BIOGRAPHICAL SKETCH

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NAME: Christopher de Graffenried

eRA COMMONS USER NAME: CDEGRAFFENRIED

POSITION TITLE: Associate Professor of Molecular Microbiology and Immunology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Carleton College, Northfield, MN	B.A.	06/1998	Chemistry
University of California, Berkeley, Berkeley, CA	Ph.D.	06/2004	Chemistry
Yale University School of Medicine, New Haven, CT	Postdoctoral	12/2007	Cell Biology
MFPL, University of Vienna, Vienna, Austria	Postdoctoral	06/2013	Cell Biology

A. Personal Statement

My diverse training in chemical biology, cell biology, and parasitology has given me a unique skillset to pursue research on the cytoskeletal biogenesis of the kinetoplastid parasites. My research program is focused on understanding how the trypanosomatid parasites undergo morphogenesis, the process they employ to shape their cells and transmit shape during cell division. This is an important question for two reasons: **1.** Trypanosomatids have evolved unique approaches for fundamental cellular processes such as morphogenesis; identifying the molecular mechanisms will provide a better understanding of evolutionary niches, such as parasitism, that arise through diversification of cellular pathways in manners that would be extremely difficult to predict; **2.** These pathways represent unique and essential aspects of trypanosomatid biology that could be exploited to develop treatments for a range of neglected tropical diseases that cause significant human suffering in developing countries I have a strong interest in training students from diverse backgrounds and have undertaken CIMR mentorship training to improve my ability to support them. Many of my trainees, both postdoctoral fellows and undergraduate and doctoral students, have completed training and are now continuing research in academia and in industry as researchers and faculty. I enjoy mentoring undergraduate and graduate students and I am committed to recruiting students from underrepresented backgrounds, disadvