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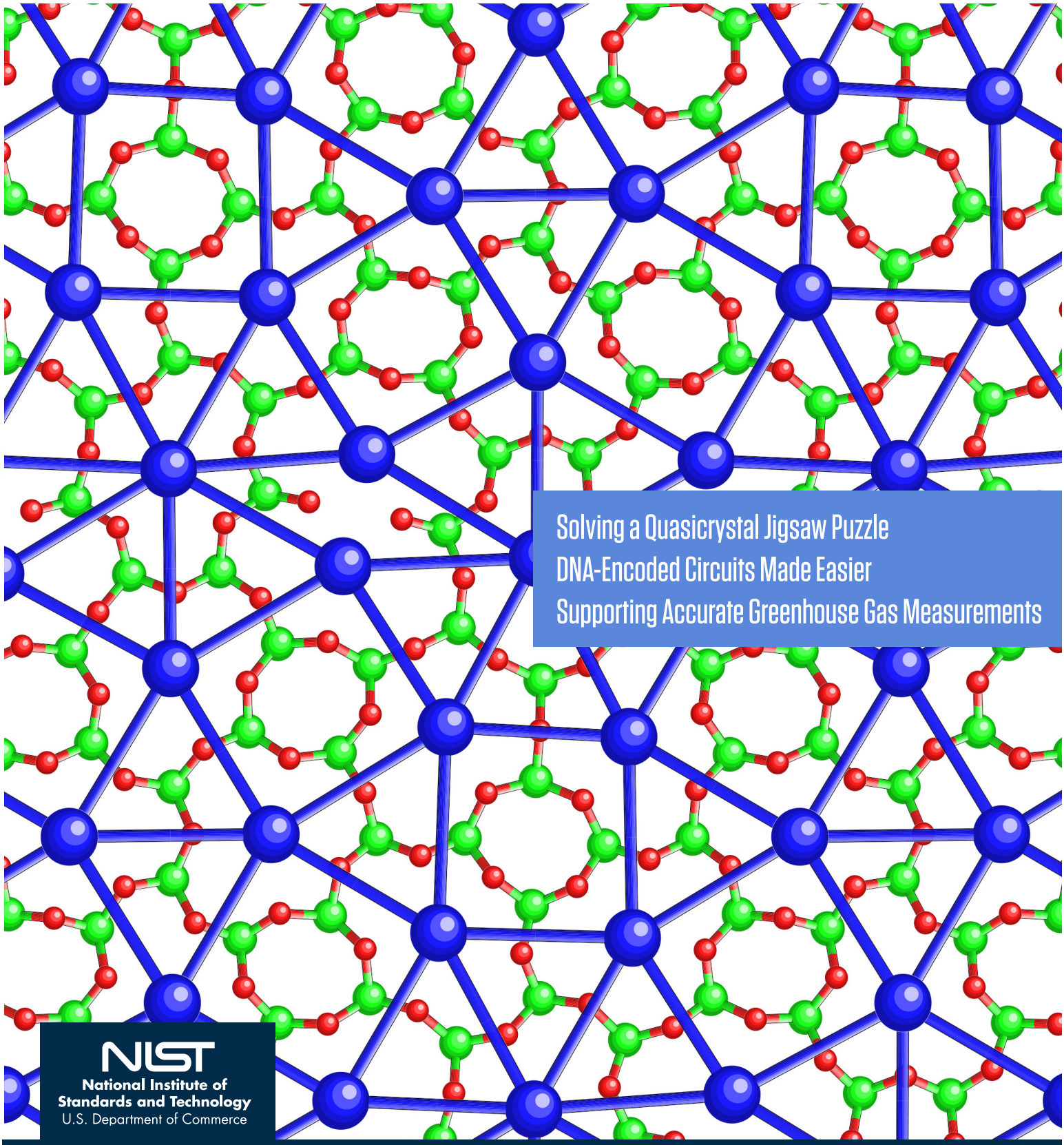
MATERIAL MATTERS

The Quarterly Magazine of NIST's Material Measurement Laboratory



Solving a Quasicrystal Jigsaw Puzzle

DNA-Encoded Circuits Made Easier

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

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A MESSAGE FROM THE MML DIRECTOR

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Historically, it has taken decades or longer to find, fine tune, and prove the utility for a new material through a series of trial-and-error experiments. There are some recent exceptions. General Electric, for example, has made new alloys for jet engines in nine years instead of the usual 15 years. The metals used in Apple watches were developed and deployed to market in just about two years. In both these cases, researchers developed new materials more quickly by working smarter and faster: They used data on the known properties of metals, along with computer modelling, to make informed choices about how to combine or process substances to get the performance they needed.

To accelerate adoption of this new approach, in 2011 President Obama announced the Materials Genome Initiative, which MML is supporting by developing a materials data infrastructure. There is no lack of data on metals, polymers, and ceramics; many research and design programs in the military and government agencies, and in universities and industry, are generating and storing data. Making that wealth of knowledge widely accessible, however, requires protocols to ensure that data can be found and is in a recognizable form, and methods are available for assessing whether the data is of sufficient quality to be useful. While NIST has a long history in making quality data and computational models widely available for the benefit of industry and other researchers, the notion that all the players might contribute to and draw from an ecosystem of data resources is rather new to materials research. Acceptance will require a powerful proof of concept.

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Delivering on that proof of concept is the Center for Hierarchical Materials Design, or ChiMaD, a NIST-funded consortium led by Northwestern University in Evanston, IL. ChiMaD uses the data and computational resources in development by the Materials Genome Initiative in actual practice to develop new materials and educate undergraduate and graduate students in the Materials Genome approach. ChiMaD's programs are informed by the many industry members who serve as partners or advisors, and by the metrology and data mining and management expertise of NIST scientists.

ChiMaD recently celebrated its second birthday with an annual review, which impresses with the breadth and depth of projects that make materials using the Materials Genome and other data, characterize materials and produce data that can be leveraged for new designs, and the center's outreach and technology transfer to the materials science community. ChiMaD investigators are working on materials that have potential to

- overcome the limitations of silicon for ever-smaller computer chips
- self-assemble into drug or gene delivery vehicles
- replace bone and teeth
- make jet engines and gas turbines more fuel efficient
- make solar cells lighter and less expensive

ChiMaD also hosts and broadcasts seminars and workshops about their work and materials design principles and, along with partner ASM International, has awarded materials design software to six universities for them to incorporate into their engineering programs. Comprehensively addressing cutting-edge science along with education and outreach, ChiMaD truly meets the criteria for a center of excellence.

Happy Birthday, ChiMaD.

NEW NIST METHOD MAY FIND ELUSIVE FLAWS IN MEDICAL IMPLANTS AND SPACECRAFT

Medical implants and spacecraft can suddenly go dead, often for the same reason: cracks in ceramic capacitors, devices that store electric charge in electronic circuits. These cracks, at first harmless and often hidden, can start conducting electricity, depleting batteries or shorting out the electronics.

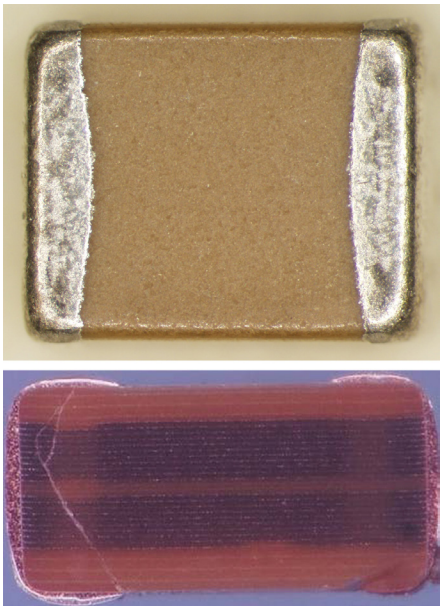
Now, after years of effort by manufacturers and researchers, NIST and collaborators have demonstrated a nondestructive approach for detecting cracks in ceramic capacitors before they go bad.

In the study, the prototype method led to the rejection of more than 90 percent of sample capacitors with visible cracks. Once further studies quantify and confirm detection levels, the new technique may help prevent failures in medical devices such as cardiac pacemakers and defibrillators and also avert electronics failures in satellites and other spacecraft. The new method may also detect structural flaws in other types of materials, researchers say.

NIST researchers invented the technique, and the study of crack detection levels was carried out with collaborators from the University of Maryland, NASA Goddard Space Flight Center, and Colorado State University.

The research grew out of an International Electronics Manufacturing Initiative (iNEMI) consortium working group. This group, which included NIST staff, focused on improving the reliability of multilayer ceramic capacitors for mission-critical electronics. The group concluded that nondestructive methods should be developed to detect cracks in capacitors before they evolve into electrically conducting pathways and cause failures.

Because they can store a lot of energy for their size, multilayer ceramic capacitors are widely used and have an annual market in the billions of dollars. But their failure rates, while low, have long been considered a problem in some applications. A NASA study notes that capacitors are the electronic component most likely to fail. Capacitors can crack during manufacturing, assembly or use because ceramics are brittle and the devices are exposed to heat and mechanical stress. Industrial screening—such as automated visual inspection, X-rays and acoustic microscopy—may not find subsurface cracks, especially near corners under capacitor endcaps, where stress can be highest.



