

AKW NIH Biosketch:**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: Wheless, Anna Kathryn

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

POSITION TITLE: Graduate Research Assistant

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Houston – Downtown (Houston, TX)	B.S.	05/2019	Microbiology
University of North Carolina – Chapel Hill (Chapel Hill, NC)	Ph.D.	In Progress	Biochemistry

A. Personal Statement

I am in the sixth year of my graduate program at UNC Chapel Hill in the Department of Biochemistry and Biophysics. While my background from my undergraduate education is in microbiology and public health, my current focus in my doctoral work is protein biochemistry and structural biology. I am an NSF Graduate Research Fellow and, in the past, have also successfully competed for T32 funds and a Pilot Study Grant from UNC. My training has so far included protein purification, enzyme activity assays, negative stain EM, mass photometry, and cryoEM data collection and processing. I am already familiar with the workflow required for a cryoEM project and with the specific proteins I am proposing to study in this application.

Throughout my tenure as a doctoral student, mentorship and community engagement have run parallel to my research efforts. I have mentored two undergraduate students in bench research projects and acted as a peer mentor to other editors during my time as Editor in Chief of the NC DNA Day CONNECT Blog. As a JEDI Leadership Fellow, I wrote a piece about how we write about race in biomedical sciences, the goal of which is to give other researchers, especially incoming students, a primer on race as a social category that inoculates them against virulent misconceptions and prevents irresponsible, uncritical communication. I believe that even in fields that are usually far-removed from human subjects, like structural biology, it is important to maintain cultural awareness and community connection. Since my application for resource usage at NCCAT was accepted last year, I have mentored others in my lab in structural biology sample preparation and data processing and will continue to disperse the knowledge I gain from my experiences off-site.

B. Positions, Scientific Appointments, and Honors

- Justice, Equity, Diversity, and Inclusion (JEDI) Leadership Fellow, Sep 2022 – May 2023
- Dismantling Racism in Academia Journal Club Planning Committee, May 2021 – May 2023

- North Carolina DNA Day CONNECT Blog, Editor in Chief, May 2021 – May 2023
- TraCS \$2k Pilot Study Grant, Jan 2022 – Jan 2023
- 2nd place poster presentation, Fredrickson Lipid Research Conference, Sep 2021.
- Graduate Research Fellowship Program (GRFP), National Science Foundation (NSF), Stipend support from Aug 2021-Jul 2024, Fellowship from Aug 2021-Jul 2026.
- UNC MiBio (Mechanistic Biology) T32 Training Fellowship Awardee, Aug 2020 – Jul 2021.
- Excellence in Biology, University of Houston – Downtown, May 2019. 1 awardee/year
- Red Rose Merit Scholarship, University of Houston – Downtown, Aug 2018 – May 2019. 26 awardees/year
- Excellence in General Chemistry, University of Houston – Downtown, May 2017. 1 awardee/year
- Scholars Academy Scholarship Program, University of Houston – Downtown, Aug 2016 – May 2019.

C. Contributions to Science

As an undergraduate working with a group in Baylor College of Medicine's Tropical Disease department, I was involved in multiple field and laboratory research efforts to study vector transmission of Chagas disease (American trypanosomiasis) in Houston, Texas. Chagas disease is categorized as an emerging tropical disease in southern Texas as the range of its insect vector, the triatomine insect, expands northward. This parasitic infection can be fatal and also subtle—it is possible for humans to have the infection for decades and die of cardiovascular disease without anyone knowing the cause was the parasite replicating in their cardiac muscle. Together with the county health department and nearby university Texas A&M, we tested surveillance methods and analyzed extracts from triatomines and from wild animals to determine the disease burden in the Houston area. The outcomes were that we contributed to surveillance for this emerging pathogen, characterized an efficient method for blood sample collection and processing, and identified a new species in the sylvatic transmission cycle of the parasite.

As a graduate student at UNC, I am studying the regulation of lipoprotein lipase (LPL), a critical enzyme for vertebrate lipid metabolism. LPL and its regulators are valuable drug targets due to their control over triglyceride metabolism and consequent association with cardiovascular disease. While working on structural studies of LPL in complex with its physiological regulators, I also co-authored and illustrated a review on the current landscape of LPL regulation research.

