

BIOGRAPHICAL SKETCH

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NAME: Mark Yeager, M.D., Ph.D.

eRA COMMONS USER NAME (credential, e.g., agency login): mark_yeager

POSITION TITLE: Andrew P. Somlyo Distinguished Professor of Molecular Physiology and Biological Physics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Carnegie-Mellon University, Pittsburgh, PA	B.S.	1971	Chemistry
Yale University, New Haven, CT	M.Phil.	1973	Molecular Biophysics and Biochemistry
Yale University, New Haven, CT	Ph.D.	1978	With Lubert Stryer, M.D.
Yale University School of Medicine, New Haven, CT	M.D.	1979	Medicine
Stanford University Medical Center, Stanford, CA	Intern and Resident	1982	Internal Medicine
Stanford University Medical Center, Stanford, CA	Chief Resident	1983	Internal Medicine with Ken Melmon, M.D. and Ferid Murad, M.D., Ph.D.
Stanford University Medical Center, Stanford, CA	Fellow	1986	Cardiology with Richard Popp, M.D.
Stanford University Medical Center, Stanford, CA	Advanced Fellow	1987	Cell Biology with Nigel Unwin, Ph.D.

A. Personal Statement

The ultimate goal of our research is to gain a deeper understanding of the molecular basis of important human diseases, such as sudden death, heart attacks, and HIV infection, which cause substantial mortality and suffering. The structural details revealed by our research may provide clues for the design of more effective and safer medicines.

At the basic science level, we are intrigued by questions at the interface between cell biology and structural biology: How do membrane proteins fold? How do membrane channels open and close? How are signals transmitted across a cellular membrane when an extracellular ligand binds to a membrane receptor? How do viruses attach to and enter host cells, replicate, and assemble infectious particles?

Our research exemplifies integrative structural biology, including the biophysical methods of cryoEM, X-ray crystallography, HDX and mass spectrometry, DEER EPR spectroscopy and molecular modeling. In 2014, we reviewed the status of integrative structural and computational biology and concluded that "Recent technical advancements in EM, X-ray crystallography and computational simulation create unprecedented synergies for integrative structural biology to reveal new insights into heretofore intractable biological systems" [Purdy, M.D., Bennett, B.C., McIntire, W.E., Khan, A.K., Kasson, P.M. and Yeager, M. Function and Dynamics of Macromolecular Complexes Explored by Integrative Structural and Computational Biology. *Curr. Op. Struct. Biol.* **27**: 138-148 (2014)].

In cryoEM, biological specimens are quick frozen in a physiological state to preserve their native structure and functional properties. A special advantage of this method is that we can capture dynamic states of functioning macromolecular assemblies, such as open and closed states of membrane channels and viruses actively transcribing RNA. Three-dimensional density maps are obtained by digital image processing of the high-resolution electron micrographs. The rich detail in the density maps exemplifies the power of this approach to reveal the structural organization of complex biological systems that can be related to the functional properties of such assemblies. In the case of lower resolution 3D maps, the molecular envelopes provided by cryoEM can be used for docking atomic resolution structures of domains and subunits, thereby

yielding molecular models for the entire complex.

In the last ~5 years, a new era in cryoEM has emerged, referred to as “The Resolution Revolution.” Technical advancements in electron cryomicroscopes, exemplified by the Titan Krios electron microscope, image processing software, exemplified by EMAN, Relion and CryoSparc, and especially the development of exquisitely sensitive direct electron detectors enabling corrections for beam-induced motion have resulted in an exponential growth in the number of near-atomic resolution 3D structures of small, asymmetric biological macromolecules and complexes. Importantly, the macromolecules are maintained in the frozen-hydrated state in physiologic buffers, as opposed to far from physiologic conditions required for 3D crystallization. Consequently, one can argue that high-resolution structures determined by cryoEM and image analysis have become the touchstone that represents the authentic, near-to-native state of biological macromolecules.

I was very fortunate to have been a post-doctoral fellow in Nigel Unwin’s lab in the 1980’s when cryoEM methods were just being developed. I can recall driving from Stanford to Pleasanton, CA to pick up the latest prototype cryostage from Gatan that we were all anxious to test. I was recruited to UVA in 2007, and one of my goals was to establish a state-of-the-art cryoEM laboratory accessible to all scientists, analogous to the access of scientists for performing X-ray crystallography at synchrotrons. The Molecular Electron Microscopy Core facility became an official School of Medicine Core in 2014, and since that time we have hosted 81 scientists from 45 labs at UVA, as well as 32 scientists from 17 outside institutions, spanning 7 states from New England to California, and also Brazil. Given the competition for access to our Titan Krios, we are applying for data collection time at the NYSBC. Our allotted time will be efficiently used because we prescreen grids on our Titan Krios, generate a grid atlas and preselect the grid squares for data collection.