NIST researchers demonstrated an approach for detecting hidden flaws in ceramic capacitors, which store energy in the electronics for medical implants and spacecraft. NIST studied 3-millimeter-long capacitors (top photo), looking for cracks similar to the one shown in the NASA photo (bottom).

A study of Food and Drug Administration data for several million pacemakers and defibrillators implanted in 1990-2002 found that about one in 150 failed, about one quarter of these failures were battery/capacitor abnormalities, and 61 people died due to device malfunctions.

The new NIST crack-detection method relies on acoustic measurements at frequencies much higher than humans can hear. Researchers briefly apply an electric field across the electrodes of a capacitor, exciting a vibration at a specific frequency. They then measure the decay over time (called ringdown) of the signal. These data are analyzed to determine slight shifts in frequency versus the magnitude of the vibration. These shifts are greater when cracks are present. This nonlinear approach—focusing on frequency shifts relative to signal strength rather than the frequency shifts alone—is especially useful because it is not affected by slight variations in size of the capacitors.

A familiar example of nonlinear acoustic effects is the way a violin's tone changes when the bow is pulled more forcefully. The ceramics in the NIST study are highly nonlinear, meaning the capacitors get less stiff and their resonant frequency drops when they vibrate more strongly. The new NIST method measures patterns in how this tone changes over time in relation to the strength of the vibrations.

Researchers measured 41 multilayer barium-titanate ceramic capacitors, each roughly 2 by 3 millimeters in size, before and after heating to high temperatures (189 °C) and quenching in ice water. This thermal treatment generated surface-breaking cracks in 27 samples. The nonlinear acoustic results were strongly correlated with the presence of visible cracks: Measurements on 25 of the 27 visibly cracked capacitors yielded results that were outside the range of those for capacitors without cracks.

The study concluded that nonlinear acoustic measurements offer a promising approach for nondestructive detection of cracks in capacitors before electrical failure occurs, and that further work should be pursued to quantify the level of detection. NIST staff are continuing this research in collaboration with a capacitor manufacturer.

W.L. Johnson, S.A. Kim, G.S. White, J. Herzberger, K.L. Peterson and P.R. Heyliger. 2015. Time-domain Analysis of Resonant Acoustic Nonlinearity Arising from Cracks in Multilayer Ceramic Capacitors. Paper presented at 2015 Review of Progress in Quantitative Nondestructive Evaluation. Posted online Feb. 2016. DOI: dx.doi.org/10.1063/1.4940511

NEW EXPERIMENTAL TEST DETECTS SIGNS OF LYME DISEASE NEAR TIME OF INFECTION

When it comes to early diagnosis of Lyme disease, the insidious tick-borne illness that afflicts about 300,000 Americans annually, finding the proverbial needle in the haystack might be a far easier challenge—until now, perhaps. An experimental method developed by federal and university researchers appears capable of detecting the stealthy culprit Lyme bacteria at the earliest time of infection, when currently available tests are often still negative.

The team suggests the approach might also be useful for early detection of other elusive bacterial infections. The collaborators—from NIST, the Institute for Bioscience and Biotechnology Research, and Johns Hopkins School of Medicine—recently reported the successful first trial of their new method.

"Our hypothesis was that Lyme bacteria shed vesicle-like particles—or fragments—derived from the cell wall of the bacteria circulating in the serum of individuals. These particles would contain membrane proteins that can be detected to provide a unique indicator of infection," explains NIST research chemist Larik Turko.

The challenge was to detect these bacterial membrane proteins among the far, far more plentiful proteins normally present in serum, the watery, cell-free component of blood. The researchers speculated that running serum samples through a high-speed centrifuge—a standard step in chemistry labs—might selectively concentrate the larger, heavier fragments containing the bacterial membrane proteins into pellets. In effect, they predicted, this step would separate the wheat—the sparse target proteins—from the chaff—the much more abundant human serum proteins.

The new method's promise was demonstrated in tests on serum samples drawn from three patients with undetected Lyme disease at the time of their initial doctor visit. By customizing standard analytical techniques for determining the types and amounts of chemicals in a sample, the team detected extremely small amounts of the target protein in all three samples. For chemistry buffs, the protein in enriched samples was present at a level of about four billionths of a millionth of a mole, the standard unit for amount of substance.

In one patient, the experimental method detected the bacteria three weeks before infection was confirmed with the standard blood tests now used. For the other two, infection was detected simultaneously by the two methods.

Deer, or black-legged, ticks can transmit Lyme disease to humans during feeding, when they insert their mouth parts into the skin. A new experimental test developed at NIST has been shown to detect the disease near the time of infection, earlier than the standard blood test now used.

"The complexity of Lyme disease, combined with lack of biomarkers to measure infection, has slowed progress," study collaborator John Aucott, head of the Johns Hopkins Lyme Disease Clinical Research Center, said in advance of a session on precision and personalized medicine this weekend at the AAAS 2016 Annual Meeting in Washington, D.C. "Now, thanks to recent advances in technology, the tiniest concentration of blood molecules can now be detected, molecules that were previously 'invisible' to scientists."

The current standard blood test for Lyme disease exposes the infection only after antibodies have accumulated to detectable levels, which can take up to 4 to 6 weeks. If patients exhibit a telltale bull's-eye rash, diagnosis and treatment can begin earlier. But the rash does not occur in 20 to 30 percent of Lyme disease patients, according to the Centers for Disease Control and Prevention.

Rather than waiting for an infected person's immune system to produce noticeable amounts of antibodies, the team chose to home in on the bacteria itself—specifically, proteins the bug sheds when attacked by the body's defenses.

"From many candidates, we chose one that is both easily distinguished from human serum proteins and an unambiguous indicator of the bacteria," Turko says. "This protein, which resides on the outer surface of membranes, became the target of our search in serum samples."

But finding that target required an important preliminary step to ensure the accuracy of their measurements: making a reference sample that contained ample amounts of the target protein. With the reference sample, the team established the unmistakable signature the bug's outer-surface membrane protein would yield when they examined samples drawn from patients. As a result of these steps, the team could detect the copies of the target protein, even though human proteins were 10 million times more plentiful.

"We believe that this approach may be universally applicable to detection of other bacterial infections in humans," the researchers write.

C.S.F. Cheung, K.W. Anderson, K.Y. Villatoro Benitez, M.J. Soloski, J.N. Aucott, K.W. Phinney, and I.V. Turko, "Quantification of Borrelia burgdorferi Membrane Proteins in Human Serum: A New Concept for Detection of Bacterial Infection." *Analytical Chemistry* (2015) 87, 11383-11388.

A JIGSAW PUZZLE WITH ATOMS: SOLVING QUASICRYSTAL PROBLEM COULD LEAD TO NEW CLASS OF IMPORTANT MATERIALS

A good puzzle can bring out the best in us. A solution requires creativity and, sometimes, a little help from our friends. For scientists at NIST and their collaborators, solving a puzzle not only brings a feeling of accomplishment, but often yields real-world benefits too.

Part of the challenge is to recognize an important new puzzle when it appears. This is what happened when MML physicist Eric Cockayne ran into one of his former Ph.D. advisors from Cornell University a couple years ago. His former professor, Christopher Henley, was an expert on a special type of solid material known as a quasicrystal. Quasicrystals were discovered at NIST in the 1980s by a visiting researcher, Dan Shechtman, who was honored for his work with the 2011 Nobel Prize in chemistry.

Ordinary crystals are made of regularly repeating patterns of atoms or molecules, like a recurring design on wallpaper. For example, ice crystals are made of repeating arrangements of water molecules, H_2O, that serve as the crystal's building block. In ordinary ice, the H_2O molecules form arrangements of hexagons that repeat endlessly. But quasicrystals are different. They also have building blocks consisting of atoms or molecules, but these building blocks never quite repeat their pattern when assembled.

Cockayne had studied quasicrystals at Cornell. At NIST, he left quasicrystal research behind to study a number of different topics, including ferroelectrics, a class of materials that respond to electric fields. Ferroelectrics are useful in a variety of applications such as tunable capacitors and medical ultrasound machines.