Publications

1. **Wheless, A., Gunn, K. H., & Neher, S. B. (2024). Macromolecular Interactions of Lipoprotein Lipase (LPL). *Sub-cellular biochemistry*, 104, 139–179.**
2. **Dye-Braumuller, K. C., Gorchakov, R., Gunter, S. M., Neilsen D. H., Roachell W. D., Wheless, A., Debboun, M., Murray K. O., Nolan M. S. "Identification of Triatomines and Their Habitats in a Highly Developed Urban Environment." *Vector-Borne and Zoonotic Diseases* (Published December 20, 2018).**
3. **Gulas-Wroblewski, B. E., Kairis, R. B., Gorchakov, R., Wheless, A., Murray K. O. "Optimization of DNA Extraction from Field-Collected Mammalian Whole Blood on Filter Paper for *Trypanosoma cruzi* (Chagas Disease) Detection." (Published August 17, 2021).**

SBN NIH Biosketch:**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: Saskia Neher

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

POSITION TITLE: Associate Professor, Biochemistry and Biophysics

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 Oregon	B.S.	06/2000	Biochemistry
Massachusetts Institute of Technology	Ph.D.	06/2005	Biology
University of California at San Francisco	Postdoc	03/2011	Biochemistry and Biophysics

A. Personal Statement

Research: I am a protein biochemist and structural biologist. My lab studies determinates of plasma lipid levels, and how these affect the risk of heart disease. Specifically, we study multiple aspects of lipase regulation, from synthesis to structure to regulation in the blood. We are interested in how the mammalian lipase LPL folds and matures in the ER, and how LMF1 controls the redox environment in the ER to allow proper LPL folding. Our lab also studies how LPL is stored in vesicles and trafficked to the cell surface in response to nutritional signaling. Finally, we study LPL's subsequent regulation by macromolecular inhibitors once it is present in the blood. We use our strength in protein biochemistry and our excellent cryoEM core at UNC to address the the structures of proteins such as LPL, ANGPTL3, ANGPTL4, and LMF1. We use single particle cryoEM and have recently been using cryo-electron tomography to image proteins in subcellular compartments. We have expertise in a broad range of biochemical techniques including measuring enzyme kinetics, determining protein structure/function relationships, mapping protein/protein interactions, and quantitative mass spectrometry. We also use cell biology and high-resolution microscopy to image LPL trafficking in the cell. We love to apply the best technique to solve our research problems, specifically mysteries in human lipid metabolism. Especially with the advent of GWAS and related studies, we have identified many open questions, that, with the application of rigorous biochemical and molecular biology methods, will new ways to treat hypertriglyceridemia and prevent heart disease.

Training: I am committed to training the next generation of scientists. I mentor trainees at all levels, from high school students to graduate students to postdoctoral fellows to junior faculty. I am committed to providing an inclusive, safe, and diverse environment. I have attended training in

lab management at UCSF and at Cold Spring Harbor, and have also taken safe zone training (LGBTQ+ awareness), mental health first aid, sexual harassment awareness training, diversity and inclusion training, and mandated reporter training through UNC. My students have gone on to success in many different science focused careers including running their own labs (Drs. Kathryn Gunn, Stony Brook and Cassandra Hayne, University of Chicago), working as teaching faculty (Dr. Nikea Pittman), working in industry (Dr. Aspen Gutgsell) and working in science communication (Dr. Ben Roberts), among other occupations. Students have earned prestigious awards including NSF fellowships, AHA fellowships, NIH K99/R00 Fellowships, Burroughs Wellcome Fund fellowships, and the ASCB's Porter Prize. As I have become more senior in my position (I am currently being promoted to full professor), I have mentored more junior faculty members. For example, I serve as the faculty advisor to our cryoEM core and help mentor the director, who is an assistant professor, in grant preparation, staff management, and setting core policies.

Ongoing projects I would like to highlight include:

R01-HL125654 (Neher) 07/01/2015 - 04/30/2025

NHLBI

Investigation of the Molecular Mechanisms of Lipoprotein Lipase Inhibitors

This project aims to 1) Develop therapeutics that target inhibitor-LPL interactions; 2) Provide molecular details

of the inhibitor-LPL complexes; 3) Explore intracellular LPL inhibitor function and biogenesis.

R01-HL163352 (Neher) 12/01/2022-11/30/2026

NHLBI

Lipoprotein Lipase Through the Secretory System

This project aims to understand LPL secretion and the structure and function of LMF1, an LPL maturation factor.

