BIOGRAPHICAL SKETCH

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NAME: Graves, Lee M.

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

POSITION TITLE: Professor

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
Iowa State University, Ames, IA	B.S.	1982	Biochemistry
University of Illinois, Urbana, IL	Ph.D.	1990	Biochemistry
University of Washington, Seattle, WA	Postdoc	1994	Pharmacology

A. Personal Statement. My overall research goal is to apply proteomics and related technologies to better understand the pharmacology of drug action with a particular focus on cell signaling. This interest originated from my time as a post-doctoral fellow studying kinases in the lab of Dr. Edwin G. Krebs (Univ. of Washington). I now have over 30 years experience applying proteomics to study kinases, cancer biology and cell signaling. Before that, I studied protein degradation in the lab of Dr. Robert L. Switzer (Univ. of Illinois). My research has now come full circle as we are exploring the interfaces between targeted protein degradation and cell signaling as described in this MIRA proposal.

My previous efforts have been successful at defining new kinase substrates, pathway cross-talk and kinase feed back responses, mechanisms of cancer drug resistance and regulation of metabolism. We have specifically focused on identifying drug mechanism of action (MOA) or how cancer cells adapt to drug exposure. To do this we have developed new proteomics technologies to accomplish our goals and built the foundation for comprehensive investigations. This includes novel kinase inhibitor bead affinity chromatography (MIBs) to study the kinome 'en masse' (kinomics), proximity ligation to measure protein-protein interactions, and peptide capture to study protein phosphorylation. All these methods are combined with quantitative mass spectrometry (MS) to accurately measure differences in peptides and post-translational modifications.

We have applied MIB/MS profiling to examine the kinome from kinase inhibitor-treated cells, Ras knockdown and drug-resistant cells. We combined these studies with a comprehensive analysis of kinase activities and protein phosphorylation through bioinformatics and statistics to define cellular adaptations. Our lab was the first to apply MIB/MS kinome profiling to study the effects of viral infection on the host kinome (Arend *et al.,* 2017). We combined MIBS with phosphoproteomics to identify anti-apoptotic proteins (Okumu *et al.,* 2017), to identify drug targets and elucidate mechanisms of kinome adaptation in cancer drug resistance (Duncan *et al*., 2012; Cooper *et. al*., 2013; Vaseva *et al*., 2018, Krulikas *et al.,* 2018; Cann *et al.,* 2019; Blake *et al.,* 2019; Kuciauskas *et al.,* 2019, McDonald *et al.* 2020, Lipner *et al*., 2020). Recently we performed the MIB-MS kinome analysis of Erk, Raf and Ras inhibition (Water *et al*., 2021; Ozkan-Dagliyan *et al.,* 2020, Klomp JA *et. al.* 2024; Klomp JE *et al.,* 2024) and analysis of SARs/COV2 infected cells (Fritch *et al.,* 2023).

We have developed an expertise in using immobilized pharmacophores for protein capture and drug-target discovery. Using an immobilized ONC201 analog(TR-80) affinity column, we were the first to discover ClpP as the target for ONC201 and related compounds (Graves *et al.,* 2019). ***This exciting new research formed the basis of my R01 and is now my lab's major focus.*** Over the past 4 years we successfully applied multi-omics analyses to investigate the proteomics, metabolomics and transcriptomic responses to ClpP activators (ClpP agonists) in cancer cells (Fennell et al., 2022; Greer et al., 2022; Mbangalo et al., 2023,; Fennell et al., 2023; Daglish et al., 2023). To better understand how the most potent ClpP agonists (TR compounds) selectively impact cancer cells, we will continue to investigate how ClpP agonists affect anti-oxidant proteins, kinome regulation, mito-nuclear signaling, and cell fate. Our previous studies revealed key biochemical insights into mechanisms of mitochondrial stress signaling. We will expand our studies to develop tools to study the effects of ROS on ClpP-mediated protein

degradation. The current proposal will extend these analyses to include focused, hypothesis-driven studies to provide new insight into the mechanism of action (MOA) of these new anti-cancer drugs. Finally, its important to note, that through my collaboration with Madera Therapeutics, I have exclusive access to a collection of the most selective and potent ClpP activators (ClpP agonists). I will continue to use these compounds to advance the research described in this proposal.