Henley presented to Cockayne some surprising recent experimental results from Germany on the important and

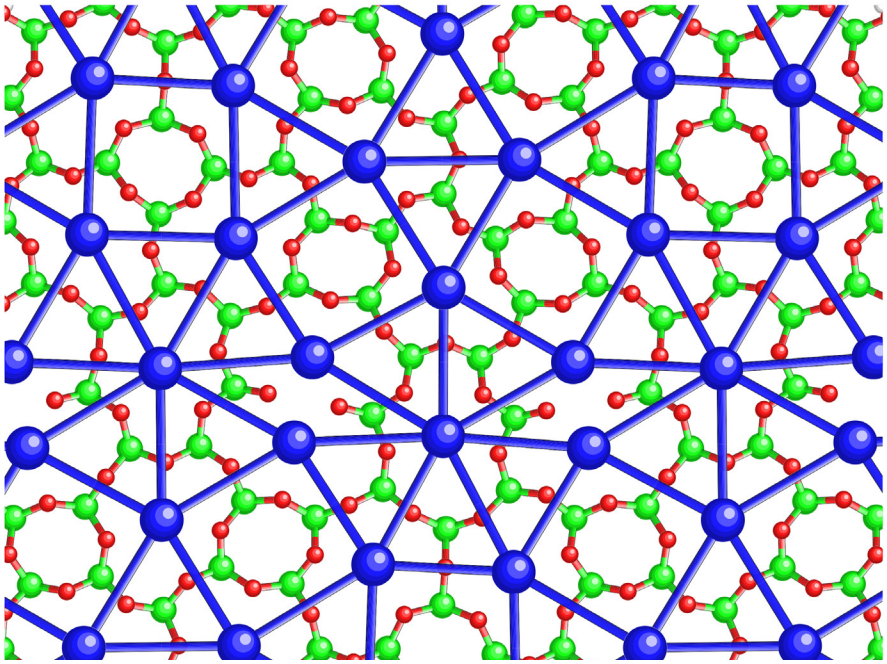


Figure 1. Tiles of atoms in the shapes of squares, triangles, and a thin rhombus could explain intriguing experimental results of a "quasicrystal" in a barium-titanium-oxygen compound on a platinum surface. Barium atoms are blue, titanium green, and oxygen red. Platinum atoms not shown.

widely studied ferroelectric material barium titanate. The experiments suggested that barium titanate deposited on a platinum surface could form a quasicrystal when subject to high temperatures. This was notable because quasicrystals had previously been found in only a select few kinds of materials such as aluminum-rich metal alloys and in "soft" materials, like polymers. Barium titanate, in contrast, is an oxide—a material that contains metal ions bound to oxygen ions.

Oxides are an important class of materials in the physical sciences. They're found in countless forms of chemical reactions in nature, and offer a broad range of applications. The oxide barium titanate is widely used as a capacitor to store electric charge and may be used as a component of cheaper, longer-lasting, and more powerful battery systems in future electric cars. The barium titanate quasicrystal the German research group had found was the first oxide quasicrystal.

"That's the kind of thing in the physics world that makes you say, 'wow,'" Cockayne says. "Two fields that don't ordinarily overlap had unexpectedly overlapped." But what was the structure of this oxide quasicrystal? What were its fundamental building blocks and how did they fit together? Knowing and understanding its structure would be the first step towards controlled studies to determine the relationships between the synthesis, structures, and properties of the new material.

Henley thus recognized the structure of quasicrystalline barium titanate as an important puzzle to solve. So Cockayne and Henley, along with Marek Mihalkovič, a researcher at the Slovak Academy of Sciences in Slovakia, set out to solve it.

Only this puzzle was unlike most. They knew the shapes of the pieces, but they didn't know what the picture was. Barium titanate ordinarily has a standard chemical formula of $BaTiO_3$. In other words, for every barium atom and titanium atom there are three oxygen atoms. But trying to make a quasicrystal with this ratio of atoms was just not working. What were the building blocks that would tile together to produce the observed experimental results?

After working on this puzzle for months alongside his many other projects, Cockayne came upon a solution that looked like it might work. The solution involved placing barium, titanium, and oxygen atoms upon (or "decorating") the three types of puzzle pieces—shaped like a square, a triangle, and a thin rhombus—and putting them together as shown in Figure 1.

So, is this the correct structure? While the researchers are confident that their model is essentially correct, direct experimental confirmation is still needed. The pictures in the experimental paper were tantalizing but didn't show all of the atoms in the materials. Interpreted in light of the modeling work of Cockayne et al. (Figure 2), the experiments could only pick up one of the types of atoms: the barium. The researchers' theoretical simulations predict what a microscope would show if it could see all of the kinds of atoms in the quasicrystal. Aside from providing a solution to the quasicrystal jigsaw puzzle, the researchers discovered similar solutions for the structures of other ultrathin film barium-titanium-oxygen structures. Their work may have novel practical applications. If free films of the titanium-oxygen portion of these structures could be obtained, they could act as ultrathin filters for separating large molecules from small ones. In addition, the barium-titanium-oxygen quasicrystal and related ultrathin film structures may be useful as insulating components in microelectronics.

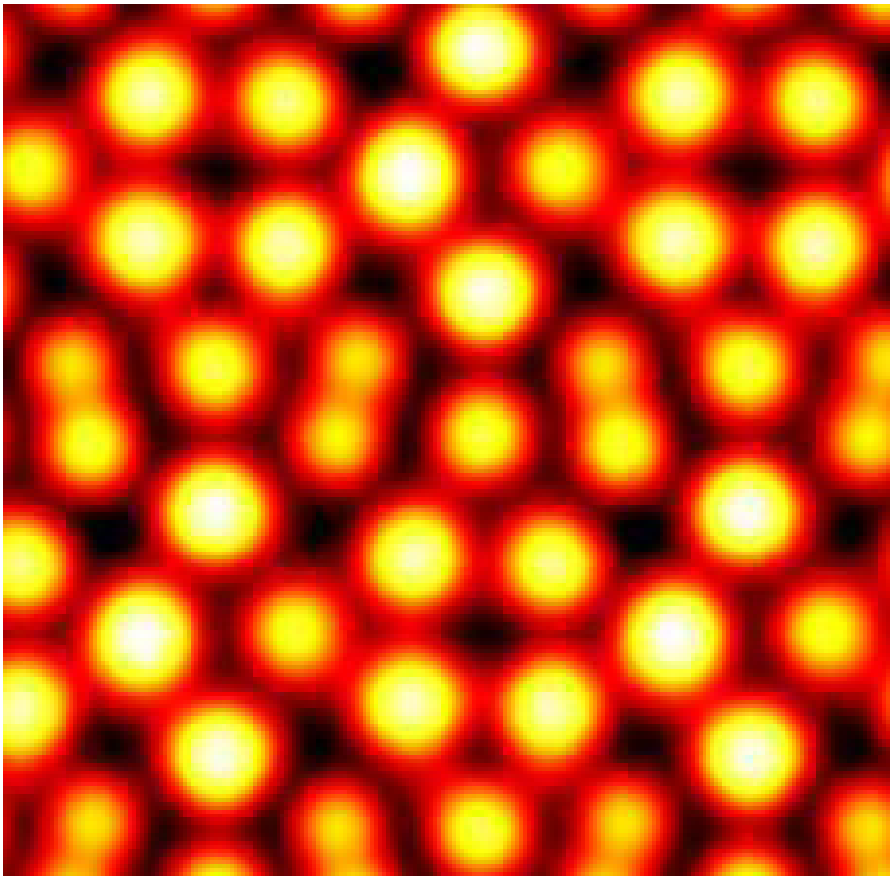


Figure 2. MML's Eric Cockayne and his colleagues produced this simulated scanning tunneling microscopy image of a barium-titanium-oxygen compound on a platinum surface. The pattern of the bright protrusions matches with experimental results. Comparison with Figure 1 shows that bright spots correspond with barium atoms.

"They open up a possible new method for making new structures and thin films of oxides," Cockayne says.

This structure also appears similar to those recently found in ultrathin silicon dioxide glass, so the barium-titanium-oxygen work may lead to advances in that field, too, potentially leading to smaller and more reliable microelectronic devices.

There is a bittersweet ending to this story. The paper describing this solution to the structure puzzle was recently published in the journal *Physical Review B*, as a Rapid Communication and Editor's Suggestion However, Cockayne's former advisor, Christopher Henley, who instigated this whole project, passed away in 2015. By fortuitous chance, this puzzle provided a reunion between a former advisor and his student for a final collaboration that may reap benefits for years to come.

E. J. Cockayne, M. Mihalkovic, C. L. Henley, "Structure of Periodic Crystals and Quasicrystals in Ultrathin Films of Ba-Ti-O" Physical Review B, Vol. 93, 4 pp. (07-Jan-2016).

ASSESSING THE BIOSIMILARITY OF PROTEIN DRUGS: NEW STUDY SHOWS METHOD'S PRECISION AT ATOMIC RESOLUTION

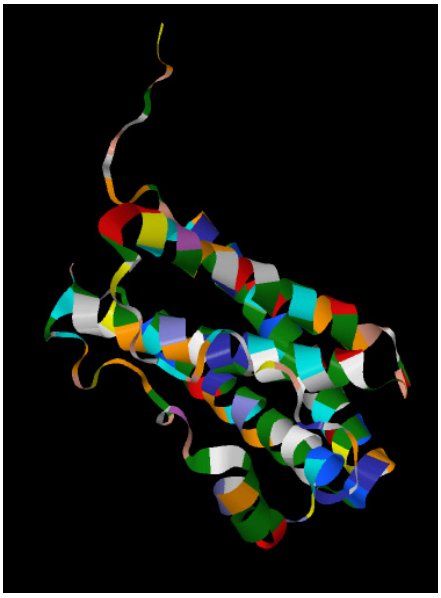
A first-ever interlaboratory study of four versions of a therapeutic protein drug—all manufactured from living cells—reports that an established analytical tool akin to magnetic resonance imaging reliably assessed the atomic structures of the biologically similar products, yielding the equivalent of a fingerprint for each.

