BIOGRAPHICAL SKETCH

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NAME: Klaus Michael Hahn

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

POSITION TITLE: Distinguished Professor of Pharmacology

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
University of Pennsylvania	BA	1981	Biochemistry, Philosophy
University of Virginia	PhD	1986	Chemistry
Carnegie Mellon University	Postdoctoral	1987-1991	Chemistry, Cell Biology
The Scripps Research Institute	Sr. Rsch. Assoc.	1992-1994	Immunology

A. Personal Statement

Our lab focuses on two synergistic areas: 1) developing methods to visualize and control protein activity in live cells and animals, and 2) applying these to address basic questions re spatio-temporal control of signaling. Our biological studies center on the integration of cytoskeletal and adhesion dynamics in metastasis, phagocytosis signaling driven by mechanical force, and the structure of dynamic adhesion organelles (eg invadopodia, podosomes, focal adhesions). We are extending our studies to examine how cancer cells alter their morphology to facilitate signaling.

While addressing specific molecules for our biological studies, we try to produce generally applicable methods for protein engineering and imaging. These include fluorescent biosensors to visualize conformational changes of endogenous proteins, and means to study structural dynamics of individual molecules in living cells. We are developing dyes that report protein conformational changes, and engineered domains that can be inserted into target proteins to control conformation using either light or small molecules.

In our biological projects, we are currently inducing macrophages to engage geometrically regular objects. Using multiplexed imaging, we simultaneously control and visualize signaling in this system. This enables us to model lines of force and molecular interactions governing target recognition and sheds light on feedback and spatio-temporal control of signaling networks. In metastatic cells we are asking how GTPases are regulated by multiple GEFs, GDIs, and GAPs with overlapping roles, and how this is affected by the tumor microenvironment.

B. Positions, Scientific Appointments, and Honors

Academic positions and appointments

2022-present CoDirector, NIH BTDD Center for Cell Signaling Analysis
2016-2021 Advisory Committee for National Cancer Institute, Frederick National Laboratory
2013-2018 Program project leader, Spatio-temporal dynamics of GEF-GTPase networks
2009-2019 UNC Center for Computational and Systems Biology
2009-2017 Founder and Director, UNC-Olympus Imaging Center
2004-present Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill
2004-present Thurman Distinguished Professor of Pharmacology, University of North Carolina, Chapel Hill
2000-2004 Associate Professor, Department of Cell Biology, Scripps Research Institute
1997-2000 Assistant Professor, Department of Cell Biology, Scripps Research Institute

1994-1997 Assistant Professor, Department of Neuropharmacology, Scripps Research Institute

NIH Peer review

2025 Innovative Molecular and Cellular Analysis Technologies for Cancer Research Review
2024 NIGMS Biomedical Technology Development and Dissemination Center Review
2022 NIH Transformative Award Review
2022 NIH Special Review Panel for RM1 Tech Development Centers
2021 NIH CSR Special Emphasis Panel
2021 Austrian Science Fund (FWF)
2015-2021 NIH Cellular and Molecular Technologies study section, standing member
2014 NIH Division of Intramural Research, site visit
2014-2105 NIH EBIT study section
2012 NCI Provocative questions review panel
2012 Director's New Innovator Awards study section, NIH
2012 Enabling bioanalytical and imaging technologies (EBIT) study section, *ad hoc* panel (chair)
2011 NIH Enabling Biophysical and Imaging Technologies *ad hoc* reviewer
2009 Bioengineering special emphasis panel, NIH
2009 ARRA Go grants study section
2009 ARRA Challenge grants study section
2009 NIGMS Special emphasis panel on cellular imaging
2009 NCI Innovative Molecular Technologies Program, ad hoc reviewer
2008 NIH Cellular Structure and Function study section ad hoc reviewer
2007 NIH Microscopic Imaging study section *ad hoc* reviewer
2006-present Italian Association for Cancer Research study section and grant review
2006-present The Wellcome Trust, England study section and grant review
2010-2012 NIH College of CSR reviewers
2006 Roadmap initiative oversight—State of Science: molecular imaging and libraries at NIH,
2006 Roadmap initiative formulation—Nanomedicine program, NIH study section
2006 NIH Roadmap study section: Molecular Libraries and High Throughput Screening
2001 NIH Biophysics study section *ad hoc* reviewer
2003 NIH study section: Cellular and molecular imaging methods
2002 Ecole Polytechnique Federale De Lausanne study section and grant review
2002 Argonne National Laboratories study section and grant review