B. Positions

- 1988-14 **Assistant Professor** (1988-1994), **Associate Professor** (1994-2000), **Professor** (2000-2014) The Scripps Research Institute, La Jolla, CA, Department of Cell Biology with a joint appointment in Molecular Biology
- 2014- **Adjunct Professor**, Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA
- 1988-07 **Invasive Cardiologist**, Department of Medicine, Division of Cardiovascular Diseases, Scripps Clinic, La Jolla, CA
- 2007- **Professor of Cardiovascular Medicine**, Department of Medicine, Division of Cardiovascular Medicine, University of Virginia Health System, Charlottesville, VA
- 2018- **Harrison Distinguished Teaching Professor**, Dept. of Molecular Physiology and Biological Physics, University of Virginia School of Medicine; Charlottesville, VA

Selected Honors and Memberships

- 1979- **Alpha Omega Alpha Honor Medical Society**, Yale University School of Medicine
- 1980- **Licensed Physician**, State of California, G044485
- 1980- **Diplomate**, National Board of Medical Examiners
- 1984- **Diplomate**, American Board of Internal Medicine
- 1984-86 **National Research Service Award Fellowship**, NIH
- 1986 **Dorothy Penrose Stout Award**, AHA, CA Affiliate, for the highest merit score
- 1986- **Diplomate**, ABIM, Subspecialty of Cardiovascular Diseases
- 1987-92 **K08 Clinical Investigator Award**, NIH
- 1989- **Cardiac Transplant Physician**, United Network for Organ Sharing
- 1991- **Member**, Biophysical Society
- 1991- **Member**, American Society of Cell Biology
- 1993-98 **Established Investigator Award**, American Heart Association and Bristol Myers-Squibb
- 1997 **Health Hero Award**, Combined Health Agencies of San Diego
- 1998-03 **Clinical Scientist Award in Translational Research**, Burroughs Wellcome Fund
- 2007-18 **Andrew P. Somlyo Distinguished Professor and Chair** of Molecular Physiology and Biological Physics, University of Virginia School of Medicine; Charlottesville, VA
- 2008- **Licensed Physician**, State of Virginia
- 2012- **Member**, Microscopy Society of America
- 2014 **Dean’s Award for Excellence in Team Science** – The Pannexin Interest Groups, University of Virginia School of Medicine
- 2015- **Fellow**, American Association for the Advancement of Science, Section on Medical Sciences

C. Contributions to Science

144 total publications; 36 publications cited > 100 times; h-index = 55; i10 index = 105

A complete list of our published work can be found at

<http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/48028565/?sort=date&direction=descending>

Structural biology of macromolecular complexes: Our laboratory uses methods of biophysics and structural biology to explore the design principles and function of macromolecular assemblies. Projects under study have included (1) membrane proteins involved in cell-to-cell communication and signaling (pannexin channels and gap junction channels, highlighted below), water transport (aquaporins), ion transport (potassium channels), and transmembrane signaling (integrins); (2) viruses responsible for significant human diseases (HIV-1 highlighted below, hepatitis B, hepatitis C, SARS, rotavirus and astrovirus); and (3) viruses used as model systems to understand mechanisms of pathogenesis (arenaviruses, reoviruses, nodaviruses, tetraviruses, tobamoviruses and sobemoviruses).

Eukaryotic membrane proteins (e.g., aquaporins and integrins)

- 1) Mitra, A.K., van Hoek, A.N., Wiener, M.C., Verkman, A.S., and Yeager, M. The CHIP28 Water Channel Visualized in Ice by Electron Crystallography. *Nature Struct. Biol.* 2: 726-729 (1995).
- 2) Adair, B.D. and Yeager, M. Three-dimensional Model of the Human Platelet Integrin $\alpha_{IIb}\beta_3$ Based on Electron Cryo-Microscopy and X-ray Crystallography. *Proc. Natl. Acad. Sci USA* 99:14059-14064 (2002).

Structural virology (e.g., rotavirus and hepatitis B)

- 3) Tihova, M., Dryden, K.A., Bellamy, A.R., Greenberg, H.B., and Yeager, M. Localization of Membrane Permeabilization and Receptor Binding Sites on the VP4 Hemagglutinin of Rotavirus: Implications for Cell Entry. *J. Mol. Biol.* 314: 985-992 (2001) [featured on the cover].
- 4) Dryden, K.A., Wieland, S.F., Whitten-Bauer, C., Chisari, F.V., and Yeager, M. Native Hepatitis B Virions and Capsids Visualized by Electron Cryo-microscopy. *Mol. Cell* 22: 843-850 (2006) [featured on the cover].

