

BIOGRAPHICAL SKETCH

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NAME Mark L. Grimes	POSITION TITLE Associate Professor		
eRA COMMONS USER NAME mgrimes			
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
Kalamazoo College, Kalamazoo, Michigan	B.A.	1974-1978	Chemistry & Biology
University of Oregon, Eugene, Oregon	Ph.D.	1979-1986	Chemistry & Molecular Biology (Advisor: Ed Herbert)
University of Oregon, Eugene, Oregon	Postdoctoral	1986 - 1987	Yeast secretion
University of California, San Francisco	Postdoctoral	1987 - 1991	Sorting in the secretory pathway
University of California, San Francisco	Postdoctoral	1991 - 1992	Signaling from endosomes

A. Personal Statement

My interest in the compartmentalization of signal transduction, which started with identification of signaling endosomes as a postdoctoral fellow with Dr. William Mobley, led to questions about the proteins that associate with receptor tyrosine kinases in different organelles and membrane fractions. This led to acquisition of phosphoproteomic data from neuroblastoma cell lines and membrane fractions including endosomes and lipid rafts. Analyzing the resulting large data set required acquiring new skills with help from collaborators in the fields of pattern recognition, computational biology and bioinformatics. We discovered that mass spectrometry data, being full of holes (missing values), requires special considerations for embedding statistical relationships into reduced-dimension data structures for clustering. Rigorous clustering provides a filter for network representations of a navigable data structure for exploratory data analysis. Applying these techniques to neuroblastoma phosphoproteomic data allows predictions about signaling pathways activated by tyrosine kinases with much greater resolution than previously possible. The considerable effort invested in learning how to analyze large mass spectrometry data sets has resulted in four recent publications and an ongoing collaboration with the NIH BD2K LINCS consortium. Importantly, these analyses provide a robust approach to generate the compelling and testable hypotheses in this proposal. Receptor tyrosine kinases in neuroblastoma cells are clustered into distinct collaborative groups distinguished by activation and intracellular localization of FYN and LYN and the SRC-family kinase scaffold protein, PAG1, all of which are among the most highly phosphorylated proteins in endosomes. We hypothesize that controlled intracellular localization of PAG1/FYN/LYN complexes is a critical mechanism to discriminate responses to different receptors.

1. Grimes, M.L., Lee, W.-J., van der Maarten, L., Shannon, P. Wrangling phosphoproteomic data to elucidate cancer signaling pathways. *PLoS ONE* 8: e52884., 2013. doi:10.1371/journal.pone.0052884.t003.
2. Xin X, Gfeller D, Cheng J, Tonikian R, Sun L, et al. SH3 interactome conserves general function over specific form. *Mol Syst Biol* 9: 652-669, 2013. doi:10.1038/msb.2013.9.
3. Shannon P, Grimes ML, Kutlu B, Bot JJ, Galas DJ. RCytoscape: Tools for Exploratory Network Analysis. *BMC Bioinformatics*: 14:217, 2013. doi:10.1186/1471-2105-14-217.
4. Palacios-Moreno, J., Foltz, L., Guo, A., Stokes, M. P., Kuehn, E. D., George, L., Comb, M., and Grimes, M. L. Neuroblastoma Tyrosine Kinase Signaling Networks Involve FYN and LYN in Endosomes and Lipid Rafts. *PLoS Comp Biol* 11, 2015. e1004130–e1004133.

B. Positions and Honors

Positions and Employment

1986 - 1987	Postdoctoral Fellow (Advisor: Tom Stevens) Chemistry Department, University of Oregon, Eugene, OR
1987 - 1991	Postdoctoral Fellow (Advisor: Regis B. Kelly) Department of Biochemistry and Biophysics, University of California, San Francisco, CA
1991 - 1992	Postdoctoral Fellow (Advisor: William C. Mobley) Department of Neurology, University of California, San Francisco, CA
1992 - 1994	Assistant Research Cell Biologist Department of Neurology, University of California, San Francisco, CA
1994 - 2001	Senior Lecturer Massey University, Palmerston North, New Zealand
2001 - 2001	Visiting Scientist Department of Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD
2002 - present	Associate Professor Division of Biological Sciences, University of Montana, Missoula, MT

