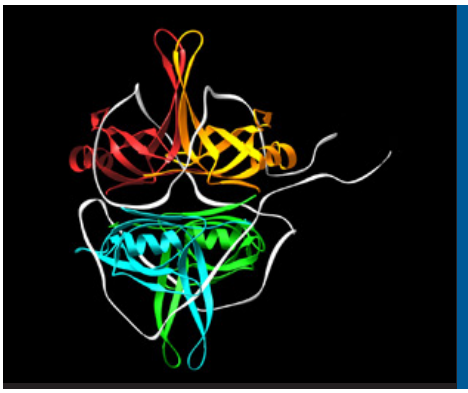

Department of
Biochemistry and Molecular Biophysics

Spring 2013

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A plaque honors former department members and Nobel Prize winners Carl and Gerty Cori.

Moving Forward: Membrane-Protein Research

Cells are composed of a complex collection of proteins, nucleic acids and small metabolites. Cells use these molecules to carry out their individual functions, as well as to grow and divide. An important goal for biomedical research is to understand how these various components give rise to and control the biological processes that define a living cell.

Biochemistry and molecular biophysics are focused on understanding the interactions among these different components at the molecular and atomic level. Our research defines the driving forces that energize biological processes and the rates and mechanisms by which the processes occur.

One exciting current area of research is understanding how proteins embedded in membranes work. Membrane-protein research has lagged behind research on soluble proteins due to the added complexity of protein-lipid interactions and the need to maintain a membrane-like environment to retain the activity of the proteins.

Assistant professor Kathrine A. Henzler-Wildman, PhD, has solved this problem through the innovative use of bicelles, a specific combination of long- and short-chain phospholipids that form a small artificial lipid bilayer into which one or a few molecules of a membrane protein can be inserted.

Bicelles allow Henzler-Wildman to characterize the function and the structure of membrane-embedded proteins. She studies how the membrane protein EmrE transports small molecules, such as drugs, across the membrane of bacteria. EmrE is

CONTINUED ON PAGE 4

Washington University in St. Louis
School of Medicine

Washington University Medical Center

Message from the Department Head

John A. Cooper, MD, PhD

Dear Colleagues and Friends:

Welcome to our first annual departmental newsletter. I am pleased to have this opportunity to welcome you to the Department of Biochemistry and Molecular Biophysics and to introduce myself as its interim head. We hope to use this newsletter to share information about our current activities and plans for the future. One important goal is to renew and maintain our connections with you and other current and past members of our extended family, including friends, family, staff and trainees.

As the interim head of the department, I am delighted to have the opportunity to work with such an outstanding collection of faculty, trainees and staff. This year has been one of fresh beginnings and looking forward. The department is positioning itself to take on even greater challenges in our missions of research and teaching.

The department has historically served as an intellectual core of Washington University School of Medicine, and we look forward to maintaining that role. The department has a broad array of strengths in research, using quantitative approaches to understand the structure and function of biological molecules at a detailed level. We study a range of processes of fundamental importance to biology and medicine, including protein folding, DNA replication, RNA function, membrane protein function, and cell signaling, among many.

The department has developed important collaborations within the medical center and the university, bringing our unique areas of expertise to bear on a wide range of issues related to health and disease, such as infections, diabetes and cancer.

The department has critical roles in the training of our medical and graduate students, through teaching courses and administering training programs. We are excited about the challenge of producing the next generation of outstanding biomedical scientists who will become world leaders in the research that will be critical for the success of our nation and planet.

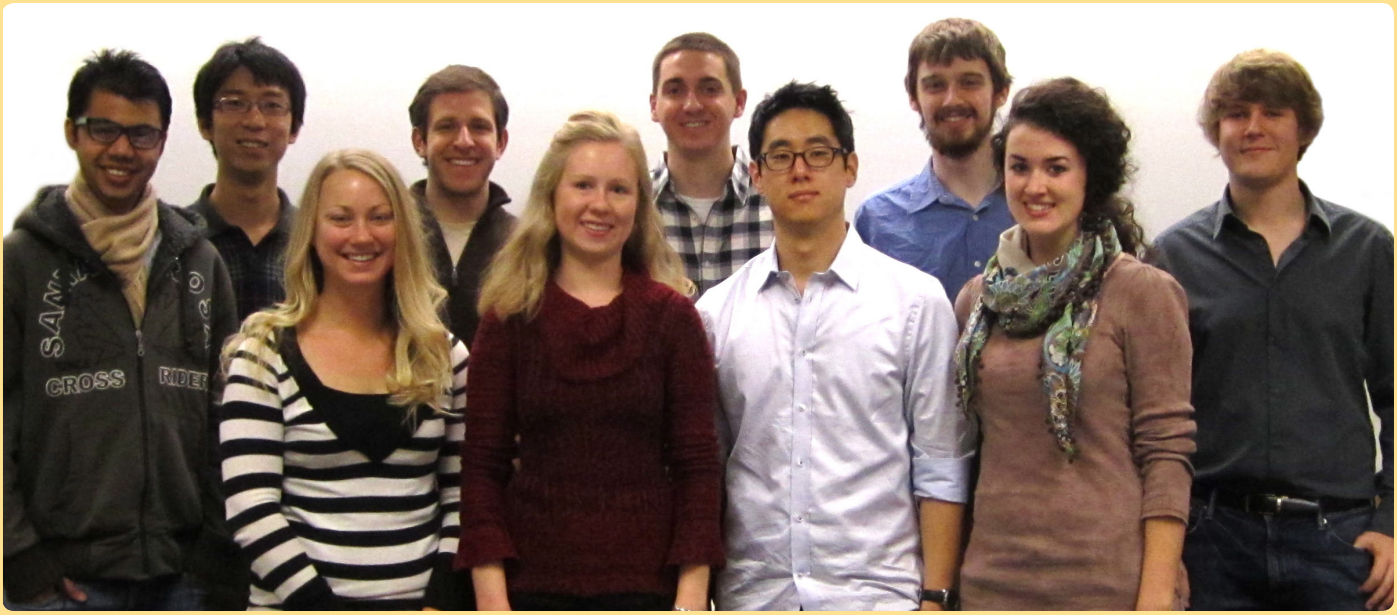
We have a new sense of momentum and excitement as we approach the challenges of biomedical research in the 21st century. Please be in touch, keep us informed of your activities, and feel free to drop in and visit.