My overall leadership goal is to use my expertise to support proteomics research on campus and beyond. As the Faculty Director of the Metabolomics and Proteomics Core, I have assembled a team of investigators and related core facilities to assist in the completion of the studies described in my proposal. I direct a large group of scientists to apply proteomics to biological research as part of the Lineberger Cancer Center and across the UNC campus. I have successfully built a network of collaborations with other Core directors, biomedical engineers, cancer researchers, virologists, immunologists, biochemists, and statisticians. I have established collaborations with clinicians, cell signaling, and mitochondria experts to support my MIRA studies (see letters of support). I have sought out and developed new collaborations to study the pharmacology of these new compounds. I am confident that our research efforts, both past and present will have an incredibly important impact on the development of this new field.

My overall mentorship goal is to recruit and train the best young scientists for the future. As a mentor, I am commited to training people and my track record strongly supports this. I have trained 9 post-doctoral fellows, 15 graduate students and countless undergraduates (>50). I am proud of my training record and many of my students have matriculated to academic positions, senior positions in the pharmaceutical industry and government. I was Co-Director and Director of the Carolina Summer Fellows (CSF) program, a program designed to provide "first-time" research opportunities for undergraduates. I was the Director of Graduate Studies (Pharmacology) for 10 years where I was influential in shaping the graduate program, including changes in coursework, committee meeting structure and grant writing program. I currently run a lab populated largely by graduate and undergraduate students. I am passionate about providing opportunites for enthusiastic students and will continue to uphold this goal throughout my career.

Key papers I would like to highlight:

1. **Graves, L.M.,** Bornfeldt, K.E., Raines, E.W., Potts, B.C., Macdonald, S.G., Ross, R. and Krebs, E.G. (1993). Protein Kinase A Antagonizes Platelet-Derived Growth Factor-Induced Mitogen-Activated Protein Kinase in Human Arterial Smooth Muscle Cells. *Proc. Natl. Acad. Sci*. 90,10300-10304. PMID: 7694289 (*Article highlighted in Science-cited over 500 times*)
2. **Graves, L.M.,** Guy, H., Dahlstrand, E.N., Lazarowski, E., Kozlowski, P., He, Y., Collins, M.A., Earp, H.S., and Evans, D.R. (2000). Regulation of Carbamoyl Phosphate Synthetase by MAP kinase. *Nature* 403, Pgs. 328-332. PMID: 10659854.(*Cited almost 300 times*)
3. Duncan J.S., Whittle M.C., Nakamura K., Abell A.N., Midland A.A., Zawistowski J.S., Johnson N.L., Granger D.A., Jordan N.V., Darr D.B., Usary J., Kuan P-F., Smalley D.M., Major B., He X., Hoadley K., Sharpless N.E., Perou C.M., Gomez S.M., Chen X., Jin J., Frye S.V., Earp H.S., **Graves L.M**., and Johnson G.L. (2012). Dynamic Reprogramming of the Kinome in Response to Targeted MEK Inhibition In Triple Negative Breast Cancer. *Cell* 149(2), 307-321. PMID: 22500798 (*Cited over 800 times*)
4. Graves PR, Aponte-Collazo LJ, Fennell EMJ, Graves AC, Hale AE, Dicheva N, Herring LE, Gilbert TSK, East MP, McDonald IM, Lockett MR, Ashamalla H, Moorman NJ, Karanewsky DS, Iwanowicz EJ, Holmuhamedov E and **Graves LM** (2019). Mitochondrial Protease ClpP is a Target for the Anticancer Compounds ONC201 and Related Analogues. *ACS Chem Biol*. 14(5):1020-1029. PMID: 31021596. (*Article highlighted in the Scientist-cited over 140 times*)