Maturation and assembly of HIV-1: Unlike viruses with icosahedral symmetry that can be crystallized and examined at high-resolution using X-ray crystallography, numerous viruses such as HIV-1, are pleomorphic. In this case, a general theme of our research program on HIV-1 has been the design and assembly of symmetric surrogates of the quasi-hexagonal and pleiomorphic lattices of the HIV-1 immature and mature capsids that enable the use of complementary biophysical approaches such as cryoEM and X-ray crystallography. For example, electron cryocrystallography of engineered 2D crystals of the HIV capsid protein CA yielded the first subnanometer resolution map that showed key interaction interfaces (Ganser-Pornillos *et al.*, 2007). In addition, the cryoEM-based C α model enabled the engineering of disulfide bonds that stabilized the hexameric and pentameric CA building blocks of the capsid (Pornillos *et al.*, 2009). These results enabled the building of the first atomic model of the conical capsid that revealed the molecular basis for the continuously variable hexagonal lattice in the pleomorphic conical capsid (Pornillos *et al.*, 2011; Yeager, 2011). Follow-on studies revealed a capsid interface for targeting drugs that would interfere with capsid assembly (Bhattacharya *et al.*, 2014). We have now used a similar hybrid approach of cryoEM and X-ray crystallography to determine the structure of a CTD-SP1 construct of the immature HIV-1 Gag lattice, which includes the final protease cleavage site that triggers assemble of mature infectious particles (Wagner *et al.*, 2016). Interestingly, the CTD-SP1 interface assembles as a 6-helix bundle, and the scissile bonds are sequestered, facing the narrow lumen of the helical bundle. HIV-1 protease is known to cleave an unfolded state of the polypeptide, and how protease gains access to the cleavage sites is an active area of our current research.

- 1) Ganser-Pornillos, B.K., Cheng, A., and Yeager, M. Structure of Full-length HIV-1 CA: A Model for the Mature Capsid Lattice. *Cell* 131: 70-79 (2007).
- 2) Pornillos, O. Ganser-Pornillos, B.K., Kelly, B.N., Hua, Y., Whitby, F.G., Stout, D., Sundquist, W.I., Hill, C.P., and Yeager, M. X-ray Structures of the Hexameric Building Block of the HIV Capsid. *Cell* 137:1282-1292 (2009).
- 3) Pornillos, O., Ganser-Pornillos, B.K., and Yeager, M. Atomic-Level Modelling of the HIV Capsid. *Nature* 469: 424-427 (2011).
- 4) Bhattacharya, A., Alam, S.L., Fricke, T., Zadrozny, K., Sedzicki, J., Taylor, A.B., Demeler, B., Pornillos, O., Ganser-Pornillos, B.K., Diaz-Griffero, F., Ivanov, D., Yeager, M. Structural Basis of HIV-1 Capsid Recognition by PF74 and CPSF6. *Proc. Natl. Acad. Sci. USA*. 111: 18625-18630 (2014).

Architecture and design of gap junction channels. We have made important contributions in understanding the structure and regulation of gap junction channels. Gap junction channels are membrane pores comprised of two hexamers, each with six connexin subunits. A hexamer in the plasma membrane of one cell docks end-to-end with a hexamer in an adjacent cell forming an intercellular conduit that enables direct cell-to-cell communication between all cells in a tissue. Indeed, gap junction channels are present in tissues throughout the body and play a critical role in coordinating the normal metabolic and signaling activity within tissues, as well as disease states such as cancer and heart disease.

A milestone in our research was determination of the first subnanometer resolution cryoEM structure of the cardiac gap junction channel (Unger *et al.*, 1997 and 1999), which revealed that each connexin subunit is formed by a 4-helix bundle. Each hexamer is therefore comprised of a beautiful bundle of 24 α -helices surrounding the central pore. The map also revealed that the lumen of the channel within the extracellular gap

is bounded by a wall of protein that provides a tight seal to exclude the exchange of substances with the extracellular milieu, which is an essential function of these intercellular channels.

During tissue injury such as acute myocardial infarction and stroke, Ca^{2+} stores are released into the cytoplasm. This state of Ca^{2+} overload is not only toxic to cells, but it also closes gap junction channels. In 1984, my postdoctoral advisor, Nigel Unwin, proposed a model based on low-resolution cryoEM that Ca^{2+} elicits large-scale, protein conformational changes that physically occlude the intercellular pore (Unwin and Ennis, *Nature* 307: 609-613). In fact, major textbooks of biochemistry, cell biology and physiology include a figure showing that Ca^{2+} gates gap junction channels by a steric “camera-iris” model in which the pore is closed by sliding and tilting of the subunits within each hexamer.

Since my postdoctoral years some 30 years ago, I have sought to explore this steric gating model at high resolution, which we recently achieved (Bennett *et al.*, 2016). Surprisingly, X-ray crystal structures of the Cx26 human gap junction channel with and without bound Ca^{2+} were almost identical, ruling out a large-scale structural change and a local constriction of the pore. (A couple technical points deserve mention. It was certainly not trivial to crystallize the channels, which ultimately required synthesis of novel facial amphiphile detergents (Lee *et al.*, 2013). As another example of hybrid methods, our 5.7-Å cryoEM map (Fleishman *et al.*, 2004) provided a phasing model for molecular replacement.) Inspection of the structures revealed that the side chains that coordinate Ca^{2+} reside at the interface between adjacent subunits, near the entrance to the extracellular gap, where local conformational rearrangements enable Ca^{2+} -coordination. Computational analysis of the two structures revealed that channel block is achieved by the dramatic positive electrostatic potential conferred by the binding of 12 Ca^{2+} ions (6 per hexamer), rather than a steric narrowing or occlusion of the pore, which is how almost all other membrane channels “gate”.