The findings, described recently in *Nature Biotechnology*, demonstrate that the method—known as two-dimensional nuclear magnetic resonance spectroscopy, or 2D-NMR—"can be a robust and powerful complementary technique for companies and regulators" when assessing these biosimilars, said Robert Brinson, an MML research chemist. This type of assessment is part of a set of comparisons required to determine whether a follow-on biological product is highly similar to an existing product, so that there is no "clinically meaningful" difference between the two.

"Other analytical methods provide useful information, but 2D-NMR is one of the few approaches that can yield complete assignment of three-dimensional structure across the entire molecule in solution at atomic-level resolution," Brinson explains. "Our study indicates that 2D-NMR data can yield a precise and unique 'fingerprint' of structural information in a biological product," he said.

Results were reported for measurements of four independently manufactured versions of filgrastim, a biological drug used to help ward off infection and anemia in cancer patients. At four laboratories, researchers used 2D-NMR to map the atomic structures of the original—or reference—filgrastim product licensed in the U.S. and three unapproved biosimilar versions.

A biosimilar, according to the Food and Drug Administration (FDA), is a biological product shown to be "highly similar to an FDA-approved biological product, and has no clinically meaningful differences in terms of safety and effectiveness." Only minor differences in clinically inactive components are allowable in biosimilar products.



Granulocyte colony-stimulating factor (GCSF), a protein consisting of about 175 amino acids (colors), stimulates the bone marrow to produce neutrophils, a type of white blood cell. Filgrastim is the recombinant DNA analog of GCSF. It is used to ward off infection and anemia in cancer patients.

Biosimilar versions of approved biological drugs at the end of their patent life are expected to cost less but be as safe and effective for licensed clinical uses. To date, the FDA has approved one biosimilar (a version of filgrastim), while the European Union has approved about 20 biosimilars over the last 10 years.

Unlike chemically synthesized drugs—aspirin, for example—biological drugs usually are composed of large, complex protein molecules and are produced by living systems. This makes producing exact duplicates impossible, even from batch to batch in the same biomanufacturing process. For specified health conditions and symptoms, the nearly exact copies that result must be shown to achieve the same clinical effects as the already-licensed biological product.

Samples of the four filgrastim products were measured on six NMR instruments made by two manufacturers and distributed across laboratories at NIST, FDA's Center for Drug Evaluation and Research, the Medical Products Agency of Sweden, and Health Canada's Center for Biologics Evaluation.

Each sample of filgrastim—a chain of 175 protein building-blocks known as amino acids—was independently measured in each participating laboratory. Rendered as a complex pattern of peaks corresponding to signals from hydrogen and nitrogen in the sample, the data were gathered on each instrument and then analyzed together. Statistical analyses determined how tightly the signals were clustered when data from all six instruments were superimposed.

Across all samples on all six instruments, measurements hardly varied. The experimentally determined precision limit of 8 parts per billion for the interlaboratory comparison, the researchers write, is "well below" the threshold beyond which structural differences due to mutations, changes in three-dimensional shape, or other causes might be obscured. The atomic structures of all four filgrastim versions were determined to be the same within the tight precision limits of the NMR data.

Separately, NIST researchers repeated measurements on all four samples one year after the interlaboratory comparison to assess their stability. Using the same 2D-NMR method, they did not find significant structural changes in any of the four biologics. NIST's Brinson stresses the importance of proper instrument calibration and control of laboratory

Continued on page 15...

DNA-ENCODED CIRCUITS MADE EASIER, FASTER, AND MORE MEASURABLE

Researchers from the Massachusetts Institute of Technology (MIT), Boston University, and NIST have demonstrated a powerful tool for programming DNA to encode new functions into living cells. The advance automates the design of genetic circuits, which process chemical signals rather than electronic ones.

As described in the April 1, 2016 issue of *Science*, the team created and tested a programming language to compile high-level functional specifications into DNA sequences that could be inserted into bacterial cells, giving them new sensing and response capabilities. Future applications for this kind of programming include designing bacterial cells that can produce a cancer drug when they detect a tumor or creating yeast cells that can halt their own fermentation process if too many toxic byproducts build up.

Until now, designing and making genetic parts — such as sensors or switches — and customizing cells to function as programmable factories have been arduous, trial-and-error tasks.

Using the MIT-developed DNA-programming language — called Cello, for cellular logic — the researchers programmed 60 circuits with different functions, and 45 of them worked correctly the first time they were tested. Many of the circuits were designed to measure one or more environmental conditions, such as oxygen level or glucose concentration, and respond accordingly. Another circuit was designed to rank three different inputs and then respond based on the priority of each one.

One of the new circuits is the largest biological circuit ever built, containing seven logic gates and about 12,000 base pairs of DNA.

"It would take years to build these types of circuits. Now you just hit the button and immediately get a DNA sequence to test," Christopher Voigt, an MIT professor of biological engineering, explained in an MIT news release.

NIST collaborators used a fluorescence-microscopy method to quantify the signal strength of the genetic circuits. The NIST approach, which entails counting individual RNA molecules, converts measurements of signal strength into standard units that make it possible to compare measurements done in different labs.

"Ultimately, our goal is to develop a suite of measurement tools that can be used across labs and enable predictive engineering of organisms," said David Ross, leader of NIST's Engineering Biology Team.

A.A.K. Nielsen, B.S. Der, J. Shin, P.Vaidyanathan2, V. Paralanov, E. A. Strychalski, D. Ross, D. Densmore, C.A. Voigt, "Genetic circuit design automation," Science 01 Apr 2016: Vol. 352, Issue 6281. DOI: 10.1126/science.aac7341

C.A. Hutchison III, R.-Y. Chuang, V.N. Noskov, N. Assad-Garcia, T.J. Deerinck, M.H. Ellisman, J. Gill, K. Kannan, B.J. Karas, L. Ma, J.F. Pelletier, Z.-Q. Qi, R.A. Richter, E.A. Strychalski, L. Sun, Y. Suzuki, B. Tsvetanova, K.S. Wise, H.O. Smith, J.I. Glass, C. Merryman, D.G. Gibson, J.C. Venter, "Design and synthesis of a minimal bacterial genome," Science 25 Mar 2016: Vol. 351, Issue 6280. DOI: 10.1126/science.aad6253

NO SMALL MEASURE

NIST's engineering biology team contributed to another important milestone in biotechnology and microbiology: a lab-made bacterium with only 473 genes, the smallest genome of any living organism, and the minimal number required for the bacterium to survive and reproduce. The work appeared in the March 25, 2016 issue of ***Science***.

Produced by a team led by the J. Craig Venter Institute, the bacterium stripped of all but the most basic functions represents a powerful new tool for biologists to understand the roles of genes in a cell. As knowledge about gene function expands, biotechnologists can design organisms for specific functions, such as bacteria that can neutralize pollutants or produce feedstocks for alternatives to fossil fuels.

As the Venter Institute scientists whittled away at the genome of their bacterium, they sent cells to MML's Elizabeth Strychalski and James Pelletier, a collaborator of Strychalski's from MIT, to measure their growth characteristics. The Venter Institute's minimal bacteria were so small that they were difficult to study with traditional cell culture and optical microscopes. They'd heard about Strychalski's expertise—using very small, fabricated environments to isolate and image biomolecules—while she was visiting the NIST-Stanford Joint Initiative for Metrology in Biology in Palo Alto, California and asked for her help. Pelletier, who studies how cells function, provided the necessary biology expertise for growing the cells in these tiny environments. Synthetic Genomics, a company founded by the non-profit J. Craig Venter Institute to commercialize its discoveries, also participated.

"The project took a lot of close communication among the collaborators," says Strychalski, "and is another great example of a successful partnership among industry, academia, and NIST that pushes forward both cutting-edge fundamental science and measurement capabilities for biology."

STUDY HIGHLIGHTS NEED FOR BETTER CHARACTERIZED GENOMES FOR CLINICAL SEQUENCING

A new study that assesses the accuracy of modern human-genome-sequencing technologies found that some medically significant portions of an individual's DNA blueprint are situated in complex, hard-to-analyze regions that are currently prone to systematic errors.

These genes and gene segments lie in yet-to-be-benchmarked regions that presently make up almost a fourth of the human genome's 3.2 billion pairs of chemical building blocks.

Stanford University and NIST researchers write that their findings should be a "call to arms for those interested in clinical grade technical accuracy for genome sequencing." As genome sequencing transitions from research to clinic, they say, it is essential to have methods to benchmark performance in all regions that are sequenced for diagnostic or other medical purposes.

Challenges in benchmarking difficult, but clinically important regions of the genome are reported in a recent issue of *Genome Medicine*. The results underscore the need to extend benchmarking references against which sequencing data and analyses can be compared and validated.

In effect, these types of standards are quality-control and quality-assurance tools. They are necessary for checking the accuracy of sequencing data and analyses—and preventing false positives and false negatives. However, genome-sequencing technologies aimed at the large health care market are advancing so quickly that efforts to develop the field's underpinning benchmarking tools must race to keep up.

Central to the Stanford-NIST study, one such tool is the genomic reference material created by NIST and its partners in the Genome in a Bottle Consortium. The NIST reference material—NIST RM 8398, Human DNA for Whole-Genome Variant Assessment—currently has about 77 percent of the genome characterized with high levels of confidence.

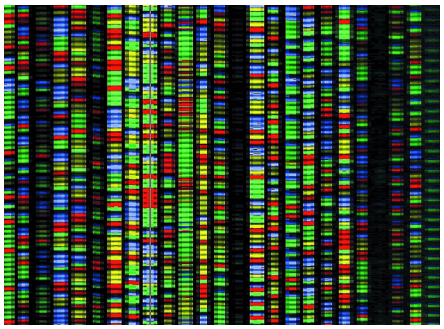
"The harder-to-characterize regions that we can't yet sequence with confidence include regions known to be clinically important," explains NIST biomedical engineer Justin Zook. "This means that our benchmark genome cannot currently be used to assess performance for more challenging genes and other difficult regions of the genome that already are being tested or for which new sequencing methods are being developed."

"The good news is that, in this case, 77 percent of the donor's genome was reliably sequenced using current methods," says lead author Rachel Goldfeder from Stanford University. "The challenge now is to focus our efforts on the other 23 percent—namely, on regions of the genome that remain elusive. Only then can we realize the full potential of precision medicine."

In their study, Stanford and NIST researchers used data from whole genome sequencing and whole exome sequencing methods. Exome sequencing focuses only on the protein-encoding portions of genes, comprising less than 2 percent of the entire genome.

Both types of these so-called next-generation sequencers follow a similar process. Paired strands of DNA are uncoupled and randomly chopped into short segments. Numerous copies of the segments are made and then are sequenced by recreating the missing paired strand for each copy. The matches are analyzed to determine their sequence of letters from the four-letter genetic alphabet: A (adenine), C (cytosine), G (guanine) and T (thymine).

Then, bioinformaticians apply complex mathematical algorithms to determine where the decoded pieces originated. The pieces can then be compared to a defined "reference sequence" to identify variations in stretches of letters and where



The end result of a DNA sequencing process. Each color represents one of the four base chemicals that make up DNA (adenine, guanine, cytosine and thymine). NIST's genome reference material is a benchmarking standard that can help labs determine how well their DNA sequencing processes are working.

letters have been deleted or inserted in specific genes. When differences are found, a "variant call" is logged.

For RM 8398, the Genome in a Bottle Consortium had catalogued high-confidence variant calls in the well-characterized regions of the benchmarking genome. The Stanford-NIST team compared these calls with variant calls made with two sequencing systems. Of particular interest were differences in 56 "medically actionable" genes that the American College of Medical Genetics and Genomics (ACMG) recommends for reporting.

Accuracy of variant calls within high-confidence regions depended on the genome region; type of difference—say, an inserted or substituted letter; extent of coverage (number of times a specific DNA segment has been read); and analytical methods.

In whole genome sequencing, for example, false negative calls—unidentified variations or mutations—resulted largely from software tools used to filter out errors in sequencing data, the researchers found. Most false negatives in whole exome sequencing stemmed from poor coverage—not enough reads to generate data of sufficient quality.

Continued on page 15...

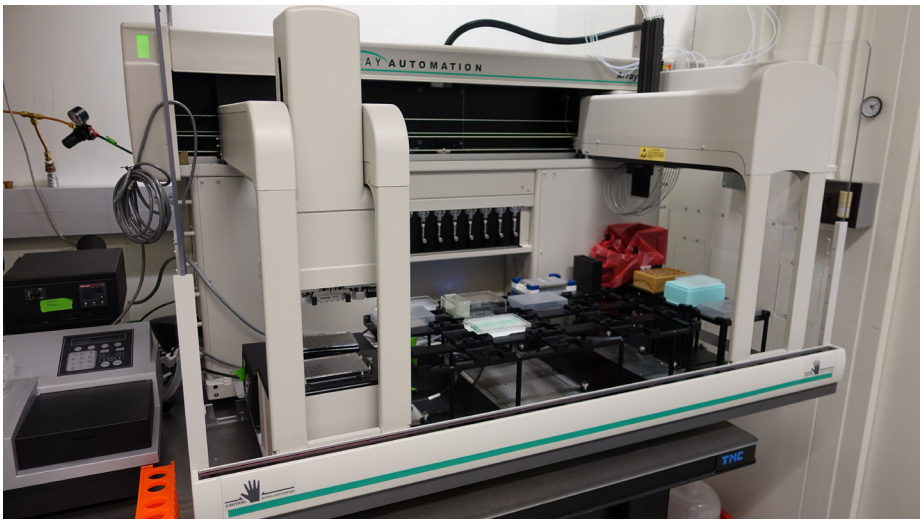
AMPING ANTIMICROBIAL DISCOVERY WITH AUTOMATION

The antimicrobial arsenal that we count on to save millions of lives each year is alarmingly thin—and these microbes are rapidly evolving resistance to our weapons. But help may be on the way: In a study posted in the *AMB Express*, MML researchers show that automated techniques commonly used to screen new drugs for mammalian cell toxicity could also dramatically speed up the challenging task of antimicrobial discovery.

In the age-old struggle between humans and microbes, bacteria seem to be regaining the offensive. Only around a dozen classes of chemicals protect us from the myriad pathogens that populate our environment. Numerous agencies, including the World Health Organization and the Centers for Disease Control and Prevention, have recently warned that evolved resistance could soon render common antibiotics useless, and that few replacement drugs are in the pipeline.

The shortage of new antimicrobials is not a result of scientists lacking candidate chemicals. The fungal and plant worlds abound with potential antimicrobials, and chemists concoct new synthetic molecules all the time. However, a major bottleneck occurs at the lab bench. Any candidate compound must be tested at multiple concentrations against multiple strains of bacteria in different forms. This remains a cumbersome process, with numerous time- and labor-intensive steps that lab workers must currently carry out by hand.

But MML researcher Samuel Forry and colleagues are convinced that the process could be vastly sped up using automation. To do so, Forry and his team looked to one of the pharmaceutical industry's most powerful tools: high-throughput screening. For several decades, companies have routinely used automated systems to test potential drugs' effects on mammalian cells in culture. In these studies, robots prepare samples of cells in arrays of small plastic wells, inject measured amounts of drugs and test whether cells live or die. The method



A robotic device designed for high-throughput screening of drug toxicity. An MML study found that this device could also accelerate antimicrobial discovery.

can quickly assess multiple chemicals at different concentrations, all in parallel and with minimal human intervention.

High-throughput screening has seen limited use for antimicrobial discovery, Forry says, because less research and development money is available and because of the large variation among microbial populations and growth conditions. Hoping to stimulate the field, Forry and his team adapted a high-throughput screening robot for antimicrobial testing. The researchers tested a set of antimicrobial compounds known as pyridinium salts against the common bacterium *Streptococcus mutans*, which causes tooth decay.

Part of the challenge in identifying useful antimicrobial compounds is that chemicals that kill free-swimming cells are often less effective against the same bacteria growing in biofilms like the plaque that can form on teeth. So Forry's team used automation to culture both free-swimming cells and biofilms, as well as an intermediate state, side-by-side in 96-well plates. The researchers measured antimicrobial activity in three different ways by identifying the concentrations that reduced bacterial activity by half, that prevented any detectable activity, and that entirely killed the bacteria. They determined the drugs' effects with high throughput by measuring light passing through the wells or using chemicals that change color to indicate metabolic activity.

The team found that the automated system delivered results indistinguishable from those obtained by doing the experiments by hand. More importantly, the robot took only a third as much time as humans do, freed up laboratory personnel for other tasks, and carried out the procedures without errors. "That's a huge improvement from the point of view of laboratory workflow and a great boon for people trying to identify and characterize antimicrobials," Forry says.

The trials weren't fully automated—for instance, the researchers moved samples from the incubator to the screening robot by hand—but Forry says his team has demonstrated the concept, and existing technology can fill in the remaining steps. He expects other research labs will adopt the technology first, followed by pharmaceutical companies. "Once a number of people start to use this and find that it works for them as well as it has worked for us, I could easily see companies and contract labs doing it."

S.P. Forry, M.C. Madonna, D. López-Pérez, N.J. Lin and M.D. Pasco, "Automation of Antimicrobial Activity Screening," *AMB Express* 2016, 6:20. DOI:10.1186/s13568-016-0191-2

HOW REFRESHING: NIST'S NATURAL AIR STANDARDS SUPPORT ACCURATE GREENHOUSE GAS MEASUREMENTS

When it comes to tallying emissions of greenhouse gases, there is no better substitute than directly measuring the atmosphere. But this important accounting can be obscured, and even confused, if measurements of the air-borne heat-trapping chemicals are inaccurate or can't be compared from one instrument or data set to the next.

To help ensure reliably accurate measurements of the big three long-lived greenhouse gases, NIST has issued two new Standard Reference Materials (SRMs) that are puffs of naturally occurring air from far-flung parts of the globe.

The new NIST-certified references—Southern Oceanic Air (SRM 1721) and Northern Continental Air (SRM 1720)—contain painstakingly measured concentrations of carbon dioxide, methane and nitrous oxide. They respond to the growing need for greenhouse gas calibration standards that extends beyond organizations participating in the World Meteorological Organization (WMO) Global Atmosphere Watch Program, including its North American network. This program is served by a dedicated set of calibration laboratories.

