DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

NATIONAL ADVISORY COUNCIL FOR BIOMEDICAL IMAGING AND BIOENGINEERING

Summary of Meeting[1]
May 21, 2012

The National Advisory Council for Biomedical Imaging and Bioengineering (NACBIB) was convened for its 29th meeting on May 21, 2012, at the Bolger Center in Potomac, Maryland. Dr. Roderic I. Pettigrew, Director of the National Institute of Biomedical Imaging and Bioengineering (NIBIB), presided as Council chairperson. In accordance with Public Law 92-463, the meeting was open to the public from 9:00 a.m. to 12:15 p.m. for review and discussion of program development, needs, and policy. The meeting was closed to the public from 1:15 p.m. to 2:00 p.m. for consideration of individual grant applications.

Council members present:
Dr. Philip Alderson, Saint Louis University, St. Louis, MO
Dr. John C. Gore, Vanderbilt University, Nashville, TN
Dr. W. Eric L. Grimson, Massachusetts Institute of Technology, Cambridge, MA
Dr. Hedvig Hricak, Memorial Sloan Kettering Cancer Center, New York, NY
Dr. Nola M. Hylton, University of California, San Francisco, CA
Dr. Cato T. Laurencin, University of Connecticut, Farmington, CT
Dr. Mark Musen, Stanford University, Stanford, CA
Dr. Etta D. Pisano, Medical University of South Carolina, Charleston, SC
Dr. Buddy Ratner, University of Washington, Seattle, WA
Dr. Michael Yaszemski, Mayo Clinic College of Medicine, Rochester, MN

Ex officio members present:
Dr. P. Hunter Peckham, U.S. Department of Veterans Affairs, Cleveland, OH
Dr. Anne Plant, National Institute of Standards and Technology, Gaithersburg, MD

Council member absent:
Dr. Cherri Pancake, Oregon State University, Corvallis, OR

Ex officio members absent:
Dr. Francis Collins, National Institutes of Health, Bethesda, MD
Dr. John McGrath, National Science Foundation, Arlington, VA
Ms. Kathleen Sebelius, U.S. Department of Health and Human Services, Washington, DC
Dr. James G. Smirniotopoulos, Uniformed Services University of the Health Sciences, Bethesda, MD

Chairperson:
Dr. Roderic I. Pettigrew

Executive Secretary:
Dr. Anthony Demsey

[1] For the record, it is noted that members absent themselves from the meeting when the Council is discussing applications (a) from their respective institutions or (b) in which a conflict of interest may occur. This procedure only applies to applications that are discussed individually, not to "en bloc" actions.

Also present:
NIBIB staff present for portions of the meeting:

Mr. Angelos Bacas
Dr. Richard A. Baird
Ms. Sheila Barrett
Ms. Angela Burks
Ms. Shirley Coney-Johnson
Dr. Richard Conroy
Ms. Christine Cooper
Ms. Nancy Curling
Ms. Marilyn Daly
Ms. Monique Day
Ms. Kate Egan
Ms. Angela Eldridge
Ms. Kathryn Ellis
Dr. Zeynep Erim
Ms. Carol Fitzpatrick
Dr. David George
Ms. Marie Gill
Ms. Pam Glikman
Dr. John Hayes
Ms. Eunica Haynes
Dr. William Heetderks
Mr. James Huff
Dr. Rosemarie Hunziker
Mr. Tom Izzard
Dr. Chris Kelley
Dr. Brenda Korte
Dr. Steven Krosnick
Mr. Eugene Lee
Dr. Christina Liu
Dr. Guoying Liu
Dr. Hector Lopez
Dr. Xiao-Zhong (James) Luo
Dr. Alan McLaughlin
Ms. Jessica Meade
Mr. Todd Merchak
Mr. Larry Morton
Mr. Joe Mosimann
Dr. Peter Moy
Dr. Vinay Pai
Dr. Grace Peng
Dr. Karen Peterson
Mr. Mohammed Rahamatullah
Dr. Mary Rodgers
Ms. Christine Rogers
Ms. Stephanie Sabourin
Dr. Antonio Sastre
Dr. Belinda P. Seto
Mr. Shaun Sims
Dr. Manana Sukhareva
Mr. Tom Tran
Ms. Florence Turska
Ms. Li-Yin Xi
Dr. Ruixia Zhou
Dr. Steven Zullo

