

G-protein-coupled receptors (GPCRs), found in the cell membrane, transmit biological signals to play critical and diverse roles in maintaining health, and are important targets for about 40 percent of all pharmaceutical drugs on the market today. This illustration depicts three phases of a new model of GPCR regulation by the protein β arrestin. To discover how these proteins interact, researchers slightly modified a GPCR, called β_2 adrenergic receptor, to facilitate their experiments, and used the powerful tools of electron microscopy and mass spectrometry, along with other techniques. (Left) The GPCR core (orange) sits in the cell membrane (row of brown circles and lines) with its “tail” (yellow) extending to the interior of a cell. (Middle) The regulatory protein β arrestin (blue) grasps the GPCR tail. (Right) The regulatory protein then changes orientation to interact with both the GPCR core and tail, blocking the receptor’s ability to transmit cellular signals. As described in this chapter, the detailed structural knowledge gained from this fundamental study may help scientists develop additional medications targeting GPCRs to improve health.

Image courtesy of Dr. Georgios Skiniotis, University of Michigan Medical School, Dr. Brian Kobilka, Stanford University School of Medicine, and Dr. Robert J. Lefkowitz, Duke University Medical Center. Adapted by permission from Macmillan Publishers Ltd: [Nature](#), AK Shukla et al. Visualization of arrestin recruitment by a G-protein-coupled receptor. 512:218–222, copyright 2014.

Cross-Cutting Science

Medical advances are not always achieved in great, intuitive leaps. More often, new prevention strategies, treatments, and cures result from a long, gradual accumulation of new knowledge from years of scientific research. Insights into the fundamental biologic building blocks and processes of an organism—its genes, the proteins they encode, the inner workings of cells, and the ways cells communicate with each other—can have broad and far-reaching implications. Indeed, many significant advances in our knowledge of disease and disease treatment can be traced to laboratory studies whose relevance to health could not have been fully known or appreciated at the time they were conducted.

With the development of innovative scientific technologies and the emergence of new scientific disciplines as talented and creative research teams join together to tackle ever more complex challenges, new opportunities to make exciting discoveries arise each day. The insights gained through this research can be expected to further scientific progress in many research areas, for today's discoveries may hold the seeds of tomorrow's cures.

Described in this chapter are several recent studies that illustrate the Institute's commitment to basic and applied research relevant across a broad spectrum of scientific disciplines. Also featured are several programs that demonstrate the breadth and collaborative nature of the NIDDK's research efforts, including innovative programs to engage K-12 and undergraduate students from rural or underrepresented groups in STEM fields—science, technology, engineering, and mathematics. Other features describe the creation of the NIDDK Information Network (dkNET), which serves to allow users to simultaneously search multiple databases to locate a biomedical resource, and the development of new NIH policies seeking balance of both males and females in animal and cell studies.

PROTEIN STRUCTURE AND THERAPEUTICS

Structure of Blood Clotting Receptor Identified:

Two recent studies have described the discovery of the three-dimensional structure of a protein receptor called P2Y₁₂ that plays a role in blood clotting. Normally, a blood clot forms to seal small cuts or breaks on blood vessel walls and stop bleeding. After the bleeding has stopped and healing has occurred, the body breaks down

and removes the clot. Excessive blood clotting can occur if the body's clotting process is altered or wrongly triggered. In addition, blood clots can form in, or travel to, the blood vessels in the brain, heart, kidney, lungs, and limbs—causing such dire medical consequences as heart attack or stroke. The P2Y₁₂ receptor is found on the surface of blood cells called platelets. When the P2Y₁₂ receptor is activated by a molecule called ADP, platelets cluster and a clot forms. The receptor is one of the most prominent clinical drug targets for the inhibition of platelet clustering. Although U.S. Food and Drug Administration (FDA)-approved drugs to limit clot formation are available, their use can cause unwanted side effects.

New research eloquently shows how the P2Y₁₂ receptor's structure undergoes a pronounced shape change when bound to the activator molecule ADP versus a potent inhibitor called AZD1283. Understanding how the P2Y₁₂ receptor responds to drug molecules is the first step toward developing the next generation of clot inhibitors. With the knowledge of a high-resolution structure of the receptor, research chemists can now use computer modeling to identify potentially more efficacious drugs with fewer side effects.