Honors

1979	Graduate Teaching Fellow, Chemistry Department, University of Oregon
1980	NIH Molecular Biology Predoctoral Training Grant GM 07759, Institute of Molecular Biology, University of Oregon
1986	American Heart Association Research Fellow, American Heart Association, Oregon Affiliate, Inc.
1987	NIH Neurobiology Postdoctoral Training Grant NS 07067-10, Department of Physiology, University of California, San Francisco
1988	National Research Service Award NS 08387-01, National Institute of Neurological and Communicative Disorders and Stroke
1991	Athena Neurosciences Special Fellowship, Athena Neurosciences, South San Francisco, CA
1992	NARSAD Young Investigator Award, National Alliance for Research on Schizophrenia and Depression, Great Neck, NY
2007	National Academies Education Fellow in the Life Sciences, National Academy of Sciences
2011	National Academies Education Mentor in the Life Sciences, National Academy of Sciences

C. Contributions to Science

1. In my graduate work with Dr. Ed Herbert, I cloned chromogranin A, a major component of neuroendocrine dense core secretory granules. An interest in secretion led me to Dr. Regis Kelly and work in which sorting of regulated and constitutive secretory granules was reconstituted *in vitro*. Interest in nerve growth factor signaling mechanisms led me to Dr. William Mobley, in whose laboratory an organelle fractionation approach was employed to define signaling endosomes containing activated TrkA, the NGF receptor tyrosine kinase. These publications have been, and still are, highly cited as the genesis of the signaling endosome hypothesis.

- a. Iacangelo A, Affolter HU, Eiden LE, Herbert E and Grimes M. Bovine chromogranin A sequence and distribution of its messenger RNA in endocrine tissues. *Nature* 323:82-6, 1986.
- b. Grimes M and Kelly RB. Intermediates in the constitutive and regulated secretory pathways released in vitro from semi-intact cells. *J. Cell Biol.* 117: 539-550, 1992.
- c. Grimes, M. L., Zhou, J., Beattie, E., Yuen, E.C., Hall, D.E., Valletta, J.S., Topp, K.S., LaVail, J. H., Bunnett, N.W., and Mobley, W.C. Endocytosis of activated TrkA: Evidence that NGF induces formation of Signalling Endosomes. *J. Neurosci.* 16:7950-7964, 1996.

- d. Grimes, M. L., Beattie, E., and Mobley, W. C. A signaling organelle containing the nerve growth factor-activated receptor tyrosine kinase, TrkA. *Proc. Nat. Acad. Sci. USA* 94: 9909-14, 1997.
2. This work fueled further studies on the compartmentalization of signal transduction and mechanisms that affect cell fate decisions, including apoptosis and differentiation. We developed high resolution organelle fractionation techniques to examine endosomes, signaling particles that are resistant to detergent and sensitive to salt, and lipid rafts. We discovered that two key effectors for many pathways, AKT and CREB, are cleaved by caspases during apoptosis. We defined the tipping point of commitment to programmed cell death, after which neuroblastoma and pheochromocytoma cells can no longer be rescued by NGF, and separated cells at different stages of apoptosis by their density. High-resolution organelle fractionation methods revealed that different receptors are in different signaling endosomes distinguished by mass and density. We discovered novel NGF-dependent interactions between TrkA, microtubules, and lipid rafts. These cell biological approaches, combined with sophisticated data analysis techniques, positions my laboratory to make unique and valuable contributions towards understanding signaling networks that control cell fate decisions.
- e. François, F, and Grimes, M. L. Phosphorylation-dependent Akt cleavage in neural cell in vitro reconstitution of apoptosis. *J. Neurochem.* 73: 1773-1776, 1999.
- f. François F, Godinho, MJ, and Grimes ML. Creb is cleaved by caspases in neural cell apoptosis. *FEBS Lett*, 486: 281-284, 2000.
- g. François, F. Godinho, M, Dragunow, M., and Grimes, M. A population of PC12 cells that is initiating apoptosis can be rescued by nerve growth factor, *Mol Cell Neurosci*, 18:347-362, 2001.
- h. Grimes, ML, and Miettinen, H. Receptor tyrosine kinase and G-protein coupled receptor signaling and sorting within endosomes. *J Neurochem*, 84: 905-918, 2003.
- i. Weible MW, Ozsarac N, Grimes ML, Hendry IA. Comparison of nerve terminal events in vivo effecting retrograde transport of vesicles containing neurotrophins or synaptic vesicle components. *J Neurosci Res* 75: 771–781, 2004. doi:10.1002/jnr.20021.
- j. MacCormick, M. Modersheim, T., van der Salm, L.W.M., Moore, A., Clements, S., McCaffrey, G., and Grimes, M.L. Distinct signalling particles containing Erk/Mek and B-Raf in PC12 cells. *Biochemical J* 387:155-164, 2005. doi:10.1042/BJ20040272
- k. Lin DC, Quevedo C, Brewer NE, Bell A, Testa JR, et al. APPL1 associates with TrkA and GIPC1 and is required for nerve growth factor-mediated signal transduction. *Mol Cell Biol* 26: 8928–8941, 2006. doi: 10.1128/MCB.00228-06.
- l. McCaffrey, G., Welker, J., Scott, J., van der Salm, L., and Grimes, M. L. High-resolution fractionation of signaling endosomes containing different receptors. *Traffic* 10, 938-950, 2009. <https://www.nihms.nih.gov/pmc/articlerender.fcgi?artid=239655>
- m. Agnihothram SS, Dancho B, Grant KW, Grimes ML, Lyles DS, et al. Assembly of arenavirus envelope glycoprotein GPC in detergent-soluble membrane microdomains. *J Virol* 83: 9890–9900, 2009. doi:10.1128/JVI.00837-09.
- n. Pryor S., McCaffrey G., Young L.R., Grimes M.L. NGF Causes TrkA to Specifically Attract Microtubules to Lipid Rafts. *PLoS ONE* 7(4): e35163, 2012. doi:10.1371/journal.pone.0035163