Non-NIBIB NIH employees:
Dr. David Filpula, Center for Scientific Review
Dr. Thomas Mampilly, Fogarty International Center
Dr. Paul Sammak, Center for Scientific Review
Dr. Ross Shonat, Center for Scientific Review

Non-NIH Federal employees:
None

Members of the public present for portions of the meeting:
Ms. Melissa Blessing, American Association of Colleges of Osteopathic Medicine
Dr. (J.J.) James Collins, Boston University and Harvard University
Mr. Sean Gallagher, American Institute for Medical and Biological Engineering
Ms. Allyson Harkey, NOVA Research Company
Ms. Lori Pellnitz, SRI International
Mr. Matt Sherman, National Capital Captioning

I. Call to Order: Dr. Anthony Demsey

Dr. Demsey called to order the 29th meeting of the National Advisory Council for Biomedical Imaging and Bioengineering. He reminded attendees that the morning session of the meeting was open to the public, welcomed attendees, and introduced Dr. Pettigrew, who formally welcomed all participants.

II. Director's Remarks: Dr. Roderic I. Pettigrew

A. Outgoing Council Members

Dr. Pettigrew thanked Drs. Philip Alderson and Cherri Pancake for their service to the Council.

B. Budget and Legislation

There have been no significant changes to the NIBIB budget since the last Council meeting.

C. NIBIB Achievements

New Policy on Principal Investigator Funding Level

Per the President's 2013 Budget Request, Institutes across the National Institutes of Health (NIH) are piloting a new evaluation procedure for competing research project grant (RPG) applications from investigators whose total NIH RPG funding exceeds $1.5 million. An investigator's total funding is determined by combining active research project grant awards, multiyear awards active in the current fiscal year, investigator-specific components of current P01 grants (e.g., project leader funds, excluding core costs), investigator-specific components of multi-Principal Investigator/Project Director (PI/PD) grants, and multiyear supplements including the out years (excluding diversity and reentry supplements). NACBIB anticipates an average of about one such application per Council meeting. Responses to Requests for Applications (RFAs) as well as applications for P01s, other multicomponent research projects, and multi-PI/PD projects would be excluded from the new evaluation procedure, unless all of the PIs/PDs exceed the $1.5 million threshold.

This special Council review is intended to provide flexibility for Institutes/Centers and Councils when establishing how to weigh factors such as inherent cost differences in certain areas of research. Councils may also advise Institutes on mechanisms that might be excluded from the policy. For example, NIBIB's Quantum Grants, which are intended to be large-scale projects with budgets that would exceed $1.5 million, could be excluded.

National Plan to Address Alzheimer's Disease

President Obama recently signed the National Alzheimer's Project Act, which calls for an aggressive and coordinated national Alzheimer's disease plan. The plan directs the expenditure of $50 million in FY2012 and $80 million in FY2013 to NIH to accelerate research efforts. Half of the FY2012 funds will be used by the National Human Genome Research Institute to evaluate a cohort of patients; the other $25 million will be distributed among the Alzheimer's Prevention Initiative (a large-scale, long-term study of 200 patients with the gene mutation for early-onset Alzheimer's disease), the Atherosclerosis Risk in Communities Study (evaluating the vascular contribution to dementia and Alzheimer's disease), a clinical trial exploring the effect of intranasal insulin on cognition and function, and an RFA on induced pluripotent stem cells.

R25 Clinician-Scientist Initiative

The R25 NIBIB training initiative is intended to foster the careers of clinician-scientist PIs through institutional support for research training of residents and clinical fellows. Trainees may come from any clinical department where the research is consistent with the NIBIB mission. The R25 requires a 75-percent effort for up to 24 months of training. PAR-12-085 was released January 27, 2012, and letters of intent were due April 23. Applications are due May 23, with the earliest start date being April 1, 2013. The PAR will be reissued annually for the next 2 years.

National Bioeconomy Blueprint and DEBUT

The White House recently published its National Bioeconomy Blueprint from the Office of Science and Technology Policy, which included a mention of NIBIB's Design by Biomedical Undergraduate Teams

(DEBUT) initiative. DEBUT awards $10,000 prizes to winning teams of undergraduate engineers. The first awards will be announced at the annual meeting of the Biomedical Engineering Society in September 2012.