A variety of other organizations outside these official monitoring networks also measure greenhouse gases and need tools for ensuring accuracy. They include state and local agencies that track emissions and atmospheric concentrations of the gases; automobile manufacturers, which are particularly interested in leaks and other unintended emissions of nitrous oxide from vehicles; and so-called megacities projects that inventory sources and levels of the gases in large metropolitan areas.

To ensure measurement accuracy, these and other types of organizations can first use their instruments to measure concentrations of the three gases in a NIST natural-air SRM. If the results differ from the SRM's certified values, they can adjust—or calibrate—their instruments accordingly before measuring gas levels in the local atmosphere.

The Baring Head, New Zealand, Clean Air Monitoring Station, where samples for Standard Reference Material 1721 (Southern Oceanic Air) were collected.

The two natural air benchmarks hold the NIST record for lowest uncertainties assigned to components in the agency's more than 60 primary gas SRMs.

The southern oceanic air hails from Baring Head, New Zealand, site of an air-monitoring station situated on a coastal cliff 79 meters above the Pacific Ocean. Samples were gathered in New Zealand at times in which prevailing winds originated from Antarctica.

Samples of northern continental air were collected during late winter and early spring seasons in the Rocky Mountains at Niwot Ridge, Colo., a forested area more than 3,500 meters (almost 11,500 feet) above sea level.

For nearly four decades, the National Oceanic and Atmospheric Administration (NOAA) has been siphoning the site's pristine air for its monitoring program and, more recently, for supplying calibrated reference samples to organizations participating in the WMO's atmospheric monitoring network and NOAA's tracking system.

NIST and NOAA independently measured concentrations of the three greenhouse gases in the volumes of northern continental air contained in aluminum gas cylinders. While the NIST and NOAA measurements were in close agreement, the SRMs' certified concentrations are taken from the actual NIST assigned values. The NOAA values are included for those users who need to use the WMO-accepted calibration.

Among long-lived greenhouse gases, the three compounds account for about 90 percent of what is known as "radiative forcing"—a measure of the compounds' influence on the balance of incoming and outgoing energy in the Earth-atmosphere system. Carbon dioxide, the most abundant greenhouse gas, accounts for 65 percent of radiative forcing. Molecule for molecule, methane (at 17 percent) and nitrous oxide (6 percent) are much stronger absorbers of the Earth's reradiated energy, but they are less abundant in the atmosphere.

Accurate measurements of known and suspected influences on climate change require a sophisticated, underpinning infrastructure. NIST research chemist George Rhoderick says certifying concentrations of the three greenhouse gases in both natural air SRMs first required developing a set of even more exacting primary standards for each gas. The NIST Gas Sensing Metrology Group, he explains, spent five years developing suites of these primary standard mixtures so that measured NIST-certified concentrations of each gas in the SRMs is linked to the global measurement system.

For both natural-air SRMs, pressurized canisters containing the mixture have been calibrated individually. Average values are 390.1 parts per million for carbon dioxide and 0.32 parts per million for nitrous oxide. Average methane values differ by about 6 percent—1.7 parts per million for the southern ocean air mixture and 1.8 parts per million for cylinders of northern continental air.

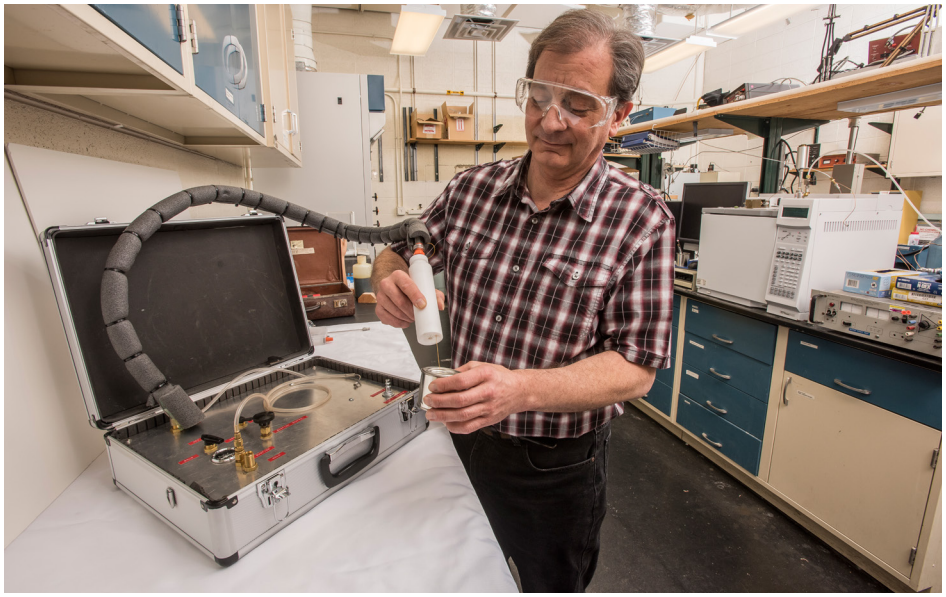
PORTABLE NIST KIT CAN RECOVER TRACES OF CHEMICAL EVIDENCE

An MML chemist has developed a portable version of his method for recovering trace chemicals such as environmental pollutants and forensic evidence including secret graves and arson fire debris.

If successfully commercialized by industry, the briefcase-sized kit could enable detectives, field inspectors and others to carry with them a convenient version of NIST's "headspace analysis" technique, which identifies solid or liquid compounds based on the makeup of vapors released into nearby air.

The underlying technique is PLOT-cryoadsorption, or PLOT-cryo—short for porous layer open tubular cryogenic adsorption. PLOT-cryo is sensitive, quantitative and more broadly useful than many competing techniques. It can identify compounds that don't readily evaporate and is not limited to samples dissolved in water, for example. The method recovers vapors by suction or by sweeping a gas across the air above a sample of interest. The laboratory version of the technique has been used to find traces of explosives, spoiled food, residues in arson debris and gravesoil.

The new portable kit collects trace chemicals while analysis is performed with other instruments such as gas chromatography and mass spectrometry, which can also be made portable. In initial demonstrations of the kit in the lab, chemist Tom Bruno recovered and reliably identified substances such as the chemical compound coumarin, the explosive TNT, and diesel fuel. Collection times as fast as 3 seconds produced definitive results. The kit detected diesel fuel—a concern with respect to illegal dumping and leaking tanks—with a sensitivity better than one part per million.



NIST chemist Tom Bruno, who invented a method for recovering trace chemicals such as environmental pollutants and forensic evidence, uses a portable version of the instrument to sample vapor inside an old paint can. The underlying technique is called PLOT-cryoadsorption, or PLOT-cryo - short for porous layer open tubular cryogenic adsorption.

There is no other portable instrument that can detect traces of as wide a range of these types of compounds, Bruno says.

The NIST kit is powered by compressed air, which enables operation without electrical power and ensures safety in potentially flammable and explosive environments. Compressed air is available on many emergency response vehicles. A key component of the kit is a vortex tube, which—without any moving parts—rotates compressed air to make hot or cold air streams.

Vapors are collected in sturdy, inexpensive tubes embedded in an epoxy wafer. The wafer can be used inside either an insulated handpiece for manual sampling or a longer probe for remote sampling of soil and spaces under buildings or in luggage or other containers. With either the handpiece or probe, the wafer can be chilled to collect vapors, and then heated to help remove them.

For now, the portable kit is less sensitive than the lab version of the method, but research continues to improve performance. Bruno has fielded interest in the basic technique from an instrument company, detectives, and film producers looking for a missing explorer. Companies interested in commercialization should contact the NIST Technology Partnerships Office at nisttech@nist.gov.

T. J. Bruno. Field Portable Low Temperature Porous Layer Open Tubular Cryoadsorption Headspace Sampling and Analysis Part I: Instrumentation. *Journal of Chromatography A*. Published online Dec. 8, 2015. DOI:10.1016/j.chroma.2015.12.013

M. Harries, S. Bukovsky-Reyes, T.J. Bruno. Field Portable Low Temperature Porous Layer Open Tubular Cryoadsorption Headspace Sampling and Analysis Part II: Applications. *Journal of Chromatography A*, published online 8 December 2015. DOI: 10.1016/j.chroma.2015.12.014

NEWLY PATENTED NIST TECHNIQUE CREATES PRECISELY SIZED NANOCONTAINERS USEFUL FOR DRUG DELIVERY

What if doctors could deliver anti-cancer drugs directly to tumors without making patients sick? Bringing this dream of targeted drug delivery closer to reality for pharmaceutical manufacturers, NIST researchers have received a patent for a method to create precisely sized nanometer-scale capsules.

The NIST method employs microfluidics, the use of fluids at the microscopic level, to create precise nanoscale spherical capsules. Made of lipids, the kinds of biomolecules that also comprise fats, the spherical capsules are known as liposomes. Essentially, liposomes are simplified artificial versions of cell membranes, the outer coverings of cells. The inside of a liposome could hold drugs, and the outside could be coated with receptors that bind to specific cancer cells.