With warm regards,
John A. Cooper, MD, PhD
Interim Head
Department of Biochemistry and Molecular Biophysics

Mission Statement

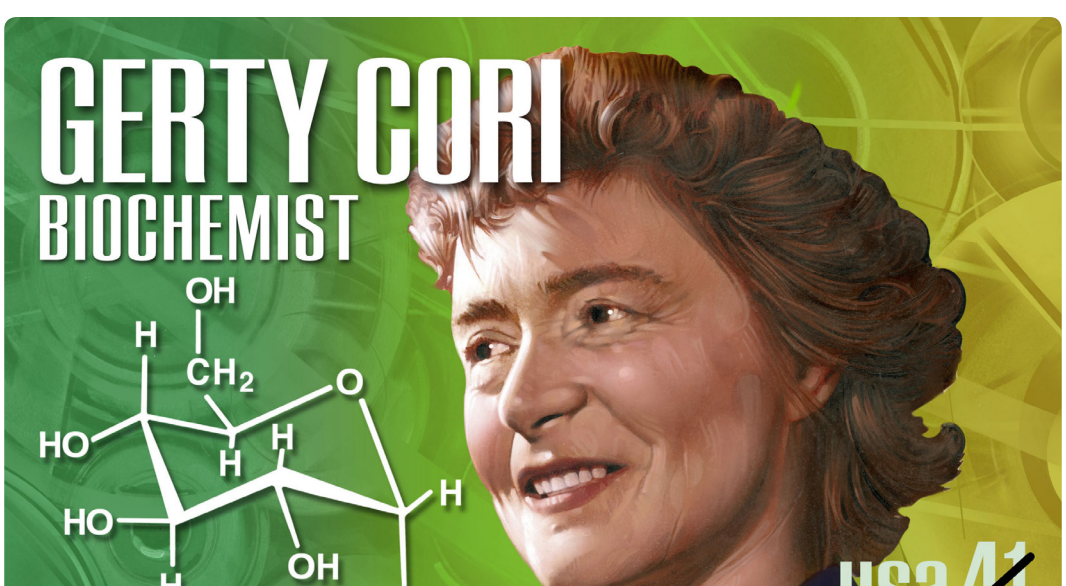
Members of the Department of Biochemistry and Molecular Biophysics are dedicated to investigating the complex relationships and mechanisms that control biological processes. These processes are defined by interactions among proteins, nucleic acids (DNA and RNA) and between proteins or nucleic acids with small metabolites.

Our investigators use experimental structural, thermodynamic, kinetic and single molecule methods as well as computational approaches to understand and quantify structural and dynamic aspects of macromolecular interactions. Our research provides fundamental knowledge that enables advances in medicine and improvements in the quality of life.



Biochemistry and Computational and Molecular Biophysics 2012 Entering Class

Front, l-r: Whitney Grither, Anne Georges, Yong Hee Chung, Mariah Lawler
Back, l-r: Apurwa Sharma, Linxuan Hao, Andrew Loza, Bryan Balthazor, Isaac Henson, Alex Holehouse
Not pictured: Britney Johnson, Yerdos Ordabayev, Vasilios Kalas



The 2008 Gerty Cori U.S. postage stamp

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involved in the development of multi-drug resistance in bacteria.

Membrane proteins, especially those embedded in the lipid bilayer, have been difficult to crystallize because the lipids or detergents needed to solubilize the protein introduce heterogeneity.

Assistant professor Weikai Li, PhD, an expert in this area, has succeeded in crystallizing an important membrane-embedded enzyme, vitamin K epoxide reductase. Vitamin K is required for the function of several blood clotting factors, and inhibitors of this enzyme are used in patients to prevent blood clots following strokes. Li is also studying another enzyme involved in vitamin K function, a γ-carboxylase that modifies several blood coagulation proteins. His studies will provide a better understanding of the function of these enzymes, and his results may identify novel approaches for the pharmacological control of blood clotting.

Single-molecule approaches allow one to observe the behavior of a single molecule over time. These approaches have provided critical new information about the molecular mechanisms of fundamental biological processes.

