first step in evaluating the efficacy of using the boron pH proxy in carbonate species for large-scale real-time monitoring of ocean acidification through NOAA's coral reef monitoring program, and future retrospective analysis through long-term archival of pH proxy samples in NIST's Marine Environmental Specimen Bank.

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Selected Recent Publications

MML researchers publish over 400 journal articles each year. Here are a few recent examples:

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