In summary, our results literally overturn a 30-year paradigm how gap junction channels are gated by calcium, which applies to states of tissue injury such as myocardial infarction and stroke. In the heart, gap junction channels mediate action potential propagation between cells and thereby play critical roles, not only maintaining the normal heartbeat but also potentially fatal arrhythmias. The action potential is propagated between cardiac myocytes via cytoplasmic K^+ , and the implication is that Ca^{2+} -mediated electrostatic block of K^+ permeation can elicit heart block, ventricular arrhythmias and sudden death.

- 1) Unger, V.M., Kumar, N.M., Gilula, N.B., and Yeager, M. Projection Structure of a Gap Junction Channel at 7Å Resolution. *Nature Struct. Biol.* 4: 39-43 (1997).
- 2) Unger, V.M., Kumar, N.M., Gilula, N.B., and Yeager, M. Three-dimensional Structure of a Recombinant Gap Junction Membrane Channel. *Science* 283: 1176-1180 (1999) [featured on the cover].
- 3) Fleishman, S.J., Unger, V.M., Yeager, M., and Ben-Tal, N. A C^α Model for the Transmembrane α -helices of Gap Junction Intercellular Channels. *Mol. Cell* 15: 879-888 (2004).
- 4) Bennett, B.C.,* Purdy, M.D.,* Baker, K.A.,* Acharya, C., McIntire, W.E., Stevens, R., Zhang, Q., Harris, A.L., Abagyan, R. and Yeager, M. An Electrostatic Mechanism for Ca^{2+} -mediated Regulation of Gap Junction Channels. *Nature Commun.* 7:8770, DOI: 10.1038/ncomms9770 (2016). * co-first authors

Complete List of Published Work in MyBibliography:

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D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Support:

1 P01 HL120840-05	08/01/2014-05/31/2019	0.76 CM
(PI Ravichandran, K.; Co-I Yeager, M.)	\$75,000 DC/yr	
NIH/NHLBI		

Pannexin Channels in Vascular Physiology and Inflammation

Pannexin 1 forms a membrane channel that supports nucleotide release for intercellular purinergic signaling that is important in inflammation, atherogenesis and blood pressure regulation. The P01 seeks to provide fundamental information on channel properties and on physiological mechanisms that control Pannexin 1 activity, which we expect will suggest ways to manipulate the channel for therapeutic purposes. Our studies will focus on the structural analysis of pannexin1 using electron microscopy and image analysis.

1 U24 GM116790-02A1 (PI Yeager, M.)	09/01/2017 – 08/31/2022	1.75 CM
NIH/NIGMS	\$200K DC/yr	

UVa Molecular Electron Microscopy Core for the Mid-Atlantic Region

The University of Virginia School of Medicine Molecular Electron Microscopy Core facility is a state-of-the-art cryoEM center for scientists at UVa and in the mid-Atlantic region to explore high-resolution structural biology of macromolecular complexes. The U24 grant provides essential support for infrastructure so that 29% of the available time can be dedicated to users in our Consortium.

5 P50 GM082545-12	09/01/2017 – 08/31/2022	1.25 CM
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(PI Sundquist, W.; Co-I, Yeager, M.)
NIH/NIGMS

\$161,800 DC/yr

Center for the Structural Biology of Cellular Host Elements in Egress, Trafficking and Assembly of HIV (CHEETAH Center)

This P50 consortium is focused on the structural biology of host proteins related to HIV1. Previously, our role was to provide core services for electron crystallography as related to all projects in the consortium. A particular project that exemplified our work is a collaboration with the Virology Core directed by Dr. Wes Sundquist on the mechanism of interaction between the restriction factor TRIM5 α and HIV-1 CA. For the next cycle of the grant, our research will focus on membrane proteins related to HIV.

5 R01 GM128507 (PI Yeager, M.)
NIH/NIGMS

09/01/2018 - 05/31/2022 2.75 CM
\$711,586 DC first year (includes \$265K for eqpt)

Structure Analysis of Viral Assembly Mechanisms

A generally accepted model for the assembly of viruses postulates that small macromolecular oligomers form a nucleating template that is required for initiation of particle formation. Moreover, it is evident in many cases that viral morphogenesis continues after completion of a closed shell. Over the last 14 years this research program has focused on the assembly and maturation of HIV-1.