Previously, one could only observe the average behavior of a large number of molecules at one time. Watching single molecules has revealed essential new information about how they behave. Assistant professor Roberto Galletto, PhD, is using single-molecule fluorescence approaches, along with conventional ensemble approaches, to study how proteins control DNA interactions. In particular, he is investigating how proteins stabilize and regulate the ends of chromosomes, so-called telomeres.

Assistant professor Eric A. Galburt, PhD, has developed another set of single-molecule approaches, termed "tweezers," in which one uses light or magnets to apply force to individual molecules. These approaches are particularly important to understand the function and replication of DNA and RNA because tension is exerted by the enzymes that operate on these nucleic acids. Galburt is investigating how the process of transcription is initiated, which is a critical element in the regulation of genes.

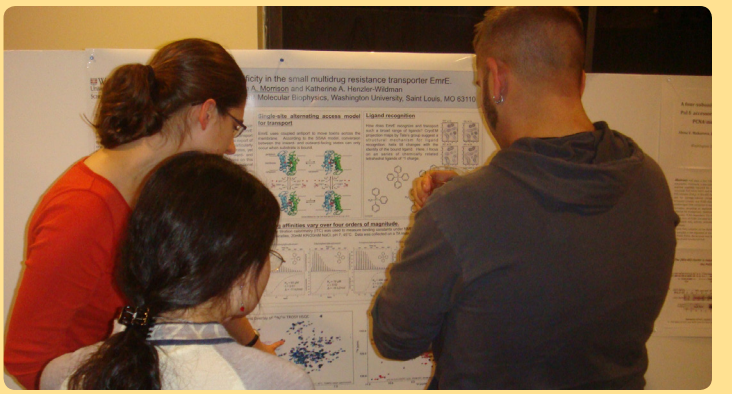
A common theme in the work done within the department is the importance of how small molecules, such as drugs, interact with larger biological molecules, such as enzymes and DNA. Two faculty members working in this area are research associate professor James W. Janetka, PhD, and research assistant Professor Scott A. Wildman, PhD. Both are chemists with strong backgrounds in the pharmaceutical industry.

Janetka is a medicinal chemist. He designs and synthesizes new chemicals designed to inhibit the growth of bacteria that cause infections and the growth of cancer cells. He uses rationale design based on the structure and dynamics of proteins.

Wildman is a computational chemist. He applies computational methods to discover new drugs. He is working collaboratively with several Washington University colleagues on projects ranging from the design of inhibitors of cell growth to the optimization of compounds that target taxol to tumors following irradiation therapy.

The addition of these new faculty with expertise in cutting-edge techniques enhances the ability of departmental faculty specifically, and the School of Medicine overall, to address significant new questions in biomedicine.

Graduate program coordinator Melissa Torres tends the fire.



Student Emma Morrison explains her poster.

Keynote speaker
Enrique de la Cruz, PhD

Keynote speaker
Wei Cheng, PhD

Students Joshua Brettmann, Shannon Ohlemacher, Joseph Stodola, JooYoung Park, Shankar Parajuli and John Robinson

Annual Graduate Programs Retreat a Great Success

The department recently sponsored and participated in the annual retreat of the Biochemistry and Computational and Molecular Biophysics graduate programs on October 26 and 27.

It was a beautiful, crisp fall day when we arrived at the Cedar Creek Conference Center in New Haven, Mo., near the Missouri river. For this year's retreat, the scientific organizers, Weikai Li, PhD, and Thomas J. Brett, PhD, had invited two outstanding young scientists to give keynote lectures on Friday night.

The first was Wei Cheng, PhD, former graduate student in our CMBP program, and now a faculty member at the University of Michigan. Wei is developing new techniques in order to improve the study of single molecules in isolation and in whole cells. In a very interesting talk, he described his development of two-photon fluorescence detection techniques of single fluorescent proteins in mammalian cells.

The second keynote speaker was Enrique De La Cruz, PhD, professor of molecular biophysics and biochemistry at Yale University. Enrique gave an outstanding talk describing the biochemical and biophysical approaches that he combined to understand how actin filaments are severed by their cofactor cofilin.

