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e_Connections

In This Issue

Synthetic bacteria just around the corner

DR. JOHN GLASS, PROFESSOR IN THE J. Craig Venter Institute's (JCVI) Synthetic Biology Group and director of the Mycoplasma Biology team at JCVI, recently gave a talk entitled "Synthetic genomics: Progress on construction of a synthetic bacterial cell" at the Virginia Bioinformatics Institute's Conference Center. The lecture, which took place on January 22, was the first talk in a new lecture series organized by GenBioOrg, a group founded by students in Virginia Tech's Genetics, Bioinformatics, and Computational Biology (GBCB) Ph.D. program.

Said Glass, "Three definitions of synthetic genomics are often encountered in the world of intelligent design. The first says synthetic biology is to genetic engineering as genomics is to genetics, which is an argument of scale. The second considers cells as machines. At JCVI, we like to define synthetic biology as the production of biological life, or essential components of living systems, by synthesis."

The JCVI research effort began by looking at how to build a minimal bacterial cell from first principles. The group hoped to determine the minimal machinery required for independent cellular life. At the outset of the work, Glass and collaborators selected *Mycoplasma genitalium* for investigation. *M. genitalium* is a sexually transmitted bacterium that has the smallest genome of any cellular organism grown in pure culture. The 582,970-base pair genome was the second microbial genome to be sequenced by JCVI (The Institute for Genomic Research as it was known in 1995).

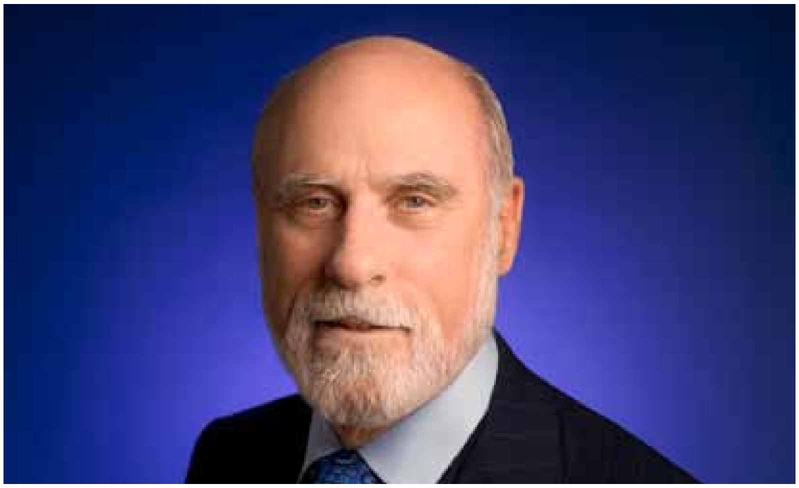
Comparative genomics suggested that members of the Mycoplasma family shared approximately 310 essential genes. Researchers confirmed that much of the *M. genitalium* genome was non-essential by transposon mutagenesis studies. Said Glass, "To move ahead on this project, we founded a new branch of synthetic biology – the design and assembly of genes and installation of the construct into a cellular milieu – or, to use a computer analogy, booting up the cell." An important change in technology made such an approach possible. "DNA synthesis is now a cost-effective commodity," commented Glass. "This has enabled efforts to produce synthetic cells right down to the level of specific nucleotides."

When the scientists started their work to synthesize the *M. genitalium* genome, a 583-kbp target was considerably larger than what had been constructed earlier (phiX174, poliovirus, coronavirus). The scientists established a 5-stage scheme to construct the circular chromosome of *M. genitalium* by cloning using bacterial artificial chromosomes in *Escherichia coli*. By the time they reached the fourth step in the assembly process they had hit a wall for *E. coli's* combining powers. Commented Glass, "We had been pushing the limits of cloning large DNAs in bacterial artificial chromosomes and we came unstuck. Amazingly we switched to yeast and, first time out, we got the whole synthetic genome, an exact match."

Once DNA assembly was a reality, the researchers turned their attention to the challenge of genome transplantation. To produce a synthetic cell, a synthetic genome must be transferred from yeast to a receptive cytoplasm. After many painstaking and meticulous experiments, the scientists honed in on an extremely narrow range of detergent and salt concentrations that permitted the transfer of a yeast-generated *Mycoplasma mycoides* genome into a related species *Mycoplasma capricolum*.

Said Glass: "There are three steps to making a synthetic cell: genome synthesis; genome transplantation; and transfer of the synthetic genome to a living organism to boot up that genome. The first two steps have been achieved and the last step is imminent. In the very near future, we will have a synthetic cell."