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2014-present	Professor, Dept. of Pharmacology, Faculty Director UNC Proteomics, University of NC at Chapel Hill, Chapel Hill, NC
2001-2013	Associate Professor, Dept. of Pharmacology, University of NC at Chapel Hill, Chapel Hill, NC
1995-2001	Assistant Professor, Dept. of Pharmacology, University of NC at Chapel Hill, Chapel Hill, NC
1994-1995	Lecturer, Department of Pharmacology, University of Washington, Seattle, WA
1990-1994	Postdoctoral Research Fellow (w/ Dr. Edwin G. Krebs), Department of Pharmacology University of Washington, Seattle, WA

Other Experience and Professional Memberships

2023-present	Associate Editor, Frontiers in Pharmacology

2017-2021	Executive Editor, Bioch. Biophys. Acta (BBA)- General Subjects
2013-2019	NIH Study Section (MIST)- regular member
2012-2013	Pharmaceuticals- Guest Editor- Protein Kinase Inhibitors
2012-2019	International Human Proteomics Organization (HUPO)
2009-2014	Associate Editor- Molecular Pharmacology (ASPET)
2002-2007	Editorial Board - Journal of Biological Chemistry (JBC, ASBMB)
2000-2001	Executive Committee ASPET - Molecular Pharmacology, 2000-2001
1998-2008	Editorial Board - Molecular Pharmacology (ASPET)
2002-present	American Society for Biochemistry and Molecular Biology (ASBMB)
2000-present	American Society for Pharmacology and Experimental Therapeutics (ASPET)

Ad Hoc Reviewer

2023	ZRG1 CTH-E (55): Mammalian Models for Translational Research
2020	Netherlands Cancer Research Grant Review
2020	French Cancer Research Grant Review
2014	Medical Research Council (MRC) UK
2013	NIH/NCI Special Emphasis Panel (Fellowship: Oncological Sciences)
2010-2012	NIH MIST Peer Review
2005	NIH/ NRSA Awards Biochemistry and Biophysics
2005	NIH/ Kirchstein NRSA Awards
2005	American Heart Association (Cell and Molecular 3)
2003-2005	Nasa Muscle Biology Group
2000-2005	NIH/NIHLBI
2000-2003	The Wellcome Trust
1999-2004	NIH/NIDDS
1997-2000	VA Medical Center

Other Service (UNC)

2011-present	Faculty Director Michael Hooker Proteomics Facility
2021	Committee Chair, 5 year review of the Center for Aging and Health
2019	NCCU Graduate Program Review Committee
2018-2020	School of Medicine (SOM)Tenure and Promotions Review Committee
2017-2018	SOM Post-tenure Review Committee
2013-2023	Director/Co-Director Carolina Summer Fellows Program (ASPET Funded)
2009-2019	Director of Graduate Studies, Pharmacology (completed departmental review 2017)

Honors

2007	University of Sydney International Research Fellowship
2006	PHRMA Sabbatical Fellowship Award-University of North Carolina
2000	American Heart Association Established Investigator Award - University of North Carolina
2000	Jefferson-Pilot Research Award - University of North Carolina

C. Contributions to Science

Contributions to Research

1. Kinome Remodeling in Response to Disease or Drug Exposure

We are applying global methodologies to study the kinome *en masse*. Our lab uses multiplexed inhibitor bead (MIB) mass spectrometry (MS) as a powerful approach to capture, identify and quantitate kinases from any sample including drug- resistant leukemias, breast and pancreatic cancer. This has been a major effort of our research and we are applying this technology to a variety of cancer projects.