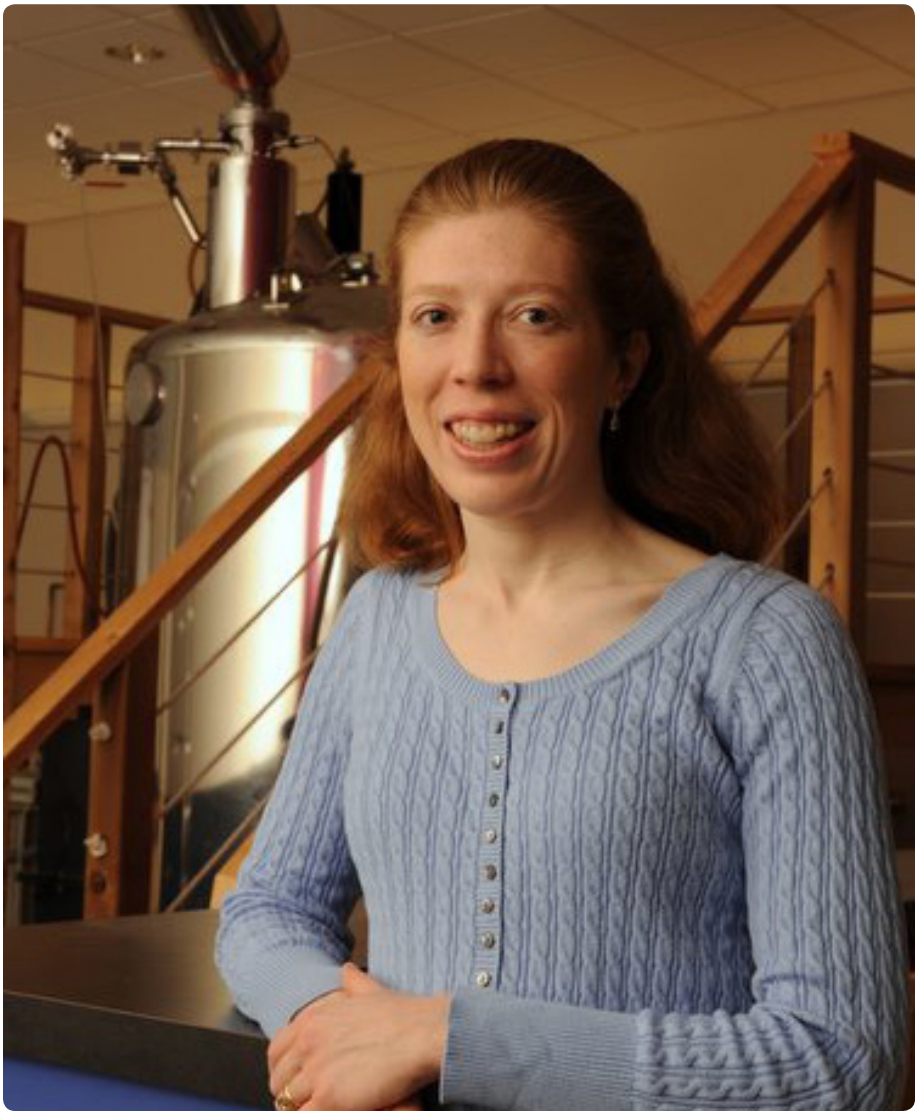
The retreat began on Friday afternoon with six high-quality talks by selected graduate students and postdocs. After dinner and the keynote speeches, it was time for the poster session, with refreshments, and a campfire to warm the chilly night. Anne Georges, a first-year student, brought a powerful telescope that she trained on the clear night sky for an excellent view of the moons of Jupiter.

The next morning, we heard five more excellent talks by students and postdocs. Most of the talks were given by graduate students in the two programs, but a few were given by students with biochemical/biophysical interests who are in other programs (chemistry, molecular microbiology). The interdepartmental participation of students and faculty lent strength to the program, the talks and the follow-up discussions. Indeed, the breadth of talks was remarkable.

Prizes were given to Emma Morrison from Katie Henzler-Wildman's lab and to Tom Kraft from Paul Hruz' lab for the best student posters, to Binh Nguyen from Tim Lohman's lab and Brett Olsen from Dan Ory's lab for the best postdoc posters, and to Megan Epperson from Daved Fremont's lab for the best talk.

Thanks for a successful retreat goes especially to Melissa Torres, coordinator for the Biochemistry and Biophysics programs, who perfectly organized every logistic aspect of the retreat. Additional thanks go to Weikai and Tom and to the many departments and divisions that provided financial contributions.

Faculty Spotlight: Katherine A. Henzler-Wildman, PhD



Katherine A. Henzler-Wildman, PhD

Katherine A. "Katie" Henzler-Wildman, PhD, assistant professor of biochemistry and molecular biophysics, comes by her interest in science naturally. Her father is an inorganic chemist, her mother is a medical physicist, and a sister and three maternal aunts have careers in science or medicine.

Growing up in Rochester, N.Y., Henzler-Wildman never questioned her own scientific calling. As an undergraduate at Cornell University, she began by studying chemical engineering. However, while conducting research in that field, she soon realized that her interest lay more in basic science.

"I wanted to get beyond an empirical understanding of how things worked to the 'how' and 'why,'" she says. "I wanted a more molecular understanding of things."

To that end, she changed her major to chemistry, which allowed time in her schedule to also take biology courses. In making the switch, Paulette Clancy, PhD, the Bodman Professor in Chemical Engineering at Cornell, sat down with Henzler-Wildman and helped her to determine exactly what line of research she wanted to pursue.

A passing reference to crystallography during high school had gotten Henzler-Wildman's attention because the field uses math, physics and chemistry to understand how molecules work at a very detailed level. When she mentioned this to Clancy, the latter immediately connected her with a colleague, Linda Nicholson, PhD, professor of molecular biology and genetics at Cornell, who studies nuclear magnetic resonance (NMR). Like crystallography, NMR allows scientists to examine the structures of proteins and their functions in detail. For the rest of her undergraduate career, Henzler-Wildman worked in Nicholson's lab.

"That's how I was introduced to NMR, and I've never looked back," she says.