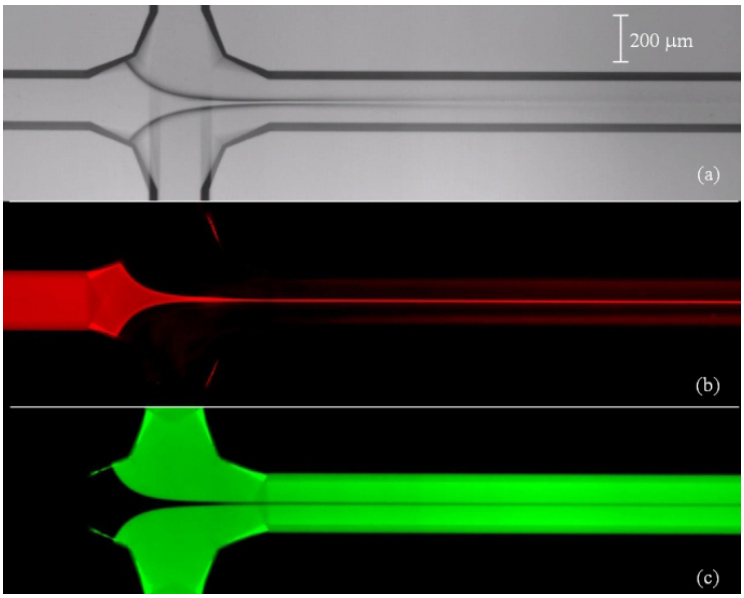
The method can produce liposomes with typical diameters of 100-400 nanometers, or billionths of a meter. This size range is useful for attaching to cells, whose size is typically 1 to 10 micrometers, or millionths of a meter.

Typical methods for making liposomes include pushing a lipid solution through a filter, a process that can lead to wide variations in the size of the resulting liposomes. Furthermore, the methods can be wasteful, and can result in large amounts of expensive drugs not being captured inside the liposomes and being discarded.

In the NIST technique, lipid material is dissolved in isopropyl alcohol. The resulting lipid-containing solution is then forced into a narrow channel and further constricted when it is squeezed by streams of water coming at it from multiple sides. Lipid molecules are repelled by water, so they clump together and coalesce into spherical liposomes. Adjusting the flow rate of the water can control the size of the liposomes that form.

"We have precision control over making liposomes and changing their size by dialing in flow rates," said Michael Gaitan, who works in NIST's Physical Measurement Laboratory.

Researchers could dissolve drugs or other molecules of interest into the water stream, Gaitan explained. Adjusting the concentrations of these molecules can determine the amount of the drug that ends up in the liposome, down to the single-molecule level.



In a recently patented NIST method for making precisely sized nanoscale capsules, a stream of fluid (isopropyl alcohol) containing dissolved biomolecules (the building blocks for the capsules) is focused by streams of water on either side. Panel (a) shows a white-light image of the focused fluid stream, and (b) and (c) show fluorescent images of the stream. The red dye indicates the presence of lipid molecules, initially dissolved in isopropyl alcohol, and the green dye indicates the presence of water. Fluid moving through channels with a width of up to 200 micrometers, or millionths of a meter, can be focused to the point at which the dissolved biomolecules coalesce into nanocapsules with diameters in the hundreds of nanometers, or billionths of a meter.

This method, which has received interest in being licensed by companies, originally developed from basic research. Gaitan and collaborator Laurie Locascio, MML Director were looking for ways to enclose individual molecules of interest in fluid-filled capsules to study their behavior in a liquid environment. Previous methods had anchored individual molecules in glass slides, which are rather unnatural environments, as opposed to the more cell-like environment of a fluid-filled liposome. Once this technique was developed, researchers were able to create a variety of liposomes of many useful sizes, and the potential drug-delivery applications became clear. The researchers were awarded a patent for this work late last year.

"This research and the resulting patent also have implications for the on-demand formulation of drugs in a way that's applicable to personalized or precision medicine," said Locascio.

"The reason that this patent is so fundamental is that this is a process patent," which is a more general form of patent, Gaitan explained. The method does not require a specific experimental configuration, but is a general approach that can be realized in many ways, he said.

Moving forward, researchers at NIST's Center for Nanoscale Science and Technology are continuing to develop this technique for more applications by creating capsules made of different types of nanoparticles. Size—and control—most definitely matter in nanotechnology. Being able to create precisely sized nanocontainers can open up many new applications, Gaitan said.

ASSESSING THE BIOSIMILARITY OF PROTEIN DRUGS: NEW STUDY SHOWS METHOD'S PRECISION AT ATOMIC RESOLUTION (CONT'D.)

conditions to ensure that results are reliable. Early on in the interlaboratory study, the research team identified deviations in data gathered with two instruments. Variations in temperatures were subsequently determined as the cause of the differences, and recalibrated measurements largely eliminated the deviations.

In addition to reporting on the utility of 2D-NMR for high-precision measurement of the detailed atomic structure of biosimilars, the new paper describes statistical methods used to assess biosimilarity. They include one for rapid analysis of many datasets, which can be generated, for example, when monitoring batch-to-batch variation during production.

In the next phase of the work, Brinson says, NIST and collaborators will c ompare 2D-NMR measurements of a monoclonal antibody—molecules able to bind to specific targets such as cancer cells—that NIST is developing as a reference material.

Monoclonal antibodies are the largest class of approved protein therapeutics in the world, and the ability to extend 2D-NMR methods to this class of therapeutic would represent an important landmark in their analytical characterization. Thirty laboratories on five continents will participate in the upcoming project. Beyond ascertaining the precision of 2D-NMR across a large network of laboratories, the effort is expected to yield a catalog of best practices to ensure the reliability and repeatability of results.

H. Ghasriani, D.J. Hodgson, R.G. Brinson, I. McEwen, L.F. Bushe, S. Kozlowski, J.P. Marino, Y. Aubin and D.A. Keire, Precision and Robustness of 2D-NMR for Structure Assessment of Filgrastim Biosimilars, *Nature Biotechnology*, published 5 February 2016. DOI: 10.1038/nbt.3474

STUDY HIGHLIGHTS NEED FOR BETTER CHARACTERIZED GENOMES FOR CLINICAL SEQUENCING (CONT'D.)

In some ways, significant parts of the genome are largely uncharted territory. Only about 5 percent of the 19,000 to 21,000 protein-encoding genes are situated entirely within portions of the human genome currently characterized with high confidence.

Unlike many research studies of groups of people, a "false call on a clinical report" can result in harmful consequences for patients, their families and even groups of people at risk for specific diseases, the researchers explain. Therefore, they say, it is critical to understand how accurately all regions of interest can be tested.

The team also points out that because current sequencing technologies are prone to systematic errors at certain genome locations, some variants reported in publicly available genome-sequencing databases may actually be false positives, or it may be difficult to distinguish between real variants and systematic sequencing errors.

The Stanford-NIST team found that, on average, about a fifth of each of the 56 disease-related genes flagged by ACMG is situated outside well-characterized, high-confidence regions of the NIST reference genome. Addressing this "sobering" state of affairs, the researchers write, requires working toward consensus across technologies or "at the very least," transparency in communicating the confidence level for every variant call.

The Genome in a Bottle Consortium is currently developing methods to integrate data from new technologies and analysis methods to characterize more challenging variants and regions of the genome, Zook says.

R.L. Goldfeder, J.R. Priest, J.M. Zook, M. Grove, D. Waggott, M. Wheeler, M. Salit and E. Ashley, "Medical implications of technical accuracy in genome sequencing." *Genome Medicine*, March 2, 2016. DOI: 10.1186/s13073-016-0269-0

OUTREACH AND PARTNERING

NIST AND FDA START NEW RESEARCH COLLABORATION

MML's Sumona Sarkar and Sheng Lin-Gibson are collaborating with the U.S. Food and Drug Administration to develop measurement assurance strategies to address regulatory needs in cell therapy and regenerative medicine products, initially focusing on improving confidence for cell viability measurements. FDA scientist Sema Rosinbum is joining NIST as a part-time guest researcher to conduct joint research and share expertise.

ISO/TC 229 TO DEVELOP PROTOCOL FOR NANOPARTICLE PHOTOCATALYTIC ACTIVITY

MML's Vytas Reipa and Nam Woong Song from the Korean Research Institute of Standards and Science are leading efforts for ISO/Technical Committee (TC) 229 International Standard "Nanotechnologies: Photocatalytic activity assay for nanoparticles in aqueous suspension." The committee's work will answer the need for a protocol to assess the hazard potential of nanoparticles with reactive oxygen species that are activated by light in biological environments. The protocol will be validated through interlaboratory comparisons and used as the basis of the TC 229 International Standard.

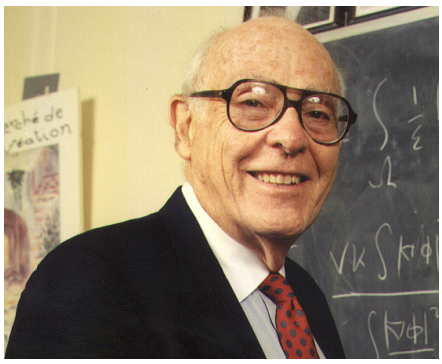
NIST TO JOIN INTERAGENCY COORDINATING COMMITTEE FOR THE VALIDATION OF ALTERNATIVE METHODS

NIST will formally join the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM), a part of the National Institute of Environmental Health Sciences' National Toxicology Program. The ICCVAM, with representatives from 15 different federal agencies, establishes guidelines and recommendations to facilitate acceptance of valid toxicological tests that minimize animal testing. MML's Elijah Petersen and John Elliott have been involved with the ICCVAM scientific advisory committee promoting strategies that provide measurement assurance to cell- and organism-based toxicology assays, particularly for nanomaterials. Petersen and Elliott expect to offer guidance on the development and analysis of assay protocols and the identification of measurement artifacts that increase confidence in nanotoxicology assay results.