Zhang K, Zhang J, Gao ZG,...Zhao Q. Structure of the human P2Y12 receptor in complex with an antithrombotic drug. *Nature* 509: 115-118, 2014.

Zhang J, Zhang K, Gao ZG,...Zhao Q. Agonist-bound structure of the human P2Y12 receptor. *Nature* 509: 119-122, 2014.

Molecular Model Sheds Light on How Antibiotics Are Synthesized: The first high-resolution snapshots of a protein “assembly line” that synthesizes a medically important class of natural products have shed light on how antibiotics and other drugs are produced naturally. This assembly line, a group of polyketide synthase (PKS) enzymes, is found in many organisms, including those that are routinely used for producing antibiotics and several other drugs. PKS enzymes are the molecular machinery responsible for synthesizing polyketides, a class of long, chain-like molecules that includes drugs such as the antibiotic erythromycin and the anti-cholesterol drug lovastatin. PKS enzymes act as modular assembly lines, with different PKS modules modifying a polyketide chain in a particular sequence. Despite the fact that PKS enzymes are the key enzymes responsible for the production of many drugs, the molecular details of how they operate were unknown.

To fill this information gap, researchers used sensitive electron microscopy to zoom in on one PKS enzyme called PikAIII and map its molecular structure both at rest and at work. These “snapshots” of PikAIII as it assembled a polyketide provided crucial clues about how PikAIII’s different modules are shaped and how they interact during polyketide synthesis. The researchers found that PikAIII is assembled as an arch around a central reaction chamber. Surprisingly, their data showed for the first time that a mobile “carrier module” inside the reaction chamber shuttles the assembling polyketide between module “workstations” to allow the growing molecular chain to be modified in a precise, orderly fashion. The sequence of these movements is determined by the dynamic movements of the different PikAIII modules as they interact with the assembling polyketide and each other. After PikAIII has completed its work, it then passes the elongated and processed polyketide to the next enzyme in the assembly pathway. These new data clearly demonstrated how PikAIII’s structure makes

it uniquely suitable for its particular function, including suggesting how PikAIII may “shield” modules from unnecessary contact with growing polyketides to reduce errors in this complex process.

This new information about PikAIII’s structure gives exciting new insight into the inner workings of PKS enzymes. Armed with this new understanding of how PKS molecules work, it may now be possible to design PKS enzymes to produce a wide range of clinically relevant drugs more efficiently. Finally, this detailed knowledge about how these drugs are synthesized naturally might also guide the development of new antibiotics and other drug candidates.

Dutta S, Whicher JR, Hansen DA,...Skiniotis G. Structure of a modular polyketide synthase. *Nature* 510: 512-517, 2014.

Whicher JR, Dutta S, Hansen DA,...Skiniotis G. Structural rearrangements of a polyketide synthase module during its catalytic cycle. *Nature* 510: 560-564, 2014.

Structural Studies Reveal Details of Early Stages of HIV Infection: NIDDK intramural scientists have described new details of the structural changes that occur at the moment that the virus that causes AIDS penetrates the outer membrane of a target cell.

The first step in viral infection is the attachment of the virus to the surface of a target cell. After latching on to the cell, the virus fuses its outer membrane with that of its target and inserts its genetic material into the cell. In HIV infection, the attachment of the virus is mediated by the protein Env, which consists of three subunits each of the proteins gp41 and gp120. Recent studies have shown that, upon binding to the target cell, Env alters the arrangement of gp41 and gp120, extending gp41 like a spear and pulling the virus and cell closer together to facilitate fusion.

Previous research has given scientists “snapshots” of what gp41 looks like before and after fusion, but the intermediate steps have been poorly understood. Researchers have now gathered new data that give a clearer picture of how gp41 “moves” during fusion. These studies suggest that, upon cell binding, the

three copies of gp41 in an Env molecule spring apart, embedding in the host cell and viral membranes. As gp41 folds back to its resting state, it pulls the membranes with it, bringing viral and cell membranes into close proximity so that membrane fusion can occur and infection can proceed.

This information may shed new light on how existing anti-HIV medications inhibit virus entry and may open the way for novel strategies to disrupt the fusion process that could have important therapeutic implications for the prevention of HIV infection.