After graduating from Cornell, Henzler-Wildman began studying the more technically demanding solid-state NMR at the University of Michigan in the laboratory of Ayyalusamy Ramamoorthy, PhD, professor of biophysics. Because solid-state NMR requires a greater understanding of quantum mechanics, she felt such study would provide a good foundation for any future NMR research. And because Michigan's chemistry program and biophysics department were closely related, Henzler-Wildman could major in chemistry but conduct research in biophysics, which was a good fit for her.

After earning her PhD, Henzler-Wildman decided to switch from studying solid-state NMR to the more biologically oriented solution-state NMR.

"I liked the biological system I was working on, but solid-state NMR wasn't really at the point then of being able to

answer very many interesting biological questions," she explains. "You weren't able to do uniform labeling, you weren't able to look at the whole molecule at once, like you could in solution-state NMR."

To that end, she conducted postdoctoral research with Dorothee Kern, PhD, professor of biochemistry at Brandeis University. Henzler-Wildman spent five years in Kern's laboratory studying how protein dynamics contribute to protein function.

That relationship is essentially what Henzler-Wildman continues to focus on, although she has reverted to the study of membrane proteins.

"My research today is a combination of the work I did as a graduate student and then as a postdoc," she explains. "But now we've backed up a little bit, because membrane proteins are a lot more difficult to work with. So what we're trying to address is 'Can we measure these kinds of detailed dynamics in integral membrane protein?'"

The answer to that question is "yes," and Henzler-Wildman and colleagues published results in the January 2012 issue of *Nature*.

"So this is my primary project — small, multi-drug resistance transporters. That's a nice model system. It's small so we can really develop the techniques, and we can really look at everything in detail," she says.

In addition, Henzler-Wildman is collaborating with two School of Medicine colleagues. She and Christopher J. Lingle, PhD, professor of anesthesiology and of neurobiology, are looking at the structure and function of the pore domain of a eukaryotic potassium channel. With Paul W. Hruz, MD, PhD, associate professor of pediatrics and of cell biology and physiology, she is attempting to translate the work her laboratory has done on small transporters to a large human glucose transporter.

The latter research has obvious disease relevance. And despite her work's focus on the most basic of science, Henzler-Wildman's research does fit into the larger "translational" picture driving research today.

"If we talk about the small multi-drug transporters, we're working on them to really understand the basic science," she says. These contribute to antibiotic resistance and bacteria which is obviously a big concern. And they're a particularly big concern because an individual transporter is a multi-drug transporter that pumps everything out of the cell, including antibiotics or chemotherapy drugs.

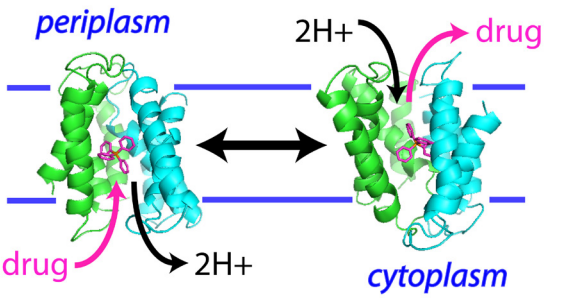
"What we'd like to be able to do is block some of these multi-drug transporters, though no one's been able yet to come up with an inhibitor for the small, multi-drug transporters. We're hoping that by really understanding the details of how it moves, we can figure out how to design a molecule that will block that motion and prevent it from functioning."

Because Henzler-Wildman is working with the smallest known active transporters, her findings also are affecting understanding of how transporters function in general. This allows researchers to really delve into the molecular details and understand how these things function with the goal of changing how they think about transporters, as well as to develop the tools that will allow them to look at these larger eukaryotic transporters, which have been very difficult to work with.

"Even a couple of years ago, people would have said it's absolutely impossible to get this kind of information on a membrane protein," says Henzler-Wildman.

But a combination of factors now has opened up the possibilities: better spectrometer technology that enables researchers to look at bigger systems; advancements in how scientists express, purify, label, produce and solubilize proteins; and advances in NMR techniques.

"Those things have come together to let the NMR technology be applied to these larger, harder-to-work-with systems," says Henzler-Wildman. "That's been the big breakthrough. Now we can start to consider additional projects by using this technology to bridge the gap between biology and the technical expertise used in NMR."



The Henzler-Wildman lab studies how small multi-drug resistance transporters convert between open-in and open-out conformations in order to pump drugs out of bacterial cells.

Henzler-Wildman joined the department in 2008. It's been a good fit: Today her laboratory is home to three graduate students, a postdoctoral student and a staff scientist shared with another NMR lab.

Although only in her mid-30s, in 2011 Henzler-Wildman received R01 funding from the National Institutes of Health (NIH). In 2010, she was honored with a Searle Scholars Award. Recently she learned that she will receive the 2013 Margaret Oakley Dayhoff Award from the Biophysical Society. Henzler-Wildman credits much of her success to mentoring received from senior faculty in the department.

She and her husband, Scott A. Wildman, PhD, research assistant professor of biochemistry and molecular biophysics, have two children: 5-month-old Thomas and 5-year-old Martha. Already, says Henzler-Wildman, her daughter is showing telltale signs of scientific interest.