SRA 22-0405 (Neher) 12/01/2021 - 11/31/2024

Pfizer

Molecular Analysis of ANGPTL3 Inhibition of Endothelial Lipase

This project covers the structure of endothelial lipase and ANGPTL3.

SRA 20-2349 (Neher) 1/24/2019-6/3/2025

Moderna

Design of Stable LPL Variants

This project funded our studies to develop a stable variant to replace LPL in inducible knockout mice.

The following citations are of special interest to this application:

- 1) Roberts BS, Yang, CQ, and **Neher, SB**. J Cell Sci 2022 Mar 1;135(5):jcs258734. Characterization of lipoprotein lipase storage vesicles in 3T3-L1 adipocytes. PMCID: PMC8403984
- 2) Structure of dimeric lipoprotein lipase reveals a pore adjacent to the active site. Gunn KH, **Neher SB**. Nat Commun. 2023 May 4;14(1):2569. PMCID: PMC10160067

- 3) Gunn KH, Roberts BS, Wang F, Strauss JD, Borgnia MJ, Egelman EH, and **Neher SB**.
The Structure of Helical Lipoprotein Lipase Reveals a New Twist in Lipase Storage. Proc
Natl Acad Sci U S A. 2020 Apr 24 PMID: PMC7229681

B. Positions, Scientific Appointments, and Honors

Research and Professional Experience

2020-Pres: Faculty Advisor, UNC CryoEM core
 2019-Pres: Associate Professor, Department of Biochemistry and Biophysics, UNC Chapel Hill
 2011-2019: Assistant Professor, Department of Biochemistry and Biophysics, UNC Chapel Hill
 2006-2011: Postdoctoral Fellow, University of California at San Francisco, Department of Biochemistry and Biophysics, Laboratory of Peter Walter
 2000-2005: Graduate Student, MIT, Department of Biology, Laboratory of Tania Baker
 1997-2000: Undergraduate Research, University of Oregon, IMB, Lab of Diane Hawley

Other Experience and Professional Membership

2024 NIH Peer Review Committee, ZRG1 F04-S 20 L Study Section, co-chair
 2023 NIH Peer Review Committee, ZRG1 F04-S 20 L Study Section, co-chair
 2023 NIH Peer Review Committee, ZRG1 F04-S 20 L Study Section
 2023 NIH Peer Review Committee, ZRG1 EMNR-K Study section
 2022 NIH Peer Review Committee, ZRG1 EMNR-K Study section
 2022 Co-Organizer, Fredrickson Lipid Conference
 2021 ANR (French National Research Agency) Proposal Review
 2016-present: NSF Proposal Review
 2020: NIH Peer Review Committee, INMP study section
 2019: Co-Chair, Triangle CryoEM Symposium
 2019-present Board member, Fredrickson Lipid Conference
 2017-2019: Member, National Lipid Association
 2014: NIH Peer Review Committee, MBPP study section, early career reviewer
 2012-present: Member, American Chemical Society (ACS)
 2011-present: Member, American Heart Association (AHA)
 2011: Cold Spring Harbor Course in X-Ray methods in Structural Biology
 2010: Cold Spring Harbor Course in Laboratory Management

Peer Review for multiple journals including British Journal of Nutrition, PNAS, Journal of Lipid Research, Nature Communications, Drug Discovery Today, Scientific Reports, BBA Molecular and Cell Biology of Lipids, Open Biology, Trends in Molecular Medicine, ACS Chemical Biology, Trends in Endocrinology & Molecular Medicine

Honors

2017 National Lipid Association Junior Faculty Awardee
 2016 UNC Jr. Faculty Development Awardee
 2014 Kavli Fellow
 2012-2016 Pew Foundation Fellow
 2010-2014 NIH K-99/R00 Transitional Award

2006-2009	Jane Coffin Childs Fellow Funding
2004	American Society for Microbiology Student Travel Award
2003	David Koch Graduate Fellowship Funding
2000	American Institute of Chemists Foundation Award
2000	Phi Beta Kappa Honorary Society
1998, 1999	University of Oregon Summer Research Fellowship

C. Contributions to Science

1. Exploring the Structural Heterogeneity of LPL

Lipoprotein lipase (LPL) is active in the blood where it hydrolyzes triglycerides from packaged lipoproteins. Over thirty years ago, the existence of a condensed and inactive LPL oligomer was proposed. We identified a helical LPL oligomer, and solved its structure to 3.8-Å resolution using cryoEM. We found that helix formation is concentration-dependent, and that helices are composed of inactive dihedral LPL dimers. Super-resolution fluorescent microscopy of endogenous LPL revealed showed that the helices were present in vesicles. We have found that these helices are present in vesicles by cryo-electron tomography, are 11 nM across, and match a new cryoEM structure of a second helical form.

1) Structure of dimeric lipoprotein lipase reveals a pore adjacent to the active site. Gunn KH, Neher SB.

Nat Commun. 2023 May 4;14(1):2569. PMCID: PMC10160067

2) Gunn KH, Roberts BS, Wang F, Strauss JD, Borgnia MJ, Egelman EH, and **Neher SB**. The Structure of Helical Lipoprotein Lipase Reveals a New Twist in Lipase Storage. Proc Natl Acad Sci U S A. 2020 Apr 24

PMCID: PMC7229681

3) Hayne CK, Yumerefendi, H, Cao, L, Gauer, JW, Lafferty MJ, Kuhlman, B, Erie, DA, and **Neher, SB**. We FRET So You Don't Have To: New Models of the Lipoprotein Lipase Dimer, Future of Biochemistry special edition. Biochemistry, 2018 16;57(2):241-254. PMCID: PMC5860654

2. ANGPTL4 Functions as a Fully Reversible, Noncompetitive Inhibitor of LPL:

LPL activity is inhibited by a protein known as ANGPTL4, which is induced in adipose tissue in response to fasting. We study the mechanism of ANGPTL4 inhibition of LPL. ANGPTL4 was previously described as an unfolding molecular chaperone of LPL that catalytically converts active LPL dimers into inactive monomers. Our studies show that ANGPTL4 is more accurately described as a reversible, noncompetitive inhibitor of LPL. We find that ANGPTL4 directly binds to LPL to inhibit it, and we have use HDX mass spectrometry to identify these binding sites. We have generated variants of LPL that are resistant to ANGPTL4 inhibition, and we have generated a peptide drug that blocks LPL-ANGPTL4 interaction. We have worked with Moderna to further develop ANGPTL4-resistant LPL as a therapeutic. Furthermore, we have generated a variant of ANGPTL4 that is dependent on divalent cations for its ability to inhibit LPL. We show that LPL inactivation by this regulatable variant of ANGPTL4 is fully reversible after treatment with a chelator. These findings are significant because, by the previous model, LPL was permanently inactivated after interaction with ANGPTL4.

1) Gunn KH, Gutgsell AR, Xu, Y, Johnson, CV, Liu, J, and **Neher SB**. Comparison of angiotensin-like protein 3 and 4 reveals structural and mechanistic similarities. Journal of Biological Chemistry, 2021 Jan 19;296:100312. PMCID: PMC7949051

2) Gutgsell, A, Ghodge, SV, Bowers, AA, and **Neher, SB**. Mapping sites of the LPL-ANGPTL4 interaction provides mechanistic insight into LPL inhibition. Journal of Biological Chemistry, 2019 Feb 22; 294(8):2678-2689. PMCID: PMC6393616.

3) Lafferty, MJ, Bradford, KC, Erie, DA, **Neher, SB**. Angiopoietin-like Protein 4 Inhibition of Lipoprotein Lipase: Evidence for Reversible Complex Formation. 2013 J Biol Chem, 288 (40) 28524-28534, PMCID: PMC3789953

3. Mechanism of Lipoprotein Lipase Folding by LMF1, an Unusual Substrate-Specific Chaperone

LPL plays a critical and complex role in lipid metabolism. In humans, deficient levels of LPL can cause hypertriglyceridemia and associated disorders, such as diabetes and atherosclerosis. LPL cannot efficiently exit the ER and achieve appropriate levels without a transmembrane protein whose identity was only recently discovered, lipase maturation factor (LMF1). In cells lacking LMF1, inactive LPL is retained in the ER as large, intermolecular disulfide bonded aggregates that are subject to degradation. We have worked to characterize how LMF1 promotes LPL exit from the ER. We find that LMF1 is present at less than 10,000 molecules per cell, a relatively low expression level compared to general chaperones like bip, calnexin, and calreticulin. However, each molecule of LMF1 can promote the maturation of dozens of molecules of LPL1. Structurally, LMF1 is a transmembrane protein with a large C-terminal domain that faces the ER. Finally, we have identified a number of LMF1-interacting partners that included chaperones, PDIs and other proteins involved in protein folding. Based on these data, our current model is that LMF1 helps to maintain redox homeostasis in the ER in order to assist in LPL folding.