Much remains to be known about how to design a genome from scratch. Glass envisages a world where it will be possible to model and design cells computationally. Scientists will be able to create novel bacterial cells that produce fuels, pharmaceuticals, textiles, and other useful materials. It may prove feasible to put new organelles into eukaryotic algae to generate fine chemicals such as pharmaceuticals and biologicals. In the meantime, the Venter Institute team, on whose behalf Glass was speaking, continues to work toward their goal of inserting synthetic chromosomes into cells and "booting up" those cells to create the first synthetic organism. ■



Google vice president discusses future for the "unfinished Internet"

IT WAS STANDING ROOM ONLY AT THE Virginia Tech Holtzman Alumni Center's Alumni Assembly Hall on Dec. 7, 2009, when Vinton G. Cerf, vice president and chief Internet evangelist for Google, who has been described as a "Father of the Internet," visited campus to deliver the talk, "The Unfinished Internet." Faculty, staff, and students filled the seats of the hall, while a long line of standing attendees gathered in the back of the room.

After receiving an introduction from Virginia Tech President Charles Steger and providing a compliment to the university for its academic work and innovations, Cerf immediately delved into the focus of his talk, stressing the need for solutions to the biggest problems the Internet will face in the coming years. He also hinted at his reputation for making predictions about how technology will affect the future. Complementing this claim, Cerf presented a list of the major changes he sees for the Internet in 2010, including:

- Internationalized domain names (to include non-Latin characters)
- Domain name system security
- Digitally-signed address registration
- Wireless sensor network connection
- Integration of smart grids (energy consumption)
- Prominence of mobile devices

"We've done very badly with mobility," said Cerf, accepting part of the blame for this shortcoming. Along with Robert Kahn, Cerf co-designed TCP/IP protocols and the basic architecture of the Internet. He told the audience he and Kahn designed the Internet architecture for mainframe computers without considering that Internet Service Provider (ISP) addresses would eventually move around. "We have to accept that many will only have mobile devices as a method of access and be ready to adapt to this," he said.

In addition to mobility, Cerf highlighted other online issues that are in need of attention. He discussed the shortcomings of cloud collaboration. Cloud computing allows users to utilize services that they don't have knowledge about or control over. These services reside in a "cloud," where they can be shared. According to Cerf, while lots of companies are using clouds, including Microsoft, Amazon.com, and Google, the clouds aren't interacting with each other. What is needed, he said, is inter-cloud interaction, which would provide a way to move data back and forth between the clouds.

There are other areas that Cerf described as "Internet research problems," including security, which he identified as a monumental challenge at all levels, and authentication, identity, and authorization. Related to these issues, he expressed concern with protecting intellectual property on the Internet. "The Internet is like a big copy machine," he explained. "We need to come up with new ways of protecting materials."

According to Cerf, there are two things he would like the Internet to be—a resource that interprets data over long periods of time, which means supporting older file types to help information last, and one that remains accessible and permissive. "I would like the Internet to stay as open as possible, so people can try out new products and applications," Cerf said.

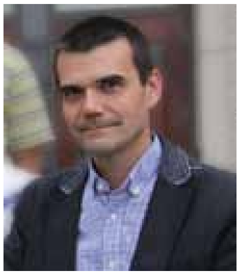
Cerf highlighted several applications that are openly available, thanks to the Internet's unrestricted access. His list of applications and their strengths, which featured several Google applications, included:

- Twitter – important tool for delivering instant information about emerging situations
- Google Wave – making all of the ways people interact available in one application
- Google Maps – using geography to represent information
- Google Earth – helpful tool for scientists to present research findings and illustrate scientific phenomena
- Android – open-source, adaptable operating system
- Google Chrome – a faster web browser that can run many applications simultaneously, including news applications
- YouTube – interactive video sharing that has helped unleash creativity and political power

Cerf holds a Ph.D. in Computer Science from the University of California, Los Angeles and more than a dozen honorary degrees. In 1997, President Clinton recognized Cerf and Kahn's work with the U.S. National Medal of Technology. In 2005, they received the highest civilian honor bestowed in the United States, the Presidential Medal of Freedom, which recognized that their work on the software code used to transmit data across the Internet put them at the forefront of a digital revolution that has transformed global commerce, communication, and entertainment. In his current position at Google, Cerf is responsible for identifying new enabling technologies and applications on the Internet and other platforms. Cerf is a fellow of the Institute of Electrical and Electronics Engineers (IEEE), the Association for Computing Machinery, the American Association for the Advancement of Science, the American Academy of Arts and Sciences, the International Engineering Consortium, the Computer History Museum, and the National Academy of Engineering. ■

VBI in the News

Peccoud chairs session at AAAS meeting on Synthetic Genomics



VBI Associate Professor Jean Peccoud chaired a session on "Customer Screening" at the meeting, "Minimizing the Risks of Synthetic DNA: Scientists' Views on the U.S. Government's Guidance on Synthetic Genomics," which was held on January 11, 2010, in Washington, D.C. and sponsored by the American Association for the Advancement of Science (AAAS). According to event organizers, the construction of the poliovirus and phiX genomes from synthetic DNA fragments, which are examples of what has come to be called synthetic genomics, have created concerns within the American biosecurity community about the use of chemically synthesized DNA to create harmful biological agents. To minimize the risks of synthetic genomics, the U.S. Government has developed draft guidance for synthetic DNA vendors. The AAAS Center for Science, Technology, and Security Policy organized this forum for representatives from different sectors of the research community to discuss and react to the draft guidance and to interact with officials from federal agencies involved in its development.