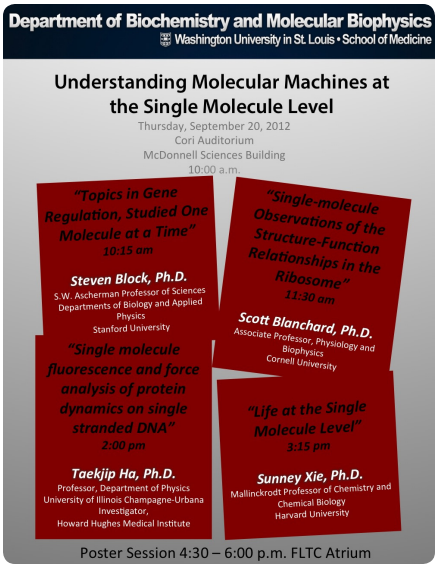
"My mother told me when I was pregnant that I'd know by the time she was age 2 if she was going to be a scientist," says Henzler-Wildman. "I laughed at the time, but she was absolutely correct. Martha is very analytical and observant, and mathematical and spatial reasoning skills come naturally to her; it's quite fascinating."

Spoken like a mom — and a scientist.

A Closer Look at Single-Molecule Approaches

Full-day symposium offers insight into state-of-the-art research

Research presented spurs discussion.



The Department of Biochemistry and Molecular Biophysics has had a longstanding focus on probing the mechanistic details of fundamental biological processes. A major part of this focus has been and continues to be to understand how things work.

How do enzymes and biological macromolecules function and how are they regulated? Probing these questions requires the use of biochemical and biophysical approaches, including structure, thermodynamics and kinetics. Until relatively recently, these approaches have involved studies of large populations or ensembles of molecules, which of course yield the average properties of a system. Outside of the field of ion channels, with the exception of a few visionaries, the concept that one could study the behavior and properties of a single enzyme, protein or nucleic acid molecule is relatively new.

The first use of a laser-based optical tweezer by Arthur Ashkin, PhD, and Steven Chu, PhD, to trap a single molecule was reported in 1986, and the first fluorescence detection of a single molecule by W. E. Moerner, PhD, and colleagues was reported in 1989. Since then, single molecule approaches have had a profound impact on mechanistic studies of biological macromolecules, allowing one to examine systems in real time without the need to synchronize an ensemble population of molecules.

By eliminating ensemble averaging, these approaches can yield distributions and fluctuations of molecular properties allowing transient intermediates to be identified and studied. Only a few years ago, single molecule experiments were performed only in laboratories in which the main focus was developing these single molecule techniques. This is no longer the case as many laboratories now use these techniques routinely indicating the incredible growth in this area of research.

In September, the department hosted a full-day symposium in Cori Auditorium titled "Understanding Molecular Machines at the Single Molecule Level." Four pioneers in the area of single molecule approaches presented seminars on their research.

Steven M. Block, PhD, from the Departments of Applied Physics and Biology at Stanford University presented a study of the co-translational folding of an RNA molecule.

Scott C. Blanchard, PhD, from the Department of Physiology and Biophysics at The Weill Cornell Medical College of Cornell University spoke on studies of the effects of antibiotics on ribosome function.

Taekjip Ha, PhD, from the Department of Physics at the University of Illinois, Urbana-Champaign and the Howard Hughes Medical Institute presented work on the translocation and DNA unwinding mechanisms of DNA helicases.

Sunney Xie, PhD, from the Department of Chemistry at Harvard University discussed studies of the regulation of gene expression in vivo using single molecule fluorescence. These individuals are leaders in the single molecule field and many of the pioneering studies and innovations that have led to the wide use of single molecule approaches to study biological systems have come from their laboratories.

The symposium was followed by a poster session and reception at which graduate students and postdoctoral associates presented their research.

The department owes a great thanks to the hard work of Jayma Mikes, Paula Reynolds, Anna Blanchard, Ginny Ribaudo and Linda Ketchens who helped to organize the symposium and made it a great experience for the entire Washington University community.

Recent Grants

Wayne M. Barnes, PhD
Fluorescent amino acid probe of template-strand bases. NIH R21HG006291. 8/1/11 – 7/31/13

Elliot L. Elson, PhD
The effects of myofibroblasts on electromechanical function of model heart tissue. NIH R01HL109505. 5/15/12 – 4/30/16.

Carl Frieden, PhD
Develop screening assays to differentiate Apoe isoforms. AHAF A2012422. 7/1/12 – 6/30/14.

Roberto Galletto, PhD
Helicase activity and its role in telomere and telomerase regulation. NIH R01GM098509. 8/1/11 – 7/31/16.

Katherine A. Henzler-Wildman, PhD
Transport mechanism of the multidrug resistance efflux protein, EmrE. NIH R01HL054390. 7/1/11 – 6/30/16.

Kathleen B. Hall, PhD
RNA-ligand interactions: simulation and experiment. NIH R01GM098102. 9/30/11 – 8/31/15.

Coevolution of snRNP U1A/U2B proteins and snRNA stemloops. NIH R01GM096444. 9/5/11 – 5/31/15.

James W. Janetka, PhD
Regulation of ligand-mediated receptor activation in metastatic breast cancer.

Susan G. Komen Foundation CCR12222792. 11/1/12 – 10/31/15.