Pending:

R01 HL142974 (PI Yeager, M.)
NIH/NHLBI

07/01/2018-06/30/2023 3.0 CM

Structure and Dynamics of Gap Junction Channels

Gap junction channels mediate intercellular signaling that is crucial in tissue development, homeostasis and pathological states such as cardiac arrhythmias, cancer and trauma. The aims of this proposal are to use electron cryomicroscopy, X-ray crystallography, EPR spectroscopy and HDX mass spectrometry to explore the molecular basis of gap junction channel regulation by Ca²⁺ and pH and to compare the structures of dodecameric gap junction channels and hexameric hemichannels. I have pursued this project since I was a cardiology fellow in 1983, and I am grateful that the project has been supported previously by the American Heart Association, an NIH K08 grant the Burroughs Wellcome Fund and philanthropic funds. This NIH grant was originally funded in 1994, and the project has culminated in papers published in *Science*, *Nature Comm.*, *Nature Struct. Biol.*, *Mol. Cell*, *J. Mol. Biol.* and *J. Struct. Biol.*

Completed:

1 S10 OD018149-01

06/01/2014 – 05/31/2015

(Co-PIs Egelman, E. and Yeager, M.)
NIH/NIGMS

Direct Electron Detector for a Titan Krios Robotic Electron Cryo-Microscope

This application provided funds to retrofit a new, state-of-the-art electron cryomicroscope commissioned in the spring of 2013 with a direct electron detector to accelerate data acquisition and the quality of recorded images.

American Diabetes Association 7-13-BS-038

07/01/2013 – 06/30/2016

(PI Daniels, M.; Collaborator Yeager, M.)
ADA

Structure-function Analysis of the Human β -cell Zinc Transporter ZnT8 (SLC30A8)

The human zinc transporter ZnT8 (SLC30A8) is expressed exclusively in pancreatic β -cells and is crucial for maintaining the concentration of blood glucose through its role in insulin storage, maturation and secretion. This transporter is associated with Type 2 diabetes (T2D) through its risk allele that encodes a major non-synonymous SNP at Arg325 and also with Type 1 diabetes (T1D) as a major auto-antigen. In this project we expressed and purified human ZnT8 from *Pichia pastoris* and SF9 insect cells and demonstrated that the purified protein is functional. EM and image analysis of negatively-stained particles revealed a dimeric structure with transmembrane and ectodomains. These results provide a foundation for higher resolution structural studies and screening experiments to identify compounds that modulate ZnT8 activity.

AZ SEML-7V59S7 (PI Yeager, M.; Salerno, M. Co-PI) 08/01/15 - 07/31/2016

AstraZeneca

Hyperlipidemic Swine Model of Microvascular Disease and Coronary Ischemia

Coronary artery disease is the major cause of mortality in the U.S., accounting for 1 in 7 deaths. In addition to obstructive, epicardial coronary artery disease, microvascular disease, has a similar adverse prognosis. The goal of this proposal is to develop a chronic ischemia, preclinical model of microvascular disease in hyperlipidemic pigs, which will be essential for drug development of novel therapeutics to meet the unmet clinical need of microvascular coronary disease.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Purdy, Michael D.

eRA COMMONS USER NAME (credential, e.g., agency login):mdpurdynih

POSITION TITLE: Assistant Professor of Research, Department of Molecular Physiology & Biological Physics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
James Madison University, Harrisonburg, VA	B.S.	12/1995	Physics
University of Virginia, Charlottesville, VA	Ph.D.	05/2002	Biophysics

A. Personal Statement

I have dedicated my training and career to the study of protein structure, and I have continually worked to develop methods to make this process more efficient and successful. As both a member of Mark Yeager's laboratory since 2011 and as a staff member in the UVA Molecular Electron Microscopy Core (MEMC) since 2016, I have gained considerable experience in cryo-EM. My responsibilities in the MEMC include advising, assisting, and training users in all aspects of cryo-EM. As part of my own research and in assisting students and collaborators in the Yeager lab I have optimized preparation of cryo-grids for challenging samples including membrane proteins, membrane protein complexes, and soluble proteins.

I have significant experience in single-particle cryo-EM data processing from my own, ongoing research projects, collaborations, advising Yeager lab students, and assisting users in the MEMC. I have been involved in cryo-EM grid preparation and screening, data collection, data processing, and structure analysis for two membrane protein projects in the Yeager lab that are now in the final stages of refinement. For one of these projects, I traveled to eBIC (Diamond Light Source, Harwell, U.K.) and NCEF (NCI, Gaithersburg, MD) where I coordinated Volta phase plate (VPP) data collection. At each facility, this was the first use of the VPP for a research project. I also collaborated with members of Dr. Tamir Gonen's laboratory to solve the structure of the CTD-SP1 fragment of the HIV-1 Gag protein in complex with the maturation inhibitor bevirimat by MicroED (electron diffraction from three-dimensional crystals).

1. Wagner, J.M., Zadrozny, K.K., Chrustowicz, J., Purdy, M.D., Yeager, M., Ganser-Pornillos, B.K., Pornillos, O. (2016) Crystal structure of an HIV assembly and maturation switch. *eLife*. 5: e17063. PMID: PMC4946879.