a) Duncan JS, Whittle MC, Nakamura K, Abell AN, Midland AA, Zawistowski JS, Johnson NL, Granger DA, Jordan NV, Darr DB, Usary J., Kuan P-F., Smalley DM, Major B, He X, Hoadley K, Sharpless NE, Perou CM, Gomez SM, Chen X, Jin J, Frye SV, Earp HS, **Graves LM**, and Johnson GL. Dynamic Reprogramming of the Kinome In Response to Targeted MEK Inhibition In Triple Negative Breast Cancer. *Cell* 2012,149(2), 307-321. PMID: 22500798

b) Cooper MJ, Cox, CN, Zimmerman EI, Dewar BJ, Duncan JS, Whittle MC, Nguyen T, Jones L, Ghoseroy S, Smalley D, Kuan P-F, Richards KL, Christopherson RI, Jin J, Frye SV, Johnson GL, Baldwin AS, and **Graves**

LM. Application of Multiplexed Kinase Inhibitor Beads to Study Kinome Adaptations in Drug Resistant Leukemia *PLOS One* 2013, 8(6), e66755. PMID: 23826126.

c) Krulikas J.K., McDonald I.M., Lee B., Okumu D.O., East M.P., Gilbert T.S.K., Herring L.E., Golitz B.T., Wells, C.I., Axtman A.D., Zuercher W.J., Willson T.M., Kireev D., Yeh J.J., Johnson G.L., Baines A.T., and **Graves LM**. Application of Integrated Drug Screening/ Kinome Analysis to Identify Inhibitor of Gemcitabine Resistant Pancreatic Cancer Cell Growth. *SLAS Discovery* 2018, 23(8), 850-861, PMID: 29742358.

d) McDonald IM, Grant GD, East MP, Gilbert TSK, Wilkerson EM, Goldfarb D, Beri J, Herring LE, Vaziri C, Cook JC, Emanuele MJ, and **Graves LM.** Mass spectrometry-based selectivity profiling reveals a highly selective MELK inhibitor that causes delayed mitotic entry in cells. *J Biol Chem.* 2020, Jan 2, RA119.011083. PMID: 31896573.

2. Regulation of Metabolic Enzymes by Phosphorylation

We have a long-standing interest in identifying phosphorylation events in metabolic enzymes that dictate their properties. We focused on enzymes involved in pyrimidine (CTP) biosynthesis- since this is a rate-limiting process. Our publications on the human CTPS 1 and 2, were the first to characterize the phosphorylation-dependent regulation of this important enzyme in human cells.

a) **Graves, L.M.,** Guy, H., Dahlstrand, E.N., Lazarowski, E., Kozlowski, P., He, Y., Collins, M.A., Earp, H.S., and Evans, D.R. (2000). Regulation of Carbamoyl Phosphate Synthetase by MAP kinase. Nature 403, Pgs. 328-332. PMID: 10659854

b) Higgins MJ, Graves PR, and **Graves LM**. Regulation of Human Cytidine Triphosphate Synthetase 1 by Glycogen Synthase Kinase 3 Beta. *J. Biol Chem*, 2007, 282(40), 29493-29503. PMID: 17681942.

c) Kassel KM, Au DR, Higgins, MJ, Hines M, and **Graves, LM**. Regulation of Human Cytidine Triphosphate Synthetase 2 by Phosphorylation. *J. Biol Chem.* 2010, 285 (44): 33727-33336. PMID: 20739275.

d) Okumu DO, Dewar BJ, Cox N, Aponte L, East MP, Tech K, McDonald I, Tikunov A.P., Holmuhamedov E, Macdonald JM, **Graves LM**. "Lyn regulates creatine uptake in an imatinib-resistant CML cell line." *Biochimica Biophysica Acta General Subject*. 2020, 1864(4):129507. PMID: 31881245

3. Proteomics and the Regulation of Cellular Functions in Viral Infection and Cancer

We have collaborated with a number of groups to apply proteomics to better understand the regulation of multiple cellular processes. This is an active area of research that interfaces with my position as Faculty Director of UNC Proteomics and is supported by a number of funded proposals.