Roche J, Louis JM, Grishaev A, Ying J, and Bax A. Dissociation of the trimeric gp41 ectodomain at the lipid-water interface suggests an active role in HIV-1 Env-mediated membrane fusion. Proc Natl Acad Sci USA 111: 3425-3430, 2014.

A Molecular Picture of Key Proteins That Send and Stop Biological Signals: Seeking new insight into the structure of proteins called G-protein-coupled receptors (GPCRs), which transmit important signals in cells throughout the body, researchers discovered how the signals may be stopped by a regulatory protein, β -arrestin. GPCRs play critical and diverse roles in maintaining health and are the targets for about 40 percent of all drugs that are in the market today. For example, a GPCR called the β_2 -adrenergic receptor plays important roles in multiple tissues and is the target of drugs for treating respiratory diseases and other conditions. Like other GPCRs, the β_2 -adrenergic receptor sits in the membrane at the outer edge of a cell. This provides an ideal vantage point from which the GPCR can bind specific hormones or factors from the nervous system and then send signals into the cell to direct a response, assisted by its namesake G-protein companion. Past research has shown that when cells need to halt or change the signals, they recruit a regulatory protein, β -arrestin. However, it has been difficult to decipher exactly how β -arrestin impedes the signaling of GPCRs such as the β_2 -adrenergic receptor. Recently, scientists devised a way to capture and study this elusive protein duo. They slightly modified the β_2 -adrenergic receptor to facilitate their experiments, and used the powerful tools of electron microscopy

and mass spectrometry, along with other techniques, for modeling and understanding how β -arrestin and the β_2 -adrenergic receptor interact at the molecular level. Their data suggest that a β -arrestin protein first grasps onto its partner β_2 -adrenergic receptor from one end, and then swings around to engage the receptor across a larger segment. In the resulting configuration, the β -arrestin is positioned as a barricade across the part of the β_2 -adrenergic receptor necessary for sending its characteristic G-protein-mediated signals. The detailed structural knowledge gained from this research may help scientists develop additional medications that modulate the signaling of GPCRs to improve health.

Shukla AK, Westfield GH, Xiao K,...Lefkowitz RJ. Visualization of arrestin recruitment by a G-protein-coupled receptor. Nature 512: 218-222, 2014.

INVESTIGATING GENE REGULATION

The Role of Chromosome Loops in Turning On Genes: By studying a protein involved in “turning on” a red blood cell gene, NIDDK intramural scientists learned more about this process and provided insight into a major question in biology. To turn on genes, a process called transcription, a cell first copies (“transcribes”) genetic information from the DNA into RNA, and then uses the RNA versions to direct production of proteins encoded by the genes. Red blood cells produce globin proteins, key components of hemoglobin, which carry oxygen in red blood cells from the lungs to the rest of the body. Globin genes are tightly regulated (turned on and off), ensuring that the subsequent production of globin proteins occurs at appropriate times during the development of red blood cells from their precursors in the bone marrow.

The mammalian β -globin genes were among the first gene clusters to provide insight into how gene regulation is influenced by long-range chromosomal interactions between DNA sequences far from and near to the protein-coding segment of a gene. Specifically, these interactions occur between a powerful element called an enhancer that helps turn on the β -globin gene, also referred to as the gene’s locus control region

(LCR), and a DNA element called a promoter, which is immediately adjacent to the gene and helps regulate whether it is on or off. Scientists continue to study enhancers such as the LCR in order to understand more fully their role in regulation of gene transcription.

Scientists previously demonstrated that the formation of a loop of chromosomal DNA, created by the interaction between the β -globin gene promoter and LCR, played an important role in β -globin gene transcription, and that this required the protein LDB1. To determine how looping was associated with turning on the β -globin gene, these scientists dissected the LDB1 protein to test the roles of different parts of the protein in this process. They added different fragments of the LDB1 protein back to mouse blood cells engineered to lack LDB1. In the cells lacking LDB1, both looping and transcription were significantly reduced. However, in adding back fragments, they identified one portion of the LDB1 protein that restored the looping, but was unable to restore activation of transcription. Their findings demonstrated that the processes of looping and transcription activation are separate, that loops can be formed in the absence of transcriptional activation, and that LDB1 plays roles in both processes, addressing fundamental issues that have been poorly understood. These results suggest that looping occurs before transcriptional activation, though the exact role of looping in the ultimate activity of genes remains to be defined.

Further insight into the regulation of gene transcription, a fundamental process in biology, is central to understanding many disease processes. The detailed characterization of the β -globin gene locus in particular may help the development of new ways to treat hematologic diseases, such as sickle cell disease, by reactivating dormant hemoglobin genes.

Krivega I, Dale RK, and Dean A. Role of LDB1 in the transition from chromatin looping to transcription activation. Genes Dev 28: 1278-1290, 2014.

Understanding Gene Silencing in Fragile X

Syndrome: New NIDDK intramural research has provided important clues in the quest to understand

the biology of fragile X syndrome, the most common cause of inherited intellectual disability and autism spectrum disorders, and has identified a key hurdle in finding a method to treat the underlying cause of the disease. People with fragile X do not create enough of a protein called FMRP, which is needed for normal brain development and is encoded by the gene *FMRI*. The first step in making FMRP—like the first step in making any other protein in a cell—is to create a “transcript” of the *FMRI* gene. (The transcript is then processed, and delivered to another part of the cell, where it essentially becomes a blueprint for making the FMRP protein.) For most people with fragile X, the reason they do not make enough FMRP is not that the *FMRI* gene itself is missing or damaged, but rather because there is extra DNA adjacent to *FMRI* that causes it to be “silenced.” That is, the extra DNA contributes to interference with that first step in the FMRP production process, the gene’s transcription. Scientists studying fragile X have long understood that silencing depends on methylation, a chemical modification of the extra DNA, and also on methylation of certain proteins that are bound to DNA in the area. Drugs that reverse the methylation either of the DNA or the proteins can at least partially undo the silencing, allowing cells to make FMRP. Unfortunately, the improvement is temporary, and the drugs available today are too toxic for use in long-term treatment of people with the disease. The new study is part of an effort to better understand how and why *FMRI* silencing occurs in fragile X, with the hope of developing approaches to achieve more practical, long-term reactivation of the gene.

The researchers used a drug that reverses the DNA methylation in cells from men with fragile X—since the disease is much more common in men than in women—and found that methylation of the DNA-bound proteins was not reduced, even as the cells began to transcribe *FMRI*. In addition, they found that silencing recurred before the DNA was re-methylated. These observations suggest that DNA methylation stabilizes the silenced state of the gene, but is not absolutely required to initiate it, and also that the protein methylation occurs first. Importantly, the scientists also found that the transcript of *FMRI*—

produced after treatment with the drug—is one of the key factors responsible for bringing the enzymes that methylate DNA and protein into proximity with the gene. Thus, the *FMR1* transcript itself contributes to *FMR1* re-silencing. As a result of this study, it is now known that a successful approach to treating fragile X syndrome will need not only to reactivate

FMR1 transcription, but also to break the link between transcription and re-silencing.

Kumari D and Usdin K. Polycomb group complexes are recruited to reactivated FMR1 alleles in Fragile X syndrome in response to FMR1 transcription. Hum Mol Genet 23: 6575-6583, 2014.

NIDDK-supported Research Training Programs for Underserved and Underrepresented Student Groups

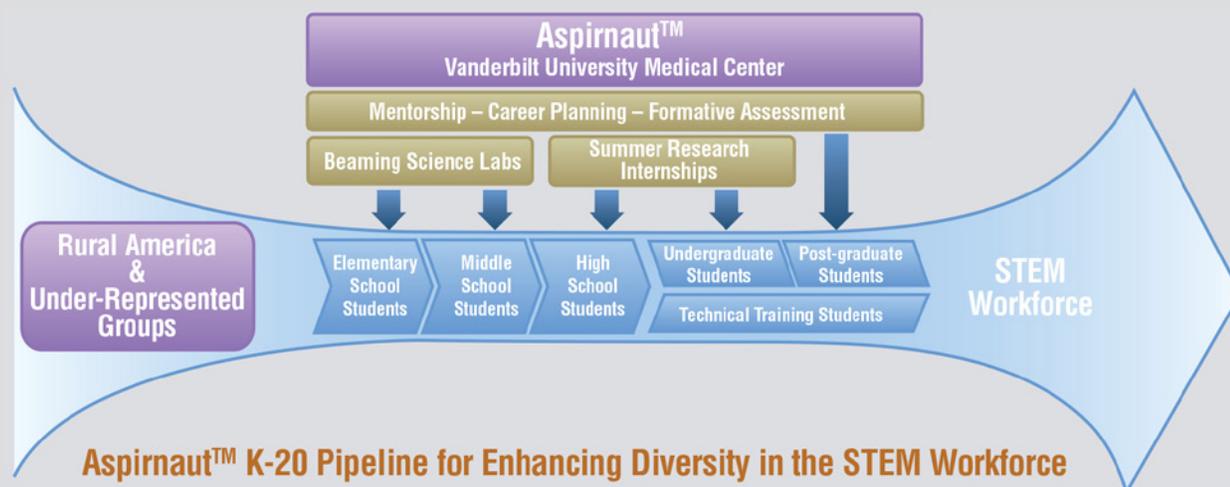
THE ASPIRNAUT INITIATIVE

In 2007, Drs. Julie and Billy Hudson co-founded the Aspirnaut Initiative at Vanderbilt University to engage K-12 and undergraduate students from rural and underrepresented groups in STEM fields—science, technology, engineering, and mathematics. Their vision is to enhance and accelerate STEM education in order to provide talented rural and other underrepresented students the opportunity to choose and excel on a STEM-related career path. Their founding project provided Wi-Fi on school buses in rural Arkansas, where students were equipped with laptop computers, turning long bus rides into productive learning time for STEM subjects. The 3-year pilot project demonstrated feasibility and gained national publicity on *NBC Nightly News*,¹ in the *New York Times*, *Wall Street Journal*, and many other TV and news agencies.

The project has now emerged as the Aspirnaut K-20 Pipeline for Enhancing the Diversity of the STEM Workforce. The program leverages the STEM resources of faculty, students, and facilities of a research university to partner with teachers in rural America. The pipeline consists of: “beaming” of live, hands-on, inquiry-based

science labs by university faculty and students to remote schools; sponsoring summer research internships; and continual mentorship and formative assessment of achievement. The project has affected over 120 high school and undergraduate interns and over 2500 elementary and middle school students from 26 states over the past 7 years (for more information, please visit www.aspirnaut.org). The Aspirnauts coauthored a recent publication in the *Proceedings of the National Academy of Sciences*: 6 were middle school students when the study was conducted, 42 were high school students, 30 were college undergraduates, and 5 were graduate students.² Specifically, the Aspirnauts researched the evolutionary origin of a specific chemical bond and determined that it traced to a common ancestor dating back more than 500 million years ago.

Dr. Billy Hudson, the Elliot V. Newman Professor of Medicine, Biochemistry, Pathology, Immunology, and Microbiology, and Director of the Center for Matrix Biology at Vanderbilt, grew up in rural south central Arkansas. As a high school dropout, he overcame poverty and childhood abuse by taking advantage of educational opportunities provided by mentors. “Bringing STEM opportunities to the ‘forgotten’ students



Aspirnaut “pipeline” figure courtesy of Drs. Julie and Billy Hudson, Vanderbilt University

in rural America can greatly increase the number and diversity of students entering STEM careers. Given the recent revolutionary advances in biology, it is essential that scientists partner with K-12 teachers to bring the excitement of discovery and career pathways to America's youth," Dr. Billy Hudson said.

Dr. Julie Hudson is Assistant Vice Chancellor of Health Affairs and Clinical Associate Professor of Anesthesiology and Pediatrics at Vanderbilt University Medical Center. She is a pediatric anesthesiologist with a background that includes development work, governmental relations, biomedical research, and science education. She and her husband have tirelessly spearheaded the Aspirnaut effort—writing grants, establishing cooperative efforts with institutions and businesses, and doing whatever is necessary for the children in the program in order to support the students' efforts to pursue careers in math and science.