Computed Tomography (CT) Radiation Dose Reduction

The 2011 Summit on Management of CT Radiation Dose: Toward the Sub-mSv Exam has resulted in two papers: "Radiation Exposure: How to Close Our Knowledge Gaps, Monitor and Safeguard Exposure" and "Achieving Routine Sub-mSv CT Scanning: Report from the Summit on Management of Radiation Dose in CT." Both are in press in the *Journal of Radiology*. The objective of the Summit was to identify the technical steps and research needed in order to provide a routine sub-millisievert CT exam and, thus, significantly reduce concerns about radiation-induced cancer. NIBIB is developing an initiative to encourage such research.

D. NIBIB 10th Anniversary Program

NIBIB's 10th Anniversary celebration will be held June 21–22, 2012. Coincidentally, the American Institute for Medical and Biological Engineering (AIMBE) will hold a Federal symposium on June 20 followed by a day of meetings and presentations to members of Congress and their staffs, including a presentation by Dr. William Heetderks and NIBIB-funded scientists, on June 21. An NIBIB-sponsored dinner on the evening of June 21 will feature presentations by Dr. C. Douglas Maynard and Mr. Robert Bazell, NBC science correspondent. The NIBIB scientific symposium on June 22 will feature notable investigators from Government, industry, and academia, including two Nobel laureates, two National Medal of Science laureates, and one National Medal of Technology laureate.

The program will showcase a number of NIBIB-supported and -developed technologies that have or will impact the nation's health care. Two patients who have benefited from such technologies will share their experiences, and Drs. Carla Pugh and Hari Shroff, two Presidential Early Career Award for Scientists and Engineers (PECASE) recipients, will present their work.

The "NIBIB In Video" segments of the program will feature NIBIB-supported innovations and scientific discoveries and their impact on health. Dr. Pettigrew previewed one of the videos that will be shown at the symposium.

E. Science Highlights

Optimizing Cardiovascular Device Thromboresistance for Eliminating Anticoagulants

Drs. Danny Bluestein and Marvin Slepian hold a Quantum Award focusing on redesigning cardiovascular devices that result in undesired thromboresistance so as to eliminate the need for anticoagulants. The use of anticoagulants increases bleeding complications that often require surgical intervention. Thrombosis begins when sheer stress activates platelets. By subtly changing the contour, angle, and position of the device blades, Dr. Bluestein dramatically reduced maximum stress levels and duration. This new design is being evaluated in subjects now.

BrainGate

Dr. John Donahue and his colleagues at the Brown Institute for Brain Science have developed BrainGate, a brain-computer interface that allows tetraplegics to control a prosthetic or robotic arm simply by thinking about the movement of their own paralyzed hand. A tiny sensor implanted on the brain's surface picks up electrical impulses and transmits them to a computer translator that controls the robotic limb. Dr. Pettigrew presented a video of the science.

III. Review of Council Procedures and Regulations: Dr. Anthony Demsey

Dr. Demsey noted for the record that a quorum was present for this Council meeting. Council member Dr. Cherri Pancake was unable to attend. Dr. Michael Yaszemski joined the closed session via telephone.

Dr. Demsey welcomed visitors and members of the science press and scientific society constituencies. He thanked Ms. Carol Fitzpatrick and Ms. Pam Glikman for planning the meeting.

A. Council Regulations, Policies, and Procedures

Dr. Demsey summarized elements of the Government in the Sunshine Act and the Federal Advisory Committee Act that govern all Advisory Council meetings. These Acts require the U.S. Department of Health and Human Services to open Advisory Council meetings to the public except when proprietary or personal information is discussed. To comply with these regulations, NACBIB meetings are open to the public for all except the review of individual grant applications. Dr. Demsey reviewed conflict-of-interest, confidentiality, and lobbying guidelines.

The Council operating procedures have been updated, with a minor change on page 6: "Consideration of Applications from Already Well-Funded Investigators." This change relates to the new evaluation policy on PI funding level, previously outlined by Dr. Pettigrew. A motion to approve the updated operating procedures was forwarded, seconded, and unanimously approved. The updated procedures will go into effect at the next Council meeting.

B. Future NACBIB Meeting Dates

The next NACBIB meeting is scheduled for Friday, September 14, 2012, with the site to be determined. Dr. Demsey asked Council members to inform him about conflicts with any of the upcoming meeting dates listed at the bottom of the agenda.

C. Approval of the January 20, 2012, NACBIB Meeting Minutes

A motion to approve minutes of the January 20, 2012, NACBIB meeting was forwarded, seconded, and approved unanimously.

IV. Elimination of Second Amended Application

Study sections reviewing grant applications routinely provide feedback to the submitting investigator, who uses this feedback to amend the application and resubmit for further review. In 2010, NIH stopped reviewing applications after the first ("single amendment") resubmission.

Although there are no hard data on the impact of this policy change at present, Dr. Pettigrew noted that emerging data seem to indicate a trend toward shorter times to award. Dr. Pettigrew encouraged Council members to discuss their experiences with the new policy in order to inform future analyses.

Discussion

Dr. Etta Pisano questioned whether the new policy has reduced the study sections' workloads as intended. The review of a second amendment is easier than that of a new submission.

Dr. John Gore stated that investigators should be allowed to resubmit what would be a second amended grant application (A-2) that received a reasonably good review as a new grant. Perhaps NIH should not restrict investigators with meritorious applications from submitting them as many times as they would like, particularly considering the current tight funding environment. Dr. Hedvig Hricak commented that it is her understanding that "new" grant applications must be at least 51 percent different from previous applications. Dr. Gore suggested a percentile cutoff; for example, if a proposal does not make it past the 50^{th} percentile in two rounds, it cannot be resubmitted without significant changes.

Dr. Eric Grimson wondered whether, when faced with resubmissions, review panels wear down and score applications well based on the fact that the investigator amended the application appropriately. That behavior could lower the bar for success.

Dr. Hricak stated that the biggest challenge is in preserving the more mature investigator and encouraging junior investigators to step up. Both NIH and academia must change to protect more experienced investigators to give them the freedom to mentor the next generation.

Dr. Cato Laurencin noted that the new system offers less feedback to investigators. Drs. Pisano, Michael Yaszemski, and Nola Hylton also expressed concern about this feedback reduction. If investigators have only one opportunity to refine applications, they need as much thorough feedback as possible to guide their revisions. In addition, junior investigators are missing the opportunity to hone their grant application skills.

Dr. Philip Alderson described an internal review panel at St. Louis University. Those who finish their applications early enough go through this internal review, which provides them with feedback and hints on what has worked in the past and how to best ready the proposal for submission.

Dr. Buddy Ratner stated that the study sections do well at discerning the upper one-third of applications from the lower two-thirds, but discerning the upper 10 percent is more difficult. Meritorious proposals that have the potential to transform human health should have the chance to work their way through the system, even if that means a third review. NIH's mission is to get science funded, not to play games with an almost arbitrary system.

Dr. Laurencin suggested that the policy makes it more difficult for the best science to be supported. The reliability and consistency of the review process should be tested, and NIH should poll outside researchers to examine their perceptions and experiences with the new review process.

Dr. Mark Musen noted that most of his colleagues resubmit proposals that have barely missed the percentile cutoff for funding. The rules were written for a different funding situation; investigators may not have radically new ideas, but they want to obtain the last five or six percentile points in order to get funded. If they were to submit completely different proposals, they would be back at the 20th percentile. Dr. Pisano reiterated that the system is not sensitive enough. Science that barely falls out of fundable range and is not funded within two cycles of review is lost.

Dr. Hricak noted that the Council must find a good way to work within the new system. One way would be to encourage mentorship through a reward system; for instance, more junior investigators winning grants would translate to more funding, resources, etc. Dr. Ratner disagreed, stating that at some point someone will appreciate that the technology being proposed will result in savings in the health system and that they will see the importance of investing both for the economy and for human health.

Dr. Ratner suggested that it would be valuable to have a future Council discussion about the budget, the study section system, and how issues might be addressed.

V. U.S.-India Workgroup Report: William Heetderks, M.D., Ph.D.

The U.S.-India Workgroup met before the Council meeting to discuss collaborative activities between the United States and India to develop a blood pressure monitoring system. At last year's workshop in India, the problem of hypertension and monitoring of blood pressure was identified as a major opportunity for development in India. Likewise, an article in the *New England Journal of Medicine* by Drs. Thomas Frieden (Director, Centers for Disease Control and Prevention) and Don Berwick (then-Administrator, Centers for Medicare & Medicaid Services) identified cardiovascular disease as the leading cause of death in the United States and called for the creation of the Million Hearts Initiative to prevent 1 million heart attacks or strokes over the next 5 years. This Initiative would implement proven, effective, inexpensive therapeutics, including blood pressure control.