a) Arend KC, Lenarcic EM, Vincent HA, Rashid N, Lazear E, McDonald IM, Gilbert TSK, East MP, Herring LE, Johnson GL, **Graves LM***, Moorman NJ*. Kinome Profiling Identifies Druggable Targets for Novel HCMV Antivirals. *Mol Cellular Proteomics* 2017, Apr16, S263-S276, PMID: 28237943. (*co-senior authors)

b) Vaseva AV, Blake DR, Gilbert TSK, Ng S, Hostetter G, Azam SH, Ozkan-Dagliyan I, Gautam P, Bryant KL, Pearce KH, Herring LE, Han H, **Graves LM**, Witkiewicz AK, Knudsen ES, Pecot CV, Rashid N, Houghton PJ, Wennerberg K, Cox AD, Der CJ. KRAS Suppression-Induced Degradation of MYC Is Antagonized by a MEK5-ERK5 Compensatory Mechanism. *Cancer Cell*. 2018, Nov 12;34(5):807-822.e7. doi: 10.1016/j.ccell.2018.10.001.PMID: 30423298

c) Blake DR, Vaseva AV, Hodge RG, Kline MP, Gilbert TSK, Tyagi V, Huang D, Whiten GC, Larson JE, Wang X, Pearce KH, Herring LE, **Graves LM**, Frye SV, Emanuele MJ, Cox AD, Der CJ. Application of a MYC degradation screen identifies sensitivity to CDK9 inhibitors in KRAS-mutant pancreatic cancer. *Sci Signal.* 2019, 12(590). PMID: 31311847

d) Klomp JE, Diehl JN, Klomp JA, Edwards AC, Yang R, Morales AJ, Taylor KE, Drizyte-Miller K, Bryant KL, Schaefer A, Johnson JL, Huntsman EM, Yaron TM, Pierobon M, Baldelli E, Prevatte AW, Barker NK, Herring LE, Petricoin EF 3rd, **Graves LM**, Cantley LC, Cox AD, Der CJ, Stalnecker CA. Determining the ERK-regulated phosphoproteome driving KRAS-mutant cancer. Science. 2024 Jun 7;384(6700):eadk0850. doi: 10.1126/science.adk0850. Epub 2024 Jun 7. PMID: 38843329

Most relevant to the MIRA Proposal

4. Small Molecule ClpP Activators as Pharmacological Agents for Cancer Treatment

Using affinity chromatography and proteomics, my lab was the first to demonstrate that small molecules based on the imipridone ONC201 and related TR compounds, were selective activators of the mitochondrial protease ClpP. Over the last 4 years we have built a network of collaborators on this project and have published important studies describing the detailed effects of ClpP activators on the proteome, transcriptome and metabome. This emergent area of research will continue to be the major focus of my lab. The following publications reflect our efforts:

a) Graves PR, Aponte-Collazo LJ, Fennell EMJ, Graves AC, Hale AE, Dicheva N, Herring LE, Gilbert TSK, East MP, McDonald IM, Lockett MR, Ashamalla H, Moorman NJ, Karanewsky DS, Iwanowicz EJ, Holmuhamedov E and **Graves LM**. Mitochondrial Protease ClpP is a Target for the Anticancer Compounds ONC201 and Related Analogues. *ACS Chem Biol*. 2019, May 1. doi: 10.1021/acschembio.9b00222. PMID: 31021596.
b) Fennell EMJ, Aponte-Collazo LJ, Wynn JD, Drizyte-Miller K, Leung E, Greer YE, Graves PR, Iwanowicz AA, Ashamalla H, Holmuhamedov E, Lang H, Karanewsky DS, Der CJ, Houry WA, Lipkowitz S, Iwanowicz EJ, **Graves LM**. Characterization of TR-107, a novel chemical activator of the human mitochondrial protease ClpP. *Pharmacol Res Perspect*. 2022 Aug;10(4):e00993. doi: 10.1002/prp2.993. PMID: 35929764
c) Mabanglo MF, Wong KS, Barghash MM, Leung E, Chuang SHW, Ardalan A, Majaesic EM, Wong CJ, Zhang S, Lang H, Karanewsky DS, Iwanowicz AA, **Graves LM**, Iwanowicz EJ, Gingras AC, Houry WA. Potent ClpP agonists with anticancer properties bind with improved structural complementarity and alter the mitochondrial N-terminome. *Structure*. 2023 Feb 2;31(2):185-200.e10. doi: 10.1016/j.str.2022.12.002. Epub 2022 Dec 30. PMID: 36586405
d) E. M.J. Fennell, L. J. Aponte-Collazo, W. Pathmasiri, B. R. Rushing, N. K. Barker, M. C. Partridge, Y. Li, C. A. White, Y.E. Greer, L. E. Herring, S. Lipkowitz, S. C.J. Sumner, E. J. Iwanowicz, **Graves LM**. Multi-omics Analyses Reveal ClpP Activators Disrupt Essential Mitochondrial Pathways in Triple-negative Breast Cancer. *Front Pharmacol.* 2023 Mar 31;14:1136317. doi: 10.3389/fphar.2023.1136317. eCollection 2023. PMID: 37063293