B. Positions and Honors

Positions and Employment

1997 – 2002	Graduate research, Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, VA
2002 – 2006	Postdoctoral research, Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, VA
2006 – 2010	Instructor of Research, Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, VA
2010 – 2011	Professional Scientific Collaborator, The Scripps Research Institute, La Jolla, CA
2016 – Present	Senior Scientist, Molecular Electron Microscopy Core, University of Virginia, Charlottesville, VA

C. Contributions to Science

1. **Project:** Investigate the mechanism of pH regulation of the Cx26 gap junction channel.
Achievements: Assisted graduate students with cryo-EM grid preparation, data collection, image processing and reconstructions, and structure determination and analysis.
 - a. Khan, A., Jagielnicki, M., Purdy, M., McIntire, W., Dharmarajan, V., Griffin, P., Yeager, M. (2018) pH gating of gap junction channels: visualization of a "ball and chain" by cryoEM. *Biophys. J.* (Meeting abstract in press).
2. **Project:** Structural basis of HIV-1 maturation inhibitor pharmacology.
Achievements: Crystallization, MicroED screening, MicroED data processing, structure determination, and analysis. HIV-1 protease (PR) cleavage of the Gag polyprotein triggers the assembly of mature, infectious particles. Final cleavage of Gag occurs at the junction helix between the capsid protein CA and the SP1 spacer peptide. We used MicroED to delineate the binding interactions of the maturation inhibitor bevirimat (BVM) using very thin frozen-hydrated, three-dimensional microcrystals of a CTD-SP1 Gag construct with and without bound BVM. The 2.9-Å MicroED structure revealed that a single BVM molecule stabilizes the 6-helix bundle via both electrostatic interactions with the dimethylsuccinyl moiety and hydrophobic interactions with the pentacyclic triterpenoid ring. These results provide insight into the mechanism of action of BVM and related maturation inhibitors that will inform further drug discovery efforts. This study also demonstrates the capabilities of MicroED for structure-based drug design.
 - a. Purdy, M.D., Shi, D., Chrustowicz, J., Hattne, J., Gonen, T., Yeager, M.. (2017) MicroED structures of HIV-1 Gag CTD-SP1 Reveal Binding Interactions with the Maturation Inhibitor Bevirimat. *bioRxiv*, doi:10.1101/2411
3. **Project:** Reveal the structural basis of G protein selectivity in the adenosine receptor family of GPCRs with cryo-EM structures of adenosine receptor-G protein complexes.
Achievements: Solved the agonist-bound structure of a GPCR using LCP crystallization. Designed constructs including receptor fusions, purified receptors, identified stabilizing detergents and ligands using fluorescence assays, prepared membrane protein complexes, and monitored sample quality by negative stain EM. Leading cryo-EM structure determination efforts. Prepared cryo-EM grids and collected cryo-EM data, performed image processing, and generated 3D reconstructions. Generated a hybrid model of the full GPCR-G protein complex by combining cryo-EM 3D maps, HDX data, X-ray structure docking, and homology modeling followed by all-atom molecular dynamics energy minimization and simulation in a lipid bilayer.
4. **Project:** Elucidate the mechanisms of calcium and pH regulation and the functional consequences of disease-causing mutations in the Cx26 human gap junction channel. Gap junction channels are formed by the extracellular docking of integral membrane proteins (connexons) in adjacent cells.
Achievements: Solved X-ray crystal structures of the Ca²⁺-bound and Ca²⁺-free Cx26 gap junction channel (PDB: 5ER7 and 5ERA) using a cryo-EM model for MR phasing. Performed MD simulations of WT and mutant Cx26. Collaborated with Dr. Ruben Abagyan (UCSD, Molsoft) to identify a novel electrostatic mechanism for channel gating and the basis of a deafness-causing mutation.
 - a. Bennett, B.C., Purdy, M.D., Baker, K.A., Acharya, C., McIntire, W.E., Stevens, R.C., Zhang, Q., Harris, A.L., Abagyan, R., Yeager, M.. (2016) An electrostatic mechanism for Ca²⁺-mediated regulation of gap junction channels. *Nat. Commun.* 7:8770. (underlined contributed equally). PMID: PMC4730032.
 - b. Purdy, M.D., Bennett, B.C., McIntire, W. E., Khan, A.K., Kasson, P.M., Yeager, M. (2014) Function and dynamics of macromolecular complexes explored by integrative structural and computational biology. *Curr. Opin. Struct. Biol.* 27:138 – 148.
5. **Project:** Methods and tools for efficient stabilization and crystallization of membrane proteins.

Achievements: In collaboration with a fellow lab member, developed an assay to rapidly screen the solubility of membrane proteins in 94 different detergents. Independently developed an assay to determine the optimal concentration for membrane protein crystallization in different detergents and an associated membrane protein crystallization screen (patents pending, licensed to Anatrace).

- a. Vergis, J.M., Purdy, M.D., Wiener, M.C.. (2010) A high-throughput differential filtration assay to screen and select detergents for membrane proteins. *Anal. Biochem.* 407(1):1-11. PMCID: PMC20667442.