Editorial and advisory boards

2022 Editor, special issue of Biophysical Journal in memory of Dr. Ken Jacobson
2016-2021 Advisory Board, Frederick National Laboratory of the National Cancer Institute
2013-2016 Editorial board, Biophysical Journal
2011-2014 Advisory Board, NIH Center: Computer Integrated Systems for Microscopy and Manipulation
2006-2008 Sigma Chemical Company biosensor advisory board
2003-2008 Scientific Advisory Board, Panomics Company
2002 Consultant, Amersham Corporation
2001-2003 Scientific Advisory Board, Q3DM Company

Organizing meetings and education

2025 Session Chair, Biophysical Society Meeting
2024-present Imaging workshop for the NIH BTDD Center for Cell Signaling Analysis
2020 Imaging Africa, workshop in Cape Town teaching imaging to students from throughout Africa
2016-2019 Annual imaging workshop, NIH P41 Center - Computer Integrated Systems for Microscopy and Manipulation
2015 Co-organizer, Keystone meeting on Optogenetics
2008-2013 Annual imaging workshop, NIH P41 Laboratory for Fluorescence Dynamics, UC Irvine
2011 ASCB annual conference program committee
2011 Session chair, Biophysical Society Meeting
2007 Program Committee, International Society for Analytical Cytology Annual Meeting
2008 Co-chair, Imaging and Biosensors, ASCB annual meeting

2007 Organizer, ASCB subgroup meeting:Image Analysis and Photomanipulative Techniques
2004 Organizer, Molecular Microscopy of Living Cells, ASCB Annual meeting
2004 Organizer, Signal Transduction Targets for Effective Therapeutics, Cambridge Healthtech

Honors

Pearse Prize of the Royal Microscopy Society; Nature Reviews Cell Bio. 10th anniversary edition "Ten breakthroughs of the decade"; Fellow of the American Association for the Advancement of Science; NIH Roadmap Transformative R01 Award; Ronald Thurman Distinguished Professor of Pharmacology; National Institutes of Health James A. Shannon Directors Award

Named and plenary lectures:Annual Meeting of the Japanese Biochemical Society, 2003; International Society for Analytical Cytology, 2006; International Conference of Systems Biology, 2009; Korean Society for Biochemistry and Molecular Biology, 2010; Leica Scientific Forum France, 2012; Snyder Institute, U. Calgary Endowed Chair lecture, 2013; ABRF national meeting 2016; Pearse Prize Lecture, Royal Microscopy Society, UK, 2019; Beijing University, China 2023

C. Contributions to Science

Biosensors. A large portion of my career has been devoted to developing and applying fluorescent biosensors. Our work has demonstrated the value of biosensors, provided approaches applicable to many proteins, and encouraged what is now a widely used technique. My laboratory has developed biosensors for GTPases, GEFs, kinases and GTPase effectors. We have used designs based on intermolecular and intramolecular FRET, environment sensing dyes, and protein analogs that can fully replicate the function of the target protein. We continue to focus on new approaches to extend biosensor imaging to the single molecule level, enable combination of biosensors and optogenetics, and study low abundance molecules by greatly reducing cell perturbation

- Kraynov, V. S., C. E. Chamberlain, G. M. Bokoch, M. A. Schwartz, S. Slabaugh and K.M. Hahn. Localized Rac Activation Dynamics Visualized in Living Cells. **Science**, *290*:333-337, 2000.
- Pertz, O., Hodgson, L., Klemke, R., and Hahn, K.M. Spatio-temporal dynamics of RhoA activity in migrating cells. **Nature**, *440*:1069-1072, 2006.
- Marston, D.J., Vilela, M., Huh, J., Ren, J., Azoitei, M., Glekas, G., Danuser, G., Sondek, J., and Hahn, K.M. Multiplexed GTPase and GEF biosensor imaging enables network connectivity analysis. **Nature Chem. Biol.,** *16(8): 826-833,* 2020. PMC7388658
- Liu, B., Stone, O.J., Pablo, M., Herron, C.J., Nogueira, A.T., Dagliyan, O., Grimm, J.B., Lavis, L.D., Elston, T.C., and Hahn, K.M. Biosensors based on peptide exposure show single molecule conformations in live cells. **Cell,** *184(22): 5670-5685*, 2021. PMC8556369