AFFILIATIONS

University of Montana Center for Structural and Functional Neuroscience

University of Montana Center for Biomolecular Structure and Dynamics

University of Washington School of Medicine, Department of Physiology & Biophysics

D. Research Support

Ongoing Research Support

Cell Signaling Technology (CST) is one of three eDSR centers (external data science research) centers initially funded by this grant. There are two main components of our contribution to the consortium: first is to develop tools for the visualization and analysis of large datasets of unique multi-layered high throughput proteomic data produced by CST, and second is to further develop the content and capability of PhosphoSitePlus (PSP) to integrate and serve to multi-dimensional mass spec data to the LINCS community.

Recent Completed Research Support

1R15NS070746-01 Grimes (PI)

04/01/2010 – 03/31/2013

National Institutes of Health – NINDS

Receptor Tyrosine Kinase Sorting and Transactivation in Endosomes

The goal of this proposal was to examine mechanisms by which different receptors sort into different signaling endosomes, and characterize in detail signaling mechanisms that arise in endosomes.

Role: PI

E. SYNERGISTIC ACTIVITIES

1. **Undergraduate Research.** A total of 32 undergraduates have engaged in research in the P.I.'s laboratory since 2002, including Native American (NIH BRIDGES to Baccalaureate) and minority students.

2. Scientific Teaching.

- a. 2007 National Academy of Sciences/HHMI Summer Institute on Undergraduate Education in Biology, Madison, WI. Dissemination to faculty at the University of Montana is ongoing. Scientific teaching methods are employed in my teaching of Cell and Molecular Biology (250-380 students). We now use audience response tools (clickers) for in-class assessment, online lecture videos, and group learning activities to uncover and challenge misconceptions and focus on learning goals. Graduate students employed as teaching assistants for this course are asked to lead group-learning activities, contribute to their design, and help evaluate their effectiveness.
- b. 2007-8 National Academies Education Fellow in the Life Sciences, National Research Council, Washington, DC.
- c. 2011 National Academies Education Mentor in the Life Sciences, National Academy of Sciences.
- d. 2012 National Academies Summer Institutes Leadership Summit 2012, Madison, WI. (invited participant)
- e. 2013 National Academies Scientific Teaching Alliance (formerly, the "Summer Institute") Entering Biology Education Research Workshop and Society for the Advancement of Biology Education Research (SABER) meeting, Minneapolis, MN (invited participant)

3. Transforming Undergraduate Education.

- a. 2008 Conference on Scientific Teaching, Howard Hughes Medical Institute, Chevy Chase, MD;
- b. 2008 AAAS /NSF BIO-ED Conversation on Science Education, Denver, CO;
- c. 2009 AAAS Transforming Undergraduate Biology Education: Mobilizing the Community for Change, Washington DC.
- d. 2013 AAAS Vision and Change in Undergraduate Biology Education: Chronicling the Change, Washington, D.C. (invited participant)

4. Community Outreach.

- a. 1997-2013 Science fair judge in New Zealand and Montana
- b. 2002-6 BRIN and INBRE Conferences on Science Education for Native Americans
- c. 2006 Hosted BRIDGES to Baccalaureate student in my laboratory
- d. 2006-13 Talks to high school and community about stem cells and scientific research, including City Club of Missoula, which was broadcast on local television.

5. Service.

- a. 1997-9 New Zealand Health Research Council Assessing Committee
- b. 1999-2001 Cancer Society of New Zealand Assessing Committee
- c. 2003-12 Rhodes Scholarship Committee of Selection for the State of Montana. Since the State selection was changed, participated in practice interviews for Rhodes candidates.
- d. 2005-10; March of Dimes Research Advisory Committee C.