Beaming Science Labs to Rural America: The Interactive Videoconference Program

Partnering with elementary and middle school teachers in rural Arkansas, Maine, and Tennessee, Vanderbilt University's undergraduate and graduate students, along with postdoctoral researchers and faculty, produce weekly live, interactive, and inquiry-based STEM lab videoconferences for students. The Hudsons welcome the opportunity to share this program with others in the hope that it can be replicated in other rural communities. The live sessions help participants learn STEM concepts and acquire critical thinking skills. "Engaging students early and often is key to increasing achievement in STEM and the number and diversity of students entering the STEM pipeline," said Dr. Julie Hudson.

The Summer Research Internships

The Aspirnaut pipeline offers summer research internships for rural and underrepresented high school and undergraduate students. Interns are given the opportunity to work side-by-side with scientists and are expected to learn and contribute to the ongoing

pace of research advancements. The students conduct research in several areas, including basic science underlying the biology and physiology of kidney function and the basis of kidney diseases such as diabetic nephropathy and hypertension.

The NIDDK began supporting the summer research programs in 2009 when Dr. Billy Hudson applied for and was awarded an American Recovery and Reinvestment Act (ARRA) supplement to his existing grant. The 2009-2010 supplemental award included funds for the Aspirnaut initiative that provided stipends to 20 "Aspirnauts" during an 8-week summer research program. Demographic data of students in the Aspirnaut summer programs for years 2009–2014 illustrates tracked diversity categories (geographic, economic, racial/ethnic, family level of education) of the 69 interns in the cohort: 33 percent met one diversity category while 26 percent met three diversity categories.³

Undergraduate Research Program

Drs. Billy and Julie Hudson and their colleagues have been able to leverage the ARRA supplement award into a successful new NIDDK award: an education project grant, entitled "Undergraduate Research Internships in Pathobiology of Diabetic Nephropathy," to help recruit, prepare, and increase the numbers of undergraduate students who pursue STEM disciplines relevant to the NIDDK mission. The 5-year award, which began in 2012, supports 10 undergraduate students each summer; 40 percent of the participants are from underrepresented racial and ethnic groups, including American Indians, and 70 percent from geographically and economically disadvantaged backgrounds. As of May 2014, 38 of 57 interns have graduated from a college degree program, and none have dropped out. Notably, 44 percent of college graduates have enrolled in STEM-related graduate education programs (D.D.S., M.D., Health Administration, M.P.H., Nurse Practitioner, Ph.D.).³ For more on the journey of two summer high school Aspirnaut students through their undergraduate years at Vanderbilt University, please see www.youtube.com/watch?v=J07gNLZ8rsg⁴

Efforts to inspire and elevate STEM achievement in rural K-12 and undergraduate students continue under the Hudsons' leadership and are aligned with the NIH's Recruitment and Retention Plan to Enhance Diversity (please see: http://grants.nih.gov/training/faq_diversity.htm) and the NIH's Physician-Scientist Workforce Working Group Report (http://acd.od.nih.gov/reports/PSW_Report_ACD_Executive_Summary_06042014.pdf).

¹ www.nbcnews.com/video/nightly-news/23526146

² Fidler AL, et al. A unique covalent bond in basement membrane is a primordial innovation for tissue evolution. *Proc Natl Acad Sci USA* 111: 331-336, 2014.

³ Data provided by Dr. Julie Hudson, Vanderbilt University.

⁴ This video has been nominated for the Mid-South Emmy Award, and winners will be announced January 31, 2015.

STEP-UP



These high school students participated in STEP-UP. (Photo credit: Bill Branson, *NIH Record*)

The NIDDK's Short-Term Research Experience for Underrepresented Persons (STEP-UP) provides summer research opportunities to talented high school and undergraduate students underrepresented in biomedical research, including students with disabilities, those from a disadvantaged background, and certain racial and ethnic minorities. The overall goal of STEP-UP is to build and sustain a biomedical, behavioral, clinical, and social science pipeline focused on NIDDK mission areas.

To accomplish this goal, STEP-UP provides research education grants to seven institutions to coordinate

four high school STEP-UP Programs and three undergraduate STEP-UP Programs that provide eligible students with 10 to 12 weeks of summer research experience and training opportunities that include exposure to the scientific method, the fundamentals of laboratory research, and production of written and oral scientific presentations of their research accomplishments. The sites engaging high school students are located at the University of Hawaii-Manoa, University of Nevada-Las Vegas, Charles R. Drew University-Los Angeles, and Stanford University. The sites engaging undergraduate students are located at Pennsylvania State University-Hershey, Children's Hospital-Los Angeles, and the American Physiological Society.