Optogenetics. Optogenetics began with the engineering of light-sensitive ion channels to control brain function, but more recently completely different approaches have enabled control of diverse protein families critical to cell physiology (eg GTPases, kinases, scaffolds). We were among the first to focus on non-channel optogenetics, controlling GTPases with light in living cells. We have developed alternate approaches for optogenetic control that are suitable for different protein families, and with complementary advantages (including light-controlled steric block of the active sitie, insertion of photoresponsive domains for allosteric control, methods to sequester molecules with light, and control of endogenous proteins). Our molecules have been valuable for us and collaborators to study information flow in motility and immune cells (our work), to control the movement of cells in living animals, and for studies in development, immunology and brain function.

- Wu, Y, Frey, D., Lungu, O. I., Jaehrig, A., Schlichting, I., Kuhlman, B. and Hahn, K.M. Genetically-encoded photoactivatable Rac reveals spatiotemporal coordination of Rac and Rho during cell motility. **Nature***, 461*: 104-110, 2009. PMC2766670
- Hayashi-Takagi, A., Yagishita, S., Nakamura, M., Shirai, F., Wu, Y.I., Loshbaugh, A.L., Kuhlman, B., Hahn, K.M., and Kasai, H. Labelling and optical erasure of synaptic memory traces in the motor cortex. **Nature** *525:*333-338, 2015. PMC4634641.
- Wang, H., Vilela, M., Winkler, A., Tarnawski, T.**,** Schlichting, I., Yumerefendi, H., Kuhlman, B., Liu, R., Danuser, G., and Hahn, K.M. LOVTRAP, An Optogenetic System for Photo-induced Protein Dissociation. **Nature Methods***, 13(9):* 755-8, 2016. PMC5137947

- Stone, O.J., Pankow, N., Liu, B., Sharma, V.P., Eddy, R.J., Wang, H., Putz, A.T., Teets, F.D., Kuhlman, B., Condeelis, J.S., and Hahn, K.M. Optogenetic control of Cofilin and αTAT in living cells using Z-lock. **Nature Chem. Biol.,** *15: 1183-1190,* 2019. PMC6873228

Engineered allosteric responses. Using insights gained from our studies of protein allostery, we have developed engineered protein domains that can be inserted into proteins to confer regulation by light or small molecules. In our work published to date, we have generated inert, catalytically inactive kinases which can be activated in living cells by adding a small molecule to the medium. These kinase analogs can be directed to interact only with specific substrates upon activation. Most recently, GEFs, GTPases and kinases were engineered for photo-inhibition. We and others have used these tools to elucidate networks that control cell morphodynamics, metastasis and immune function. These techniques provide almost absolute specificity, so have been useful to differentiate the functions of very similar proteins, altering their function without the compensation seen when using genetic manipulation.

- Karginov, A., Ding, F., Kota, P., Dokholyan, N.V., and Hahn, K.M. Engineered allosteric activation of kinases in living cells. **Nature Biotech**., *28(7):* 743-7, 2010. PMC2902629
- Karginov, A., Tsygankov, D., Berginski, M., Chu, P-H., Trudeau, E., Yi, J.J., Gomez, Shawn, Elston, T.C. and Hahn, K.M. Dissecting motility signaling through activation of specific Src-effector complexes*.* **Nat. Chem. Bio.** *10(4):*286-90, 2014*.* PMC4064790
- Dagliyan, O., Tarnawski, M., Chu, P-H., Shirvanyants, D., Schlichting, I., Dokholyan, N.V., and Hahn, K.M. Engineering extrinsic disorder to control protein activity in living cells. **Science***. 354(6318):*1441-1444, 2016. PMC5362825
- Daglilyan, O., Krokhotin, A., Ozkan-Dagliyan, I., Deiters, A., Der, C.J., Hahn, K.M., and Dokholyan, N.V. Computational design of chemogenetic and optogenetic split proteins. **Nature Communications**, *9(1):4042,* 2018. PMC6168510