Design and synthesis of C-mannosides to treat UTIs. Bear Cub Fund. 3/20/12 – 3/19/13.

Timothy M. Lohman, PhD
Helicase catalyzed DNA unwinding. NIH R01GM045948. 5/15/12 – 4/30/16.

Linda J. Pike, PhD
Heterodimerization in ERBB receptors. NIH R01GM099695. 7/1/12 – 6/30/16.

Allocations

Katherine A. Henzler-Wildman, PhD
Solution NMR structure and dynamics of facilitative glucose transport proteins. Children's Discovery Institute, Interdisciplinary Research Initiative MD-II-2012-234. PI Paul Hruz. 2/1/12 – 1/31/15.

Evaluation of BK channel pore-gate-domain peptides for solution NMR. CIMED. PI Chris Lingle. 2/1/12 – 1/31/13.

James W. Janetka, PhD
Targeting essential ROP kinases in toxoplasma. NIH R01AI082423. PI David Sibley. 8/1/11 – 7/31/16.

Scott A. Wildman, PhD
Targeting essential ROP kinases in toxoplasma. NIH R01AI082423. PI David Sibley. 8/1/11 – 7/31/16.

Cancer research innovations. Siteman, Alvin J Cancer Research Fund. PI Dennis Hallahan. 8/1/11 – 7/31/13.

Accolades

Peter M. Burgers, PhD
Editorial Board, *Journal of Biological Chemistry*

John A. Cooper, MD, PhD
Public Information Committee, American Society for Cell Biology
Editorial Board, *Cellular Logistics*
Editorial Board, *Experimental Cell Research*
Co-editor, special issue on Chromosome Biology, *Experimental Cell Research*
Editorial Board, *Cell Motility and the Cytoskeleton*

Carl Frieden, PhD
Editorial Board, *Biochemistry*
Editorial Board, *Protein Science*

Kathleen B. Hall, PhD
Editorial Board, *Biochemistry*
Associate Editor, *Biophysics Journal*
Co-chair Public Affairs Committee, *Biophysical Society*

Katherine A. Henzler-Wildman, PhD
Margaret Oakley Dayhoff Award, *Biophysical Society*
Outstanding St. Louis Scientist Award, *Academy of Science, Saint Louis*
Distinguished Investigator Award, *Washington University*

James W. Janetka, PhD
Editorial Board, *Current Drug Discovery Techniques*
Editorial Board, *Chemistry in Cancer Research*

Linda J. Pike, PhD
Distinguished Service Teaching Award, Washington University School of Medicine
Associate Editor, *Journal of Lipid Research*

Scott A. Wildman, PhD
Program Chair, National Meeting Division, American Cancer Society

Selected Publications

Netz, DJA, Stith, CM, Stümpfig, M, Köpf, G, Vogel, D, Genau, HM, Stodola, JL, Lill, R, Burgers, PM and Pierik, AJ. Eukaryotic replicative DNA polymerases require aniron-sulfur cluster for complex formation. *Nat Chem Biol* 8:125-32 (2011).

Kim, T, Ravilious, GE, Sept, D and Cooper, JA. Mechanism for CARMIL protein inhibition of heterodimeric actin-capping protein. *J Biol Chem* 287:15251-62 (2012).

Soto-Pantoja, DR, Miller, TW, Frazier, WA and Roberts, DD. Inhibitory signaling through signal regulatory protein-alpha is not sufficient to explain the antitumor activities of CD47 antibodies. *Proc Natl Acad Sci USA* 109:E2842 (2012).

Frieden, C and Garai, K. Structural differences between apoE3 and apoE4 may be useful in developing therapeutic agents for Alzheimer's disease. *Proc Natl Acad Sci USA* 109:8913-8 (2012).
ApoE4 is the major risk factor for late-onset Alzheimer's Disease. ApoE4 differs from apoE3, which is not related to Alzheimer's Disease, by a single amino acid (of 299). This paper defines structural differences between apoE4 and apoE3 which may relate to their functional differences.

Galburt, EA, Parrondo, JMR and Grill, SW. RNA polymerase pushing. *Biophys Chem* 157:43-7 (2011).

Barranco-Medina, S and Galletto, R. DNA binding induces dimerization of *S. cerevisiae* Pif1. *Biochemistry* 49: 8445-54 (2010).
In S. cerevisiae *Pif1 is involved in a wide range of DNA transactions. It operates both in mitochondria and in the nucleus, where it has telomeric and non-telomeric functions. All of the activities of Pif1 rely on its ability to bind to DNA. We have determined the mode of Pif1 binding to different DNA substrates. While Pif1 is a monomer in solution, we show that binding of ssDNA to Pif1 induces protein dimerization. DNA-induced dimerization of Pif1 is also observed on tailed- and forked-dsDNA substrates, suggesting that on the latter formation of a Pif1 dimer prevents binding of additional Pif1 molecules. A dimer of Pif1 also forms on ssDNA of random composition and in the presence of saturating concentrations of non-hydrolyzable ATP analogs. The observation that a Pif1 dimer is formed on unwinding substrates in the presence of ATP analogs suggests that a dimeric form of the enzyme might constitute the pre-initiation complex leading to its unwinding activity.*

Williams, SG and Hall, KB. Coevolution of Drosophila *snf* protein and its snRNA targets. *Biochemistry* 49:4571-82 (2010).