1) Koerner CM, Roberts BS, **Neher SB**. Endoplasmic reticulum quality control in lipoprotein metabolism. Mol Cell Endocrinol. 2019 Aug 20;110547. PMCID: PMC6814580

2) Roberts BS, Babilonia-Rosa, MA.; Broadwell, LJ.; Wu, MJ., and **Neher, SB**. 2018. Lipase Maturation Factor 1 Affects Redox Homeostasis in the Endoplasmic Reticulum. EMBO Journal, 2018 Oct 1;37(19) PMCID: PMC6166125

3) Wu, MJ, Wolska, A, Roberts, BS, Pearson, EM, Gutgsell, A, Remaley, AR, and **Neher, SB**. Co-expression of Novel Furin-Resistant LPL Variants with LMF1 Enhances LPL Secretion and Activity, J Lipid Res. 2018 Dec;59(12):2456-2465. PMCID: PMC6277163

4) Babilonia-Rosa MA, **Neher SB**. Purification, cellular levels, and functional domains of lipase maturation factor 1. Biochem Biophys Res Commun; 2014 Jul 18;450(1):423-8 PMCID: PMC4114500

4. Studies to Improve LPL function and Stability:

LPL is an important therapeutic target. It could be delivered to LPL-deficient individuals as a protein, RNA or gene therapy drug. We have undertaken studies to enhance its function. First, we aimed to understand how one mutation, LPL^{S447X}, causes a gain-of-function. This mutation truncates two amino acids from LPL's C-terminus. Carriers of LPL^{S447X} have decreased VLDL levels and increased HDL levels, a cardioprotective phenotype. LPL^{S447X} is used in Alipogene tiparvovec, the gene therapy product for individuals with familial LPL deficiency. It was not known why LPL^{S447X} results in a more favorable serum lipid profile than LPL. *In vitro* reports vary as to whether LPL^{S447X} is more active than LPL. We undertook a comprehensive, biochemical comparison of purified LPL^{S447X} and LPL dimers. We found that LPL^{S447X} enhanced remnant lipoprotein uptake to a greater degree than LPL. An LPL structural model shows that the LPL^{S447X} truncation exposes residues implicated in LPL binding to uptake receptors, solving this longstanding mystery. We also revealed ways to enhance LPL production and stability for use as

a protein therapeutic. Finally, we have recently worked to generate a variant of LPL that is resistant to inhibition by ANGPTL4 and ANGPTL3. We have tested this variant in inducible LPL knockout mice, and a publication describing these results is in preparation.

1) Risti R, Gunn KH, Hiis-Hommuk K, Seeba NN, Karimi H, Villo L, Vendelin M, **Neher SB**, Lõokene A.

Combined action of albumin and heparin regulates lipoprotein lipase oligomerization, stability, and ligand interactions. PLoS One. 2023 Apr 12;18(4):e0283358. PMCID: PMC10096250

2) Piskiewicz S, Gunn KH, Warmuth O, Propst A, Mehta A, Nguyen KH, Kuhlman E, Guseman AJ, Stadtmiller SS, Boothby TC, **Neher SB**, Pielak GJ. Protecting activity of desiccated enzymes. Protein Science. 2019 May; 28(5):941-951. PMCID: PMC6459994

3) Wu, MJ, Wolska, A, Roberts, BS, Pearson, EM, Gutgsell, A, Remaley, AR, and **Neher, SB**. Co-expression of Novel Furin-Resistant LPL Variants with LMF1 Enhances LPL Secretion and Activity, Journal of Lipid Research. 2018, Dec;59(12):2456-2465. PMCID: PMC6277163

4) Hayne CK, Lafferty MJ, Eglinger BJ, Kane JP, **Neher SB**. Biochemical Analysis of the Lipoprotein Lipase Truncation Variant, LPL^{S447X}, Reveals Increased Lipoprotein Uptake. Biochemistry; 2017, Jan 24;56(3):525-533. PMCID: PMC5848218

URL to My Bibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/saskia.neher.1/bibliography/47394252/public/?sort=date&direction=ascending>.