The Workgroup has been discussing how to improve blood pressure control in those hypertensive individuals who are not currently well controlled. Dr. Heetderks suggested three areas to consider: developing better sensors for measuring blood pressure; improving the flow of information from the sensors to the patient and his or her electronic health record; and allowing patients and physicians to act on that information. The sensors must be integrated into a system for blood pressure control. The Workgroup recommends

proceeding in phases, beginning with sensor development. One possibility is to develop low-cost devices for use by individuals; another is to develop sensors that may not be low cost themselves but would have low per-measurement cost (i.e., one large device with many users).

The Workgroup proposed proceeding with an initiative to focus on development of sensing devices. Dr. Pettigrew added that wearable devices could be quite successful even in rural India, where cell phone service is ubiquitous.

Dr. Pisano wondered why the problem is being approached from a technological standpoint as opposed to a public health standpoint. The problem seems to be access to care. Dr. Heetderks responded that effortless sensing of blood pressure is a significant part of the problem, but that the larger issue is one of public health.

A recommendation to proceed with the proposed initiative was forwarded, seconded, and unanimously approved.

VI. Synthetic Biology: Biomedical Applications Come of Age: James J. Collins, Ph.D.

Dr. Pettigrew introduced Dr. James J. Collins, Ph.D., the William F. Warren Distinguished Professor of Biomedical Engineering at Boston University, and core founding faculty member of the Wyss Institute for Biologically Inspired Engineering at Harvard University. Dr. Collins received his A.B. in physics at the College of the Holy Cross and his Ph.D. in medical engineering at University of Oxford. He has received a MacArthur Fellowship and Boston University's Metcalf Cup and has been inducted into the National Academy of Engineering and the National Academy of Arts and Sciences.

Dr. Collins described his work in synthetic biology, a field that develops ways to reprogram organisms to perform a variety of useful tasks by manipulating gene networks. The field, only 12 years old, was founded on the work of the Human Genome Project (HGP) and necessitates collaboration between engineers and molecular biologists. Molecular biologists were interested in engaging biomedical engineers to reconstruct the inner workings of cells (i.e., a reverse engineering approach), but the dearth of functional data at the time made this nearly impossible. Instead, Dr. Collins and his colleagues applied a forward engineering approach, taking apart the various elements and reorganizing them in novel networks to endow cells with new functions.

Dr. Collins' earliest work in this field, published with then-postdoctoral student Dr. Tim Gardner, was designing a genetic toggle switch with two repressor genes in a mutually inhibitory network. The system was designed to exist in one of two stable states: State A (gene 1 is on, gene 2 is off) or State B (gene 2 is on, gene 1 is off). One would then be able to flip between those stable states by delivering a stimulus (e.g., a chemical, environmental change). Dr. Gardner designed multiple bistable E. coli toggle switches with lacI and temperature-sensitive cI.

By 2000, the notion of biocomputing—specifically, programming cells for insertion in the body to carry out different functions—was beginning to take hold. Drs. Collins and Gardner inserted a synthetic gene network into cells and coupled the network to natural input and output pathways. Dr. Collins and his colleagues used these engineered gene circuits to create highly sensitive, whole-cell biosensors to detect DNA damage. They coupled the toggle to switch on in the presence of DNA damage and record a memory element of the event. The switches were highly stable, remaining in the off state indefinitely until a stimulus was delivered, in this case mitomycin C or ultraviolet radiation. More sensitive than commercially available DNA damage sensors, the switches responded to very low concentrations of stimulus, switching on and indicating via rheostat readout how much DNA damage was in the space. A fluorescing biosensor to biofilm readout was also possible.

Dr. Ron Weiss, Massachusetts Institute of Technology, has engineered these sensors to enable intercell communication toward creating a population of cells that can, for example, be used to identify the source of a heavy metal in the environment. Under a grant from the Office of Naval Research, he and Dr. Collins are currently attempting to reengineer microbes to detect explosives and other chemicals in marine environments that would then communicate with micro-robots to remediate the action.

Another application of this system is of special interest to the Department of Energy and the bioenergy industry. There is interest in using bacteria to generate energy (e.g., convert sunlight or cellulose into fuel); however, the high cost of the chemical inducers needed to kickstart these processes at the right time limited the utility of this approach. Energy industry representatives have challenged Drs. Collins and Gardner to develop programmable bacteria that need no chemical inducers. Drs. Collins' and Gardner's bacteria are programmed to switch on when a certain density is reached. This works beautifully inside bioreactors, and they currently are working with several biotechnology firms to incorporate it as a cost-saving measure inside their bioreactors.

Most synthetic switches in the literature are transcriptional, but now researchers are working to engineer protein-DNA interactions. Dr. Collins' colleagues have been attempting to harness the regulatory properties of RNA to create novel switches. Drs. Farren Isaacs and Daniel Dwyer designed a switch that uses an engineered cis-repression sequence that constitutively blocks protein expression and a short, non-coding, transactivating RNA that interacts with the cis-repression sequence to allow protein expression. The system has a very fast response time (under 5 minutes) and strong on/off control. The RNA switch also can be adjusted to allow varying levels of protein expression and be partnered with a number of switches for sequence specificity and simultaneous, independent intracellular operation.

A number of ethical and social concerns have arisen regarding synthetic biology. To address concerns about the potential effects of the long-term presence of engineered cells in living organisms and/or the environment, Dr. Collins and his colleagues considered how to program bacteria to die after they had been in a patient's body for a certain period of time. For this purpose, they designed two different types of switches: a daisy chain cascade and a kill switch. The daisy chain cascade is used with both RNA switches and recombinases to "count down" to the end of a programmed bacteria's life. The kill switch is activated by the simultaneous production of two different proteins, each of which is expressed in response to a distinct stimulus; thus, the bacteria survive normally when neither or one of the stimuli are present, but die rapidly when exposed to both stimuli.

In 2009, public concern about man's ability to "create" life and control it came to a head when Dr. Craig Venter announced the creation of a synthetic bacterial cell. This work earned significant response from the public and industrial sectors. President Obama charged the Presidential Commission for the Study of Bioethical Issues with evaluating the field of synthetic biology. They held three public hearings and highlighted Dr. Collins' work in their final report as a much-needed safeguard for engineered organisms that are used in a laboratory or inserted into patients' bodies. The Defense Advanced Research Projects Agency launched a new program built around this and related schemes for security purposes. In efforts to protect against industrial espionage, biotech companies have asked Dr. Collins to engineer a switch that will kill the programmed microbe and shred the DNA if the proprietary material is stolen. Dr. Collins is currently working on the first of those two requirements.

Synthetic biology shows great promise in the medical realm, from identifying new drug targets and their mechanisms of action to identifying new drugs, delivery methods, and production techniques. Drs. Collins and Gardner realized that they could use their toggle switch to reverse engineer natural networks, flipping genes on and off and measuring responses to understand the structure of the network. As the pharmaceutical industry is moving away from development of new antibiotics—despite growing numbers of bacterial strains resistant to existing antibiotics—Dr. Collins and his associates decided to focus on enhancing the existing arsenal of antibiotics. They collected hundreds of gene expression profiles for E. coli and reconstructed networks on a genome scale. They used systems analysis coupled with synthetic biology to examine quinolones, a class of antibiotics that target DNA gyrase. They also used their RNA switch to control expression of CcdB, an endogenous protein that also inhibits gyrase but has been difficult to study using traditional approaches. Using network analysis, they discovered that the DNA damage response, oxidative damage response, iron uptake and utilization, and iron-sulfur cluster synthesis networks all were activated when gyrase was inhibited either by quinolones or increased expression of CcdB.

Two of Dr. Collins' students, Drs. Dan Dwyer and Michael Kohanski, conducted phenotypic studies that uncovered that quinolones induce a common oxidative damage cell death pathway that involves a burst of superoxide that (1) leeches iron from internal iron-sulfur clusters, (2) produces a Fenton's reaction, and (3)

produces lethal hydroxyl radicals that damage DNA, lipids, proteins, and many of the nucleotides inside a cell, causing death. Further studies of other major classes of antibiotics revealed that cell-killing antibodies all induce the same common oxidative damage cell death pathway; they also disrupt central metabolic feedback, which leads to hyperactivation of the electron transport chain, a shift in the NAD-NADH ratio, Fenton's reaction, production of radicals and hydroxyl radicals, and cell death.

Drs. Collins, Kohanski, and Dwyer worked to harness this newly discovered pathway to enhance existing antibiotics. If all cell-killing antibiotics work by damaging DNA, then blocking protective responses to DNA damage (e.g., by interfering with gateway protein RecA) should enhance killing efficacy of antibiotics—and possibly help resensitize antibiotic-resistant disease strains, thereby slowing the emergence of future resistance. Using an NIH-supported natural products library at Boston University and the Department of Defense Screening Library at Harvard, Drs. Guillaume Cottarel and Jamey Wierzbowski set up a high-throughput RecA assay in the Collins Laboratory. They examined more than 50,000 bioactive compounds and found several small molecules that are candidates for blocking RecA.