Morrison, EA, DeKoster, GT, Dutta, S, Vafabakhsh, R, Clarkson, MW, Bahl, A, Kern, D, Ha, T and Henzler-Wildman, K. Anti-parallel EmrE exports drugs by exchanging between asymmetric structures. *Nature* 481:45-50 (2011).
This is the first quantitative, atomic-resolution measurement of the kinetics and relative populations of a transporter converting between the inward- and outward- facing states. This conformational exchange has long been believed to occur and is the key step in the transport cycle, effectively moving substrate from one side of the membrane to another. However, this study provides the most detailed direct experimental observation of this process. Furthermore, by establishing the antiparallel topology of the two monomers in the homodimer while it is actively exchanging, this work resolves the long-standing controversy surrounding the topology of EmrE in its functional form. These experiments also demonstrated that the inward- and outward-facing states of EmrE are identical, with the two monomers in the antiparallel asymmetric homodimer simply swapping conformations. This finding of "dynamic symmetry" nicely

reconciles the asymmetry observed in cryo-electron microscopy and crystal structures of EmrE with the functional symmetry of residues in the active site that is apparent in biochemical studies.

Cusumano, CK, Pinkner, JS, Han, Z, Greene, SE, Ford, BA, Crowley, JR, Henderson, JP, Janetka, JW and Hultgren, SJ. Treatment and prevention of urinary tract infection with orally active FimH inhibitors. *Sci Transl Med* 3:1-10 (2011).

Li, W and Li, F. Cross-crystal averaging with search models to improve molecular replacement phases. *Structure* 19:155-161 (2011).

Wu, CG, Xie, F and Lohman, TM. The primary and secondary translocase activities within *E. coli* RecBC helicase are tightly coupled to ATP hydrolysis by the RecB motor. *J Mol Biol* 423:303-14 (2012).
This study demonstrates that a novel "secondary" translocase activity within the RecBC helicase is driven by the canonical RecB ATPase motor.

Zheng, X, Wu, C, Ponder, JW and Marshall, GR. Molecular dynamics of highly stabilized beta-hairpin models of epigenetic recognition motifs. *J Am Chem Soc* 134:15970-8 (2012).
The conformations and stabilities of the β-hairpin model peptides have been experimentally characterized as a function of lysine ε-methylation. These models were developed to explore molecular recognition of known epigenetic recognition motifs. This system offered an opportunity to computationally examine the role of cation-π interactions, desolvation of the ε-methylated ammonium groups, and aromatic/aromatic interactions on the observed differences in NMR spectra. AMOEBA, a second-generation force field was chosen as it includes both multipole electrostatics and polarizability thought to be essential to accurately characterize such interactions. The ability of MD simulations to reproduce the observed NOEs for the four peptides was further estimated for the monopole-based force fields, AMBER, CHARMM and OPLSAA. AMOEBA correctly predicted over 80 percent of the observed NOEs for all four peptides, while the three-monopole force fields were 40 to 50 percent predictive in only two cases and approximately 10 percent in the other 10 examples. Significance: This paper demonstrates the inability of force fields using monopole electrostatics to even approximate the dynamics of peptide/proteins in solution. Thus, the results of almost all MD simulations reported to date are suspect!

Macdonald-Obermann, J, Yang, KS, Piwnica-Worms, D and Pike, LJ. Mechanics of EGF receptor/ErbB2 kinase activation revealed by luciferase fragment complementation imaging. *Proc Natl Acad Sci USA* 109:137-42 (2012).
This manuscript addresses a mechanistic question regarding the activation of the EGF receptor kinase. These studies show that activation of the kinases within the singly-ligated EGFR dimer is not random but rather is determined by which subunit binds the ligand. The data demonstrate that the kinase domain of the subunit that binds ligand becomes activated and phosphorylates its partner subunit which then mediates the downstream effects of the ligand.

Take a Break: Departmental Offerings

Come in, grab a cup of coffee, look at the graduate student plaques, discuss science or just relax and enjoy the art.

Walking toward the break room you are immediately aware of a large eyeball staring straight at you. This and all of the photos on the south wall of the break room feature photographs taken by Carl Frieden, PhD.

Frieden has been taking photos since he was in high school and has graciously agreed to display several of his photos with one photo changing each month. Photos include bridges in Forest Park, sculptures in Laumeier Park and scenes from the Missouri Botanical Garden.

The west wall of the break room is now our departmental ArtWall. Recently the wall displayed a quilt, "And Dragons, Too," hand-crafted by Suzanne Marshall, an internationally known quilt-maker. Examples of her work are displayed in museums all over the country, as well as in books. She is married to Garland Marshall, PhD, professor of biochemistry and molecular biophysics.

Be sure to stop by often to view the next exciting art work. If you are an artist from the departmental community willing to display your work, please contact Kathleen Hall at **kathleenhal@gmail.com**.

Walnut plaques displaying the names of students who completed their graduate work in the department hang on the east wall of the break room. In addition, many of their theses are available on the shelves directly to the left of the plaques.

Suzanne Marshall's "And Dragons, Too"

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