Complete List of Published Work in MyBibliography:

<https://www.ncbi.nlm.nih.gov/sites/myncbi/1RyYAozxiSXA-/bibliography/51656671/public/?sort=date&direction=ascending>

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

1 U24 GM116790-01A1 (PI Yeager, M.)

09/01/2017 – 07/31/2022

NIH/NIGMS

UVa Molecular Electron Microscopy Core for the Mid-Atlantic Region)

The University of Virginia School of Medicine Molecular Electron Microscopy Core facility is a state-of-the-art cryoEM center for scientists at UVa and in the mid-Atlantic region to explore high-resolution structural biology of macromolecular complexes. The U24 grant provides essential support for infrastructure so that 29% of the available time can be dedicated to users in our Consortium, which includes Vanderbilt University, the University of Pittsburgh, Virginia Commonwealth University, Virginia Tech Carilion Research Institute and the NIH. The respective Co-PIs are Drs. Melanie Ohi, Peijun Zhang, Montserrat Samso, Debbie Kelly and Jennie Hinshaw.

5 P50 GM082545-11

08/01/2007 – 07/31/2022

(PI Sundquist, W.; Co-I, Yeager, M.)

NIH/NIGMS

Center for the Structural Biology of Cellular Host Elements in Egress, Trafficking and Assembly of HIV (CHEETAH Center)

This P50 consortium is focused on the structural biology of host proteins related to HIV1. Previously, our role was to provide core services for electron crystallography as related to all projects in the consortium. A particular project that exemplified our work is a collaboration with the Virology Core directed by Dr. Wes Sundquist on the mechanism of interaction between the restriction factor TRIM5 α and HIV-1 CA. For the next cycle of the grant, our research will focus on membrane proteins related to HIV.

5 R01 GM128507 (PI Yeager, M.)

09/01/2018 - 03/31/22

NIH/NIGMS

Structure Analysis of Viral Assembly Mechanisms

I have pursued this research program since I was a postdoctoral fellow in 1983, and I am especially grateful for support provided by NIH RO1 AI31535 (1992-97) and NIH R01 GM066087 (2003-17). For the last two cycles of the grant, our work was focused on mechanisms of assembly and maturation of HIV-1, which we propose to continue in R01 GM128507. In addition, to HIV-1, our research on virus assembly has included other human

pathogenic viruses (e.g., hepatitis B, hepatitis C, SARS, rotavirus and astrovirus, as well as viruses used as model systems to understand mechanisms of pathogenesis (arenaviruses, reoviruses, nodaviruses, tetraviruses, tobamoviruses and sobemoviruses). The project has culminated in papers published in Science, Nature Comm., Nature Struct. Biol., Mol. Cell, J. Mol. Biol. and J. Struct. Biol.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Susan A. Leonhardt

eRA COMMONS USER NAME (credential, e.g., agency login): sl2xfNIH

POSITION TITLE: Research Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Cook College-Rutgers University, New Brunswick, NJ	B.S.	05/1988	Biological Sciences
University of Massachusetts, Amherst, MA	Ph.D.	09/1995	Molecular & Cellular
University of Colorado, Denver, CO		02/2004	Molecular Endocrinology

A. Personal Statement

We have recently begun studies on the SERINC3 and SERINC5 proteins, multiple membrane spanning proteins that have been implicated as HIV restriction factors. I have been a member of the Yeager lab for over seven years and have extensive experience in the expression and biophysical characterization of eukaryotic membrane proteins for structural studies. I have used insect cell systems for large-scale expression and purification of adenosine receptor(s) and G-proteins for our adenosine receptor-G protein complex project as well as for the channel proteins connexins and pannexins. I have also used mammalian cells (HEK293T and HEK293S) for large scale production of pannexin concatemers for structural studies. I will be able to use both insect and mammalian expression systems to express the SERINC proteins for structural studies. I will use my expertise in the expression and biophysical characterization of membrane proteins to further our goal of understanding the structure of SERINC3 and SERINC5 to enable novel therapeutics that leverage the restriction activity of these proteins to help combat the spread of HIV-AIDS.

B. Positions and Honors

Positions and Employment

1986	Laboratory Assistant, Diabetes Group, Sandoz Pharmaceuticals, East Hanover, NJ. Synthesis and analysis of organic compounds
1988 – 1996	Undergraduate Independent Project under Dr. Lee D. Simon at the Waksman Institute of Microbiology, Rutgers University. Title: "Effects of hflA and hflB mutations on abnormal protein degradation in Escherichia coli." Experiments included the use of P1 transductions to generate E.coli strains containing mutations in hflA, hflB, lon and dnaK genes. Abnormal protein turnover in these strains was measured by biochemical analysis of puromycyl polypeptides and by assaying the stability of the missense mutant protein lacI(Ts) by α -galactosidase assays.
1988 – 1995	Laboratory Assistant to Dr. Mark Swanson, Molecular Biology Group, Sandoz Pharmaceuticals, East Hanover, NJ. Application of recombinant molecular biology techniques to isolate human genes involved in cholesterol and glucose metabolism.