Bacteriophages (viruses that only attack bacteria) also can be engineered. Dr. Tim Lu engineered bacteriophages to overexpress *lexA*, which represses the SOS (DNA damage) response. In bacteria, RecA detects DNA damage and cleaves LexA, beginning the SOS response. Over expression of *lexA* prevents the repair response, thus enhancing cell killing. Dr. Lu conducted a mouse study that showed that this therapy could substantially boost the clinical efficacy of treatments for different infections. This therapy currently is being studied and adapted to treat U.S. veterans who are returning from Iraq and Afghanistan with skin infections.

Dr. Gardner also has reengineered bacteriophages to attack biofilm, a community of bacteria that is surrounded by a matrix of extracellular polymeric substance and attached to a surface. Because of their structure, biofilms are more resistant to antibiotics than are free-swimming bacteria. He designed a lytic DspB-expressing T7 phage with an active enzyme on its surface capable of degrading the biofilm matrix, allowing the reproduced phage to attack lower levels of the biofilm. Dr. Gardner also created a Trojan-horse version that expresses the enzyme both on the surface of the bacteriophage and inside the host cells. These techniques have obtained nearly total reduction and dispersion.

This work has commercial applications as well. Two of Dr. Collins' former students launched Novophage, a company that initially focused on industrial biofilm and now is tackling pathogen detection. With potential markets in the food and hospital industries, Novophage's goal is to engineer phages that detect as few as 10 cells in under an hour, for under $10.

Dr. Collins' colleagues also are working on novel antibiotic development. The Microbial War Project, run by Drs. Ruben Morones and Peter Belenky, pits microbe against microbe in battles to the death. The goal is to identify new natural products that could stimulate different environments to create synthetic ecosystems. They aim to develop a platform of 1,000 microbes such that when a new microbe or emerging strain of an existing microbe arises, it can be pitted against the platform in order to quickly identify novel molecules.

Current efforts in synthetic biology are shifting from microbes to higher organisms. Attempts to create a mammalian toggle switch similar to those engineered in bacteria and yeast failed because there was too much leakage when the switch was "off." One of Dr. Collins' students, Dr. Tara Deans, instead developed a two-part "off" switch using a transcriptional repressor and RNAi; the engineered RNAi target tagged to the gene of interest knocks down any leakage. The switch works from effective off to any level, can be used with any gene of interest, and can be used to explore threshold effects downstream in complex networks; it is now commercially available.

In the arena of regenerative medicine, researchers are attempting to reprogram patient cells into pluripotent stem cells and redifferentiate them into cells that could be placed back into the patient as tissue or used to explore how a drug affects that specific patient. Dr. Collins and his colleagues are striving to achieve reprogramming without using viral vectors that could change the genome of the cell and lead to cancer and other unwanted outcomes. They created synthetic mRNA that can be delivered directly to the cells. Recently, Dr. Collins has been using synthetic vesicles—or engineered exosomes—to deliver synthetic networks to targeted sites in mammals. This work presents interesting opportunities for *in vivo*

reprogramming. He is using the exosomes to target granulomas in tuberculosis. The exosomes fuse with the granulomas, thus delivering antimicrobial peptides. Dr. Collins' unpublished systems analyses of latent tuberculosis show that the molecular pathways responsible for uptake of peptides from the environment are upregulated in otherwise dormant cells. It may be possible to harness these upregulated pathways to deliver synthetic material to these cells.

Synthetic biology offers insights into small networks, computationally and experimentally. Dr. Collins has developed a commercial platform that allows researchers to assemble even large, complex networks. Analyses of synthetic biology provide exploration of, for instance, the effects of fluctuations in mammalian systems. Stem cells may allow regular tissues to explore the landscape of different states, and modeling grants the investigator opportunities to discover which cells play which roles in networks. Dr. Collins and his colleagues developed a molecular biology switch tuner that allows users to systematically turn up the "noise" in cells in order to drive differentiation and reprogramming.

There is growing interest in synthetic biology to study the human microbiome (i.e., microbial cells living within the human body). Some believe that diseases and conditions such as allergies, asthma, and obesity may be linked to changes in our microbiome. Dr. Collins and his associates are exploring ways to reprogram the microbiome using synthetic circuits. They recently received funding from the Gates Foundation to engineer lactobacilli to sense and remediate cholera infection. A decision circuit would respond to cholera infection signals by triggering synthetic probiotic to deliver antimicrobial peptides to the cholera bacteria.