Dr. Lawrence Agodoa, Director of the NIDDK's Office of Minority Health Research Coordination, which manages the program, has said that having a diverse pool of researchers to tackle some of science's most pressing issues is crucial. "People of all walks of life need to come together and think about how to solve these problems," he said. "Many chronic diseases such as diabetes affect minority communities disproportionately. Having friends and family who are affected by a disease often gives people extra motivation to pursue biomedical research."

STEP-UP also provides research opportunities for high school students who live in the Pacific region. "Students in the Pacific region often live thousands of miles away from facilities that can support cutting-edge

research,” said Dr. Agodoa. To overcome this barrier, STEP-UP labs have opened in America Samoa, the Commonwealth of the Northern Mariana Islands, the Republic of the Marshall Islands, the Federated States of Micronesia, and the Republic of Palau. “By providing laboratories and training local science teachers as mentors, we expose students to the newest biomedical research techniques without them needing to travel far from home,” Dr. Agodoa noted.

At the end of the summer, the STEP-UP Summer Research Symposium is held on the NIH Bethesda campus. This culminating event provides students the opportunity to showcase their summer research in a formal oral and poster presentation. In addition, the Symposium engages students in a true scientific, convivial manner and exposes them to students from diverse backgrounds.



STEP-UP lab opens in Palau. Local science teachers are being trained to use the equipment.
(Photo credit: Dr. George Hui, University of Hawaii)

dkNET: One-stop Shopping for Biomedical Resources



Want a one-stop Web-based “shop” for finding biomedical resources such as data, reagents, organisms, and tools? The NIDDK has created one: the NIDDK Information Network (dkNET), which allows users to simultaneously search multiple databases for information that may be difficult to find using regular search engines. The information in dkNET is relevant to anyone interested in doing research on kidney, urologic, hematologic, digestive, metabolic, and endocrine diseases; diabetes; or nutrition.

Scientists generate enormous amounts of data, but there is often no good way to track and search all of the existing digital resources for information specific to an important question. dkNET helps solve this problem by providing selected NIDDK-supported data in an online “data-mart” that allows users to take advantage of advanced informatics tools.

Launched in April 2014, dkNET was created by the NIDDK, along with researchers at the University of California, San Diego, and elsewhere, as a catalog of

NIDDK-supported online resources. dkNET contains information on an increasing number of resources, currently exceeding 1900, including links to such digital networks as the Nuclear Receptor Signaling Atlas, the GenitoUrinary Development Molecular Anatomy Project, and the Diabetic Complications Consortium. Links to related sources of data take the user to the broader universe of online scientific networks such as the Antibody Registry and Addgene, among others.

The dkNET site is built upon the Web structure SciCrunch, which allows researchers to cost-effectively build and share data repositories. As the online connectivity of research increases, dkNET will gradually scale to serve as a vehicle for future NIDDK-supported data and resources.

The Institute’s intent is that sharing data and tools will enrich the research landscape in serendipitous ways. For instance, researchers can use dkNET to search for resources, compare their data with existing data sets, add to the existing store of knowledge, and generate new ideas.

The dkNET site is easy to navigate, includes tutorials, and even has online “office hours” to answer user questions. To find out more, go to www.dknet.org

(Adapted from a piece originally published in the summer 2014 NIDDK Director’s Update.)

NIH Seeking Balanced Approach to Representation of Both Males and Females in Animal and Cell Studies

Recent research has indicated that biological sex—determined in humans and many animals by the complement of X and Y chromosomes—can have profound and sometimes unexpected effects on health and disease. Some progress has been made in identifying sex and gender¹ differences in clinical research studies, such as finding that certain drugs require different dosing or have different side effects or efficacy in women and men, and that certain diseases and conditions progress differently in women than in men. However, knowledge gaps still exist that could be remedied by expanding consideration of biological sex in pre-clinical research studies conducted in animal models and cells. The NIH is taking steps to ensure that pre-clinical studies it supports include assessment of the impact of biological sex in ways most relevant and beneficial to human health.

