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	Ph.D.	1986	Chemistry
Carnegie Mellon University	Postdoctoral	1987-1991	Chemistry, Cell Biology
The Scripps Research Institute	Sr. Rsch. Assoc.	1992-1994	Immunology

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 tools to address basic questions re spatio-temporal control of signaling. Our biological studies center on the role of cytoskeletal and adhesion dynamics in signaling crosstalk, directed motility, and immune cell function. We are extending our cell biology studies to examine metastasis and macrophage motility in 3D models and *in vivo*.

While addressing specific molecules for our biological studies, we have produced generally applicable approaches to visualize and control signaling. These include new fluorescent biosensor designs to quantify conformational changes of endogenous proteins, and to visualize the conformational changes of individual molecules in living cells. This work includes the development of bright dyes that report protein conformational changes. We are developing engineered domains that can be inserted into target proteins to control protein function using either light or small molecules. Other new methods selectively activate specific protein interactions. We benefit greatly from collaborations with other labs who focus on computational image analysis, modeling of signaling dynamics, and developing novel microscopes.

In our biological projects, we are inducing macrophages to engage geometrically regular objects, while controlling protein activity at precise times and positions. Using multiplexed imaging, we simultaneously control and visualize signaling in this sytem. This enables us to model lines of force and molecular interactions governing target recognition and sheds light on feedback control of GTPase 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.

Positions and Employment

1994-1997 Assistant Professor, Department of Neuropharmacology, Scripps Research Institute
1997-2000 Assistant Professor, Department of Cell Biology, Scripps Research Institute
2000-2004 Associate Professor, Department of Cell Biology, Scripps Research Institute
2004-present Thurman Distinguished Professor of Pharmacology, University of North Carolina, Chapel Hill
2004-present Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill
2009-2017 Founder and Director, UNC-Olympus Imaging Center
2009-present UNC Center for Computational and Systems Biology
2015-2019 Co-Director, NIH P41 Center: Computer Integrated Systems for Microscopy and Manipulation

Honors

1998	National Institutes of Health James A. Shannon Directors Award
2004	Ronald Thurman Distinguished Professor of Pharmacology
2009	NIH Roadmap Transformative R01 Award
2010	Fellow of the American Association for the Advancement of Science
2010	Nature Reviews - Molecular Cell Biology: "10 breakthroughs of the decade"
2019	Pearse Prize of the Royal Microscopy Society

Plenary and Keynote lectures: Annual Meeting of the Japanese Biochemical Society, 2003; International Society for Analytical Cytology, 2006; NIH Conference on Imaging Probes, 2007; International Conference of Systems Biology, 2009; Korean Society for Biochemistry and Molecular Biology, 2010; Twelfth International Conference on Methods and Applications of Fluorescence 2011; Leica Scientific Forum France, 2012; Snyder Institute, U. Calgary Endowed Chair lecture, 2013; Labex France Signalife, 2014; SPIE Photonics West, 2015; Future of Imaging Symposium, U. Calgary 2015, ABRF national meeting 2016; Pearse Prize Lecture, Royal Microscopy Society Meeting UK, 2019.

Other Professional Activities

Editorial board, Biophysical Journal, 2013-2016
Advisory Board, Frederick National Laboratory of the National Cancer Institute, 2016-present

NIH study sections: NIH Biophysics study section *ad hoc* reviewer, 2001; NIH study section: Cellular and molecular imaging methods, 2003; NIH Roadmap study section: Molecular Libraries and High Throughput Screening, 2006; Roadmap initiative formulation—Nanomedicine program, 2006; Roadmap initiative oversight—State of Science: molecular imaging and libraries at NIH, 2006; NIH Microscopic Imaging study section *ad hoc* reviewer, 2007; NIH Cellular Structure and Function study section *ad hoc* reviewer, 2008; NCI Innovative Molecular Technologies Program, ad hoc reviewer, 2009; NIGMS Special emphasis panel on cellular imaging, 2009; ARRA Challenge grants study section, 2009; ARRA Go grants study section 2009; Bioengineering special emphasis panel, 2009; NIH College of CSR reviewers 2010-2012; NIH Enabling Biophysical and Imaging Technologies ad hoc reviewer 2011; Director's New Innovator Awards study section, 2012; Enabling bioanalytical and imaging technologies (EBIT) study section, ad hoc panel, 2012 (chair); NCI Provocative questions review panel, 2012; NIH EBIT study section 2014; NIH Division of Intramural Research, site visit 2014; NIH Cellular and Molecular Technologies study section standing member, 2015-present.

Other study sections and grant review: Argonne National Laboratories, 2002; Ecole Polytechnique Federale De Lausanne, 2002; The Wellcome Trust, England, 2006-present; Italian Association for Cancer Research, 2006-present.

Organization of scientific meetings: Organizer, Signal Transduction Targets for Effective Therapeutics, Cambridge Healthtech Institute, 2004; Organizer, Molecular Microscopy of Living Cells, ASCB Annual meeting, 2004; Program Committee, International Society for Analytical Cytology Annual Meeting, 2007; Organizer, ASCB subgroup meeting on High Performance Image Analysis and Photomanipulative Techniques for Cell Biology, 2007; Co-chair, Imaging and Biosensors, ASCB annual meeting, 2008; Session chair, Biophysical Society Meeting, 2011; ASCB annual conference program committee, 2011; Co-organizer, Keystone meeting on Optogenetics, 2015.

Consulting and advisory boards: Scientific Advisory Board, Q3DM Company, 2001-2003; Consultant, Amersham Corporation, 2002; Scientific Advisory Board, Panomics Company, 2003-2008; Sigma Chemical Company biosensor advisory board, 2006-2008; Scientific Advisory Board, NIH Center for Computer Integrated Systems in Microscopy and Manipulation, 2011-2014.

Professional memberships: American Society for Cell Biology; American Chemical Society; Biophysical Society; International Society for Analytical Cytology; American Association for the Advancement of Science.

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.

- Hahn, K.M., R. DeBiasio and D.L. Taylor. Patterns of elevated free calcium and calmodulin activation in living cells. **Nature**, *359*: 736-738, 1992.
- 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.,** *in press.*

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.
- 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 be activated in living cells and animals by adding a small molecule to the medium or circulation. These kinase analogs can be directed to interact only with specific substrates upon activation. GEFs, GTPases and kinases were also 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*.* PMC40647t90
- 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.

- 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.

- Toutchkine, A.,V. Kraynov, and K. M. Hahn. Solvent-Sensitive Dyes to Report Protein Conformational Changes in Living Cells, **J. Amer. Chem. Soc**., 125:4132-4145, 2003.
- 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., 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
http://www.ncbi.nlm.nih.gov/sites/myncbi/klaus.hahn.1/bibliography/40336082/public/?sort=date&direction=ascending