Fluorescent dyes that report protein function *in vivo*. My lab has developed environment-sensing fluorescent dyes to interrogate signaling activity in living cells and animals. When attached to proteins, their fluorescence responds to protein conformational changes or post-translational modifications. Biosensors based on the dyes provide greater sensitivity than FRET because they are directly excited, are very bright (ε > 150,000, QY > 0.7), and fluoresce at wavelengths > 600 nm. We and others have used the dyes to quantify the conformational state of endogenous proteins. Our studies of photobleaching and dye response mechanisms have been valuable in the design of fluorophores by other laboratories. New dyes are being designed for single molecule and super-resolution microscopy of conformational change within living cells, and for biosensors that enhance quantitative work because they produce ratiometric output without bleach correction.

- Nalbant, P., L. Hodgson, V. Kraynov, A. Toutchkine, K. M. Hahn. Activation of Endogenous Cdc42 Visualized in Living Cells. **Science**, 305:1615-1619, 2004.
- Gulyani, A., Vitriol, E., Allen, R., Wu, J., Gremyachinskiy, D., Lewis, S., Dewar, B., Graves, L.M., Kay, B.K., Kuhlman, B., Elston T., and Hahn, K.M. A biosensor generated via high-throughput screening quantifies cell edge Src dynamics. **Nature Chem. Bio***., 7*: 437-444, 2011. PMC3135387
- MacNevin, C.J., Toutchkine, A., Marston, D.J., Hsu, C.W., Tsygankov, D., Li, L., Liu, B., Qi, T., Nguyen, D.V. and Hahn, K.M. Ratiometric imaging using a single dye enables simultaneous visualization of Rac1 and Cdc42 activation. **J. Am. Chem. Soc.,** *138(8): 2571-2575,* 2016. PMC4825053
- MacNevin, C.J., Watanabe, T., Weitzman, M., Gulyani, A., Fuehrer, S., Pinkin, N.K., Tian, X., Liu, F., Jin, J., and Hahn, K.M. Membrane-Permeant, Environment-Sensitive Dyes Generate Biosensors within Living Cells. **J. Am. Chem. Soc.** *141(18):7275-7282,* 2019. PMC6572722

GTPase signaling. My lab has used the above tools together with "traditional approaches" to elucidate the function of signaling circuits that incorporate Rho family GTPases. These studies elucidated the pathways mediating apoptosis in response to cell damage and receptor ligation, dissected the role of GTPases in the control of cytoskeletal dynamics, and now are being used to elucidate platelet formation, phagocytosis and the role of the tumor microenvironment.

- Subauste, M., O. Pertz, E. Adamson, C. E. Turner, S. Junger, K. M. Hahn. Vinculin modulation of Paxillin-FAK interactions regulates ERK to control survival and motility. **J. Cell Biol**., *165*:171-181, 2004. PMC2172187

- Machacek, M., Hodson, L., Welch, C., Elliot, H., Pertz, O., Nalbant, P., Abell, A., Johnson, G., Hahn, K.M.* and Danuser, G.* Coordination of Rho GTPase activities during cell protrusion. **Nature**, *461*: 99-103, 2009. PMC2885353
- Hodgson, L., Spiering, D., Sabouri-Ghomi, M., Dagliyan, O., DerMardirossian, C., Danuser, G. and Hahn, K.M. FRET binding antenna reports spatiotemporal dynamics of GDI-Cdc42 GTPase interactions. **Nature Chem. Biol.,** *12(10): 802-9,* 2016. PMC5030135
- Azoitei, M.L., Noh, J., Marston, D.J., Roudot, P., Marshall, C.B., Daugird, T.A., Lisanza, S.L., Sandí, M.J., Ikura, M., Sondek, J., Rottapel, R., *Hahn, K.M., and *Danuser, G. Spatiotemporal dynamics of GEF-H1 activation controlled by microtubule- and Src-mediated pathways. **J. Cell Biol.,** *218(9): 3077-3097,* 2019. PMC6719461

Complete List of Published Work in MyBibliography
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