EXPERTISE EXCHANGED WITH KOREAN INSTITUTE OF OCEAN SCIENCE AND TECHNOLOGY

MML's Jessica Reiner and Stacy Vander Pol traveled to Oahu, Hawaii in December 2015 to continue a collaboration with scientists from the Korean Institute of Ocean Science and Technology (KIOST). The visit was partially funded through an agreement between the Ministry of Oceans and Fisheries, Korea and the U.S. National Oceanic and Atmospheric Administration meant to foster knowledge transfer on ocean science. During the visit to Hawaii, Vander Pol demonstrated to KIOST scientists procedures for the sampling, homogenization, and storage of seabird egg samples. The egg samples will be archived as a part of the Seabird Tissue Archival and Monitoring Project at the NIST Marine Environmental Specimen Bank facility in Charleston, South Carolina. The KIOST scientists taught NIST staff sampling methods for macro- and microplastics from beaches. Sand samples from various beaches on Oahu were brought back to the NIST Hollings Marine Laboratory, where they will be used to explore development of a method for isolation of microplastics. NIST and KIOST scientists plan to continue their collaboration on microplastic pollution in marine environments by evaluating new isolation and measurement techniques and developing reference materials.

IN MEMORIAM



JOHN W. CAHN, 1928-2016

Senior NIST Fellow Emeritus John W. Cahn passed away on March 14, 2016 in Seattle, Washington, where he had moved with his wife Anne to be closer to their children and grandchildren. Cahn, a renowned theoretician in metallurgy and materials science, worked at NIST from 1977 to 2006. Cahn earned many honors while at NIST, including a Department of Commerce Gold Medal, the Franklin Institute's Bower Award, the National Medal of Science presented by President Bill Clinton, and the Kyoto Prize for Advanced Technology.

AWARDS

CREUZIGER, LOVESTEAD RECEIVE PRESIDENTIAL EARLY CAREER AWARDS FOR SCIENTISTS AND ENGINEERS

MML's Tara Lovestead and Adam Creuziger have received the Presidential Early Career Awards for Scientists and Engineers, the highest honor bestowed by the United States Government on science and engineering professionals in the early stages of their independent research careers.

Lovestead is honored for developing new methods for the detection of trace levels of chemicals. Her work with the Department of Homeland Security has improved detection of compounds used in improvised explosive devices and provided data for the certification of field detectors. Lovestead has also used these methods for the rapid detection of the chemicals emitted by spoiled food, allergens in seafood, and changes in grave soil—a method applicable to crime investigations. In addition, Lovestead has made significant contributions to thermodynamic models of alternative fuel mixtures, models that are used to optimize engine designs and predict the performance of engines burning new fuels.

Creuziger is honored for developing new neutron measurement methods of crucial crystal structures in advanced high-strength steel, which the automotive and other industries are evaluating as part of efforts to "lightweight" new cars to make them more fuel efficient. In addition to contributing to the success of the NIST Center for Automotive Lightweighting, Creuziger participated in a Materials Genome Initiative project led by General Motors to develop the next generation of advanced high-strength steel. He is also the co-developer of a neutron diffraction technique that may form the basis of an expanded ASTM standard method for measuring fractions of different crystal structures.

Creuziger (left) and Lovestead (fourth from left) with U.S. Department of Commerce Secretary Penny Pritzker (fourth from right) and other DoC and NIST staff at the NIST-NOAA PECASE Awards Luncheon. Credit: Derek Parks, NOAA

In addition to their outstanding technical work and contributions to the NIST mission, Lovestead and Creuziger actively contribute to the health of the scientific community through mentoring, education, and volunteer work.

SIEBER RECEIVES ASTM AWARD OF MERIT

MML research chemist John R. Sieber is a recipient of the 2016 ASTM International Award of Merit following his nomination by Committee E01 on Analytical Chemistry of Metals, Ores and Related Materials. The Award of Merit is the highest award granted by the society to a member for distinguished service and outstanding participation in ASTM International committee activities. Each recipient is elected as a Fellow of the Society.

MML PAPER HIGHLIGHTED IN ACS TOXICOLOGY JOURNAL

A paper by MML scientists published in the ACS journal *Chemical Research* in Toxicology was selected as one of the editorial board's top papers of the last two years. The January 2015 paper, entitled "Use of Cause-and-Effect Analysis to Design a High-Quality Nanocytotoxicity Assay," authored by NIST scientists John Elliott, Marc Salit, and Elijah Petersen with colleagues from EMPA (Switzerland), describes the use of cause-and-effect analysis to design a novel 96-well plate layout with process control measurements to quantify sources of variability and increase confidence in a nanocytotoxicity assay results. This work will help reduce variability often observed in nanocytotoxicity studies and improve data for assessing the potential hazards associated with engineered nanomaterials.

NIST STANDARD STORY

NIST scientists have thoroughly measured and characterized more than 1,300 physical products, NIST Standard Reference Materials®, to help people in industry, academia, and government agencies calibrate instruments, verify their test methods, and develop new measurement methods. NIST reference materials, for example, help manufacturers make interoperable parts in far-flung facilities, verify the accuracy of cholesterol and other clinical tests, and monitor environmental threats.

WILL THE LADY FROM NIST PLEASE COME UP HERE?

NIST analytical chemist Elizabeth Mackey had been to a lot of American Chemical Society meetings, but this was the first time she had attended a session focused on chemicals used in agriculture. An expert in measuring the elements in a variety of materials, Mackey was drawn in by the mention of trace elements in the title.

When the fertilizer company chemists in the room noticed Mackey's NIST affiliation on the sign-in sheet, they must have sighed in relief. They'd been struggling to respond to proposed state regulations on arsenic, cadmium, and other potential toxins in fertilizers, unintentional contaminants in the metals and ores companies used to fortify their products with nutrients like iron and zinc that make plants flourish.

Back then, around 2004, fertilizer company labs were accustomed to measuring beneficial nutrients, but most of them were not attuned to checking for additional elements in the mix. Many labs lacked methods to extract and measure small amounts of arsenic, cadmium, and other contaminants in the jumble of chemicals—many with different requirements for sample preparation and measurement—used to produce fertilizers.

Mackey was surprised when the session moderator asked, "Will the lady from NIST please come up here?" The moderator recognized the need for better analytical methods and reference materials to validate those new methods, and knew NIST's reputation for just that sort of work. The dialogue kicked off a collaboration among NIST and industry, state, and university test labs. "It was industry at its best," says Mackey. "Sometimes there's a concept that industry is trying to beat regulation, but these people worked closely with their regulators to identify problems and wanted the best products for their customers."

But first, fertilizer industry members needed guidance. Their initial request was for a list of 30 different reference materials. Mackey suggested that they develop a single reference material that could help labs test for the largest number of both nutrient and contaminant elements. A multi-nutrient blended fertilizer, donated by a fertilizer company, was just such a material. Mackey and her NIST colleagues used a series of advanced analytical methods to test for the fertilizer industry's list of "elements of interest" based on actual incidences of contamination and regulations. Because a typical fertilizer company lab is unlikely to use NIST's more time-consuming and costly analytical methods in day-to-day practice, an industry working group developed a test method within the capabilities of most labs, and 10 industry, state, and university labs participated in a "round robin," using the same methods on the same samples to determine how well the method worked.

Mackey and her collaborators spread the word about the new reference material and industry test method through participation at industry association meetings, and journal and trade publication articles. SRM 695 has been purchased by labs around the world, from Alabama to Wollongong.

Learn More: http://go.usa.gov/cuzPG

Technical Contact: Thomas Vetter, thomas.vetter@nist.gov

WHAT

Standard Reference Material 695, Trace Elements in Multi-Nutrient Fertilizer: About 70 grams of pulverized fertilizer pellets, donated by The Mosaic Company, that has undergone analysis by NIST scientists using a half dozen laboratory techniques or instruments.

WHY

Incidences of contamination of agricultural fertilizer with toxins arsenic and cadmium led to new regulations and fertilizer companies needing to test their products for compliance.

WHO

A collaboration among NIST and members of the Association of American Plant Food Control Officials and The Fertilizer Institute.

HOW

A lab analyzes NIST SRM 695 as if it were a typical sample, then checks to see if their results agree with the values on the NIST Certificate of Analysis, the result of rigorous measurement techniques. This check gives industry scientists and regulators confidence that their testing protocols are detecting contaminants and other elements of interest.

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K. W. Anderson, N. Mast, J. W. Hudgens, J. Lin, I. V. Turko, I. A. Pikuleva, "*Cholesterol Hydroxylase CYP46A1: Mapping of the Allosteric Site for Efavirenz, a Drug that Stimulates Enzyme Activity*" Journal of Biological Chemistry (07-Apr-2016)

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