In the May 15, 2014, issue of the scientific journal, *Nature*, the Director of the NIH Office of Research on Women's Health (ORWH) and Associate Director for Research on Women's Health, Dr. Janine Clayton, and the Director of the NIH, Dr. Francis Collins, outlined some of the key scientific challenges posed by a lack of knowledge about sex-based differences and pointed out the need for change.² For example, while over half of NIH-funded clinical research participants are now women—a change wrought largely through the NIH Revitalization Act of 1993—in studies using animal models, such as mice, researchers have continued to use male animals predominantly even when the condition or question under study is not exclusive to males. In studies using cells, the biological sex of the original animal or human has also been largely overlooked as a factor that could affect experimental results.

These practices may not only have unintentionally led to gaps in knowledge, but also inadvertently hindered the hunt for treatments and cures. For example, by using only male animals, fundamental differences between males and females that could guide later clinical

studies in humans may have been missed. Discovery of such differences earlier in the research pipeline may help avoid such costly and challenging problems as sex-based adverse drug reactions. Moreover, it is possible that by focusing on male animals, novel drug targets and treatment approaches that might be more effective in females have remained as yet undiscovered. Finally, inadequate analysis and reporting of sex differences in scientific journals could be contributing to some of the irreproducibility of published research results that has been observed in recent years and which the NIH is actively working to address.

The NIH is now developing policies that will require NIH applicants to consider sex as a scientific variable in research involving animals, tissues, or cells. This change is being pursued through a deliberate and thoughtful approach, spearheaded by a trans-NIH working group, and will be implemented in phases. For example, to ensure that the views and concerns of the public and scientific community were considered in development of these policies, the NIH solicited comments on “Consideration of Sex as a Biological Variable in Biomedical Research” through a request for information in September 2014. Other efforts are informing policy development, as well. For example, on October 20, 2014, the ORWH hosted a public workshop to discuss scientific concepts, methods, challenges, techniques, and strategies for integrating biological sex as a variable in pre-clinical research. The ORWH also continues to offer, in partnership with the U.S. Food and Drug Administration, online training in the science of sex and gender differences, as well as other online resources that will help to support this change toward studying basic biology and disease processes in both males and females to strengthen science overall.

To facilitate the phasing in of the new policies, the NIH will be issuing notices in the *NIH Guide for Grants and Contracts* that will explain what new information should be included in applications and progress reports to

address sex differences and the timing of these new requirements. The NIH is also developing guidelines for how grant application reviewers should consider information given about the sex of animals when they evaluate proposals. In addition, the NIH is working with others in the scientific community to encourage consideration of the importance of sex differences in research efforts it supports. For example, the NIH is working with editors of scientific journals to encourage the reporting of analyses of both males and females in animal and human studies when these studies are published. For more details and updates about NIH policy development, as well as links to resources in this research area, see the ORWH webpage “Studying Sex to Strengthen Science (S4)” at <http://orwh.od.nih.gov/sexinscience/>

The NIDDK fully supports these efforts to enhance our knowledge of human health. Reflecting the spirit of these new policies, the NIDDK has updated its approach to reporting on research it supports within this annual publication. Already, summaries of research studies conducted in people usually note whether studies were performed in men, women, or both. Now, whenever the information is provided in

the original research article, summaries of studies using mice, rats, and certain other animal models will indicate whether male, female, or both sexes were used. The results of any sex differences analyses that were performed will also be captured. In this way, the Institute hopes to model—and encourage—the routine reporting of biological sex as a scientific variable just as significant as whether an experiment was performed in the liver versus the pancreas.

¹ *The terms “sex” and “gender” are often used interchangeably when referring to males and females. However, according to definitions recommended by the Institute of Medicine, biological sex differs from gender in that gender (e.g., “woman” or “man”) refers to how a person presents themselves to others as male or female, or how that person is responded to by social institutions on the basis of the individual’s gender presentation. Gender is thus a human term rooted in biology and shaped by environment and experience, whereas sex is simply an innate biological feature shared by many organisms, including humans.*

² Clayton JA and Collins FC. Policy: NIH to balance sex in cell and animal studies. *Nature* 509: 282-283, 2014.