Shulaev named Virginia Tech Scholar of the Week

The Office of the Vice President of Research at Virginia Tech selected VBI Professor Vladimir Shulaev as its "Virginia Tech Scholar of the Week" for the week of December 14, 2009. The Scholar of the Week is featured on the main page of the university's Office of the Vice President of Research's website. The goals of the feature are to recognize individuals working at Virginia Tech while also telling people about research and scholarly achievements at the university. Recognition is based on research and/or scholarship. Shulaev's Research Group at VBI is interested in developing metabolomics technologies and applying high-throughput metabolite profiling to study stress response in microorganisms, plants and animals.

Decoding the turkey's genetic gobbledygook: Va. Tech research could lead to 'happier' birds, bigger breasts or legs

Washington (Washington Post) -- If ever there were a candidate for genetic engineering, surely it is the pale, flavor-challenged bird that will adorn millions of American dinner tables Thursday as a matter of Thanksgiving ritual.

And here is a reason to give thanks: The day of the super-turkey might be nigh. Virginia Tech scientists announced this week that they have secured funding to complete the genetic map of *Meleagris gallopavo*, the domesticated turkey. The U.S. Department of Agriculture has awarded a two-year, $908,000 grant to Tech and the University of Minnesota to finish decoding the turkey, one of a few species to be mapped at the genetic level. Turkeys are the fourth-leading source of meat on dinner tables. Cows, chickens and pigs have been genetically catalogued.

The possibilities for genetic manipulation seem endless. At a minimum, the turkey might be genetically engineered to convey a bit more flavor. University scientists say genetic mapping will help turkeys lead healthier lives. Breeders will come to know how the turkey immune system works and how to fight off such pathogens as bird flu.

"The traits you might want to improve are sometimes complex and not defined by a single gene," said Otto Folkerts, associate director of technology development at the Virginia Bioinformatics Institute at Tech. "Sometimes people might want a turkey to taste more like a wild turkey. You can start addressing flavor traits, texture traits."

A group called the Turkey Genome Sequencing Consortium, including researchers from the University of Maryland and the USDA as well as Tech and Minnesota, picked up the genetic drumstick a year ago.

Source: Abridged from The Washington Post, November 26, 2009

VBI scientific publications

Modulation of hepatic PPAR expression during Ft LVS LPS-induced protection from *Francisella tularensis* LVS infection. Mohapatra SK, Cole LE, Evans C, Sobral BW, Bassaganya-Riera J, Hontecillas R, Vogel SN, Crasta OR. *BMC Infectious Diseases* 2010 **10:**10 doi:10.1186/1471-2334-10-10.

A new paper in *BMC Infectious Diseases* suggests that lipopolysaccharide (LPS)-induced blunting of the pro-inflammatory response in mouse is, in part, mediated by peroxisome proliferator activated receptors or PPARs (alpha and gamma). Previously it had been shown that administration of *Francisella tularensis* (Ft) Live Vaccine Strain (LVS) lipopolysaccharide (LPS) protects mice against a further challenge with Ft LVS and blunts the pro-inflammatory cytokine response. The researchers used Affymetrix arrays to profile global hepatic gene expression following Ft LVS LPS or saline pretreatment and subsequent Ft LVS challenge. A large number of genes (more than 3,000) were differentially expressed at 48 hours post-infection. The degree of modulation of inflammatory genes by infection was clearly attenuated by pretreatment with Ft LVS LPS in the surviving mice. However, Ft LVS LPS alone had a subtle effect on the gene expression profile of the uninfected mice. By employing gene-set enrichment analysis, the scientists discovered significant up-regulation of the fatty acid metabolism pathway, which is regulated by PPARs.

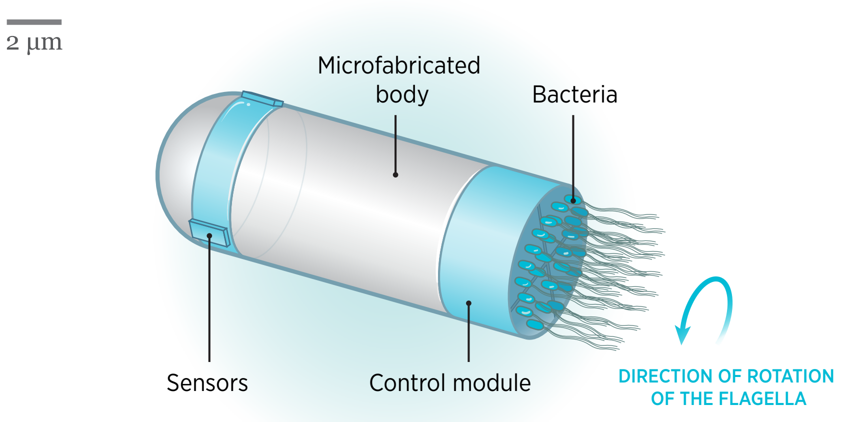
VBI welcomes mechanical engineering faculty

TWO VIRGINIA TECH MECHANICAL engineering faculty are bringing new areas of expertise to the Virginia Bioinformatics Institute (VBI) on the Virginia Tech campus. Ranga Pitchumani and Bahareh Behkam recently moved into laboratory space at VBI's main building to pursue their research interests in advanced materials science and biomechanical engineering.

Ranga Pitchumani, John R. Jones III Professor of Mechanical Engineering and Associate Department Head for Research in Mechanical Engineering at Virginia Tech, is interested in five main areas of research including advanced material processing; microsystems and microfabrication; energy (fuel cells as well as solar, wind, and energy-storage technologies); biofluidics; and uncertainty quantification and analysis. In the area of biofluidics, Dr. Pitchumani is trying to understand how complex physical phenomena, for example the flow of liquids, take place in small- and large-scale systems. Bahareh Behkam, Assistant Professor in the Department of Mechanical Engineering at Virginia Tech, is the director of the university's MicroN BASE Laboratory. Her research interests include the design, modeling, and fabrication of biologically integrated micro/nano systems, biomimetic micro-robotics, directed self-assembly, and the biophysics of bacterial motility and adhesion.

In seminars at VBI, Pitchumani and Behkam outlined some of their work that touches on applications in the life sciences. Dr. Pitchumani described how physical-based simulations could be used as guidelines for biological design in two projects that he is working on. The first project, a collaboration with Sandia National Laboratories, looks at the fabrication of high aspect ratio microstructures (HARMS). Said Pitchumani, "In this investigation, we are using fundamental science to widen the materials used in the manufacture of electroforming micro molds from silicon to ceramics and other materials. This should help to improve the quality and consistency of the manufacturing process." Electroforming is a process that allows a "metal skin" to be built on a metal surface. This is achieved by the application of an electric current to a paint that contains metal particles. Micro molds are used to manufacture very small microscale components and products that are used in industries like electronics, biomedicine and information technology.

CONCEPTUAL DRAWING OF A HYBRID SWIMMING ROBOT



The second project, which represents a new research path for Dr. Pitchumani, is a bioapplication design initiative that looks at the optimal design of bypasses for carotid and coronary arteries. This work is helping to generate guidelines that help, for example, to minimize recirculation zones when bypasses are present in blood arteries. Recirculation zones produce inadequate cleansing of the arterial walls and may lead to health problems such as restenosis. Said Pitchumani, "Physics-based simulations can give useful guidelines for bypass design. By seeking a physical understanding of the process, we hope to be able to tailor the design of bypasses in such a way that will bring clinical benefits to specific groups of patients."

In a separate seminar, Dr. Behkam outlined her work on mobile bio-hybrid microrobots that can access small spaces and which offer potential applications for minimally invasive diagnosis and the localized treatment of diseases, environmental monitoring, and homeland security. Employed in large numbers, microrobots can serve as inexpensive agents of distributed systems for swarm robotic applications.

Said Behkam, "While the potential impact of these microrobotic systems is high, particularly for biomedical applications, many challenges remain in developing such microrobots." One of most significant obstacles to make mobile robots work at micron-length dimensions is the miniaturization of on-board actuators and power sources required for mobility. Actuators are mechanical devices for moving or controlling a mechanism or system.

Added Behkam, "To overcome some of these limitations, we have focused on interfacing live microorganisms, for example bacteria, with a microfabricated robot body to develop bio-hybrid microrobotic systems. We are currently using bacteria for controlled actuation of microscale robots. The ultimate goal is to use these bacteria to achieve more sophisticated levels of control and added functionalities such as sensing." ■

VBI e_Connections is a quarterly publication of the Virginia Bioinformatics Institute produced by the Public Relations team. The newsletter includes feature articles, technology updates as well as interviews that may be of interest to VBI's audiences. Contributions are welcomed. Please direct submissions to newsletter-editor@vbi.vt.edu. Newsletter team: Susan Bland: Editor; Barry Whyte: Editor.

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