Questions and Discussion

Dr. Belinda Seto wondered whether a switch could be developed to inhibit protein-protein interactions that form protein aggregates in neurodegenerative disease. Dr. Collins responded that there has not been much development of protein-based switches. He is currently working with prion expert Dr. Susan Lindquist on protein aggregation interference and exploring how synthetic biology can harness prions.

Dr. Ratner inquired about mass transport and diffusion issues with the cellulase approach to biofilm. Dr. Collins responded that Novophage researchers are working on this issue. A further challenge is that many biofilms contain multiple types of bacteria; Dr. Collins and his colleagues are exploring combinations of phages that can effectively penetrate and diffuse these biofilms. Thus far, the Environmental Protection Agency has been cautious about filings.

Dr. Alderson asked Dr. Collins where he and his colleagues fit within various disciplines. Dr. Collins stated that many are comfortable in almost any of the basic sciences, engineering, and medical school. There is growing demand for this field—which was formed by smart amateurs and misfits, computer scientists, and physicists, all of whom were outside of microbiology—and the supply is still low. The student population is just beginning to take off.

Dr. P. Hunter Peckham asked about curriculum and training goals in Dr. Collins' department. Dr. Collins reported that there are no textbooks, tutorials, or other materials. The curriculum feeds off of microbiology classes, experimental biology classes, and some early bioengineering and biotechnology studies. Boston University recently hired two new professors to offer synthetic biology classes.

Dr. Pettigrew wondered how Dr. Collins was able to accomplish this work beginning as a physicist. Dr. Collins stated that he began at a high level at first but quickly realized that he needed to start more simply. He hired outstanding students with open minds. Unhampered by what had been tried and failed, they benefitted as systems engineers from not having many of the details of existing disciplines. They are able to take a broader view of what is possible versus impossible.

Dr. Laurencin wondered about moving this work into the clinic and the response from the Food and Drug Administration (FDA). Dr. Collins responded that the field has not yet approached FDA, although FDA has been represented at some meetings and seems open to future development. The field is now beginning to interact with clinicians who also have been very open to this work.

Asked about dual use, Dr. Collins reported that he has been in meetings with the Federal Bureau of Investigation, Department of Defense, and Defense Intelligence Agency to discuss dual use of his work. Though the field is not yet at the point of engineering a supervirus, weaponizing natural biological elements is possible. Companies that practice synthetic biology have a policy of sharing information so that there are multiple checks against a company attempting to collect and/or distribute anthrax, for example.

Dr. Seto asked whether Dr. Collins deposits these microorganisms with the American Type Culture Collection (ATCC). Dr. Collins responded that he obtains many of the microorganisms from ATCC. Most synthetic biology is biosafety level 1, though some of Dr. Collins' work is biosafety level 2. He is not aware of anyone doing synthetic biology work in biosafety levels 3 or 4.

Dr. Ratner asked how Dr. Collins maintains stability of the programmed bacteria. Dr. Collins stated that stability is the top concern for the Presidential Commission for the Study of Bioethical Issues. The toggle switch, for example, often becomes nonfunctional after 2 weeks. Dr. Collins is working to reduce the mutation rate and increase redundancy in the system. This is very difficult; most of the parts of the system do not work well. There is not yet enough biological insight for synthetic biology to be a predictable science.

VII. Adjournment

The open session of the NACBIB meeting was adjourned at 12:15 p.m.

VIII. Closed Session

The grant application review portion of the meeting was closed to the public in accordance with provisions set forth in Section 552b(c)(4) and 552b(c)(6), Title 5, U.S. Code, and 10(d) of the Federal Advisory Committee Act, as amended (5 U.S.C. appendix 2). The closed session was adjourned at 2:00 p.m.

Certification:

We certify that, to the best of our knowledge, the foregoing minutes are accurate and complete.[2]

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Anthony Demsey, Ph.D.
Executive Secretary,
National Advisory Council for Biomedical Imaging and Bioengineering
Director,
Office of Research Administration
National Institute of Biomedical Imaging and Bioengineering

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Roderic I. Pettigrew, Ph.D., M.D.
Chairperson,
National Advisory Council for Biomedical Imaging and Bioengineering
Director,
National Institute of Biomedical Imaging and Bioengineering

[2] These minutes will be approved formally by the Council at the next meeting on September 14, 2012, and corrections or notations will be stated in the minutes of that meeting.