1988-1989	Teaching Assistant, Department of Microbiology, University of Massachusetts at Amherst, Amherst, MA. Taught the laboratory sections of Microbiology and Immunology.
1988-1995	Graduate Student in the Program of Molecular and Cellular Biology at the University of Massachusetts at Amherst under Dr. Thomas L. Mason. Title: "Structure, function and regulation of HSP78, a nuclear gene encoding a mitochondrial heat shock protein in <i>Saccharomyces cerevisiae</i> ." Use of yeast genetics and molecular biology to characterize the regulation and function of a gene encoding a stress-induced protein in yeast mitochondria.
1995-2004	Postdoctoral Fellow in Dr. Dean P. Edwards's Laboratory, University of Colorado Health Sciences Center, Denver, CO. Defined fundamental mechanisms of how progesterone antagonists inactivate the progesterone receptor.
2008-2015	Laboratory Specialist II in Dr. Mark Yeager's Laboratory, University of Virginia Charlottesville, VA. Directs and resolves issues in the expression and purification of membrane proteins related to projects in the laboratory, with a focus on generating stable Adenosine Receptor/G protein complexes.
2014-present	Scientific Consultant (2014-present) BERYLLIUM, Bainbridge Island, WA and Boston, MA.
2015-present	Research Scientist

Selected Honors and Professional Memberships

1. Program in Molecular and Cellular Biology Teaching Assistant Award, University of Massachusetts
1. Member of the Genetics Society of America
1. Platform Presentation-Program in Molecular and Cellular Biology Annual Retreat, University of Massachusetts
- 1997-2000 National Research Service Award Fellowship (NIH)
- 1998-2003 Member of the American Association for the Advancement of Science

C. Contributions to Science

1. For my graduate work I used yeast genetics and molecular biology to be the first characterize the regulation and function of a gene encoding a stress-induced protein in yeast mitochondria. The protein, Hsp78, is involved in conferring thermotolerance to the mitochondrial compartment and also participating in the removal of damaged polypeptides.
 - a. **Leonhardt, S.A.**, Fearon, K., Danese, P.N. and Mason, T.L. 1993. *HSP78* encodes a yeast mitochondrial heat shock protein in the Clp family of ATP-dependent proteases. *Mol. Cell. Biol.* **13**:6304-6313. PMID: PMC364689.
2. My postdoctoral work directly defined fundamental mechanisms of how progesterone antagonists inactivate the progesterone receptor. These compounds have application in the treatment of uterine myoma, endometriosis, dysfunctional uterine bleeding, as potential contraceptives and in steroid responsive tumors.
 - a. Gass, E.K., **Leonhardt, S.A.**, Nordeen, S.K., and Edwards, D.P. 1998. The antagonists RU486 and ZK98299 stimulate progesterone receptor binding to deoxyribonucleic acid *in vitro* and *in vivo*, but have distinct effects on receptor conformation. *Endocrinology*. **139**: 1905-1919.
 - b. **Leonhardt, S.A.**, Altmann, M. and Edwards, D.P. 1998. Agonist and antagonists induce homodimerization and mixed ligand heterodimerization of human progesterone receptors *in vivo* by a mammalian two-hybrid assay. *Mol. Endocrinol.* **12**: 1914-1930.
 - c. Tetel, M.J., Giangrande, P.H., **Leonhardt, S.A.**, McDonnell, D.P. and Edwards, D.P. 1999. Hormone-Dependent Interaction between the Amino- and Carboxyl-Terminal Domains of Progesterone Receptor *In Vitro* and *In Vivo*. *Mol. Endocrinol.* **13**: 910-924
3. My work in the Yeager lab has contributed to understanding how Pannexin 1 channels are activated. Pannexin 1 subunits form oligomeric plasma membrane channels that mediate nucleotide release for purinergic signaling, which is involved in diverse physiological processes such as apoptosis, inflammation, blood pressure regulation, adipocyte glucose uptake and cancer progression and metastasis.
 - a. Yu-Hsin C, Jin, X., Medina, C.B., **Leonhardt, S.A.**, Kiessling, V., Bennett, B.C., Shu, S., Tamm, L., Yeager, M., Ravichandran, K.S., Bayliss, D.A. 2017. A quantized mechanism for activation of pannexin channels. *Nature Commun.* Jan 30;8:14324.

Complete List of Published Work in MyBibliography:

<https://www.ncbi.nlm.nih.gov/sites/myncbi/1lymqn97x4J5t/bibliography/51656584/public/?sort=date&direction=ascending>.

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

Active

5PO1HL120840-03 (Ravichandran, Kodi) 08/01/2014-5/31/2019 3.0 CM
Sub-Project ID: 7342 (Bayliss, Doug)
Mechanisms of Pannexin channel activation in physiology and cell death

The goal of the proposed research is to understand the molecular mechanisms of pannexin channel activation in physiological and diseased states. My role on this project is to express and purify pannexin I channels for functional and structural studies.

5 P50 GM082545-11 08/01/2007 – 07/31/2022 6.0 CM
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5 R01 GM128507 (PI Yeager, M.) 09/01/2018 - 03/31/22 3.0 CM
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Completed Research Support

None