Contributions to Mentorship and Training

I have made a major commitment to training students in my lab and throughout the department and university. I have trained >15 students in my own group and served on the PhD committees of >90 students. I just recently graduated 2 PHD students and currently have 2 new PHD students and 4 undergraduates. I typically have 2-3 undergraduates per year, plus summer students, making my number of undergraduate trainees very large (>50). I have also trained and continue to train post-doctoral fellows. My philosophy is to train students in rigorous science, both in practive and thought. I require my students to be actively involved in the design and application of their projects and to take "ownership" from the very beginning. I emphasize writing and encourage them to outline their manuscripts as they are developing. Each student writes their own paper with my support and frequent editing. Similar goals are encouraged with my undergraduates. Krulikas *et al.* was a paper entirely performed and written by an undergraduate (Linas Krulikas) in my lab. I find this strategy has been a great way to develop successful trainees. My most recent graduate student Emily Fennell, published 3 first author papers and is now a post-doctoral fellow at the Salk Institute.

My students and post-docs have gone on to prominent positions in academia and industry. A few examples:
Dr. Olivia Gardner (Kutnu)- Senior Director, Compound Development (Oncology), Johnson and Johnson
Dr. Tom Hilder- Senior Research Scientist II, Grifols
Dr. Brian Dewar- Associate Professor and Co-Chair of Biology, Taylor University
Dr. Eric Wauson- Associate Professor, Des Moines University
Dr. Eric Zimmerman- Director, Clinical Pharmacology, Pfizer
Dr. Ian McDonald- Senior Manager, Bain Consulting
Dr. Lucas Aponte-Collazo- Graduate Student Success Manager, Scripps Research
Dr. Emily Fennell- Post-doctoral Fellow, Salk Research Institute

List of Published Work in MyBibliography: https://pubmed.ncbi.nlm.nih.gov/?term=graves%2C+LM

D. Highlighted Funding

1. R01GM138520 Elucidating the mechanism of action of novel ClpP activators in activation of the mitochondrial unfolded protein response. Graves (PI) 09/15/2020-06/30/2024
2. R35CA232113 Targeting undruggable RAS for cancer treatment. Der (PI); Role: Research Personnel 09/01/2018-08/31/2025
3. R37CA251877 Mechanistic dissection and inhibitor targeting of autophagy in RAS driven cancers. Bryant (PI); Role: Research Personnel 07/01/2020-06/30/2025
4. R01CA247436-01A1 Tuning CAR-T Cell Functions. Dotti (PI); Role: Research Personnel 01/01/2021-12/31/2025
5. U01TR003715 A consortium effort to translate therapies for neurological diseases via an ex vivo organotypic platform. Hingtgen (PI); Role: Research Personnel 04/01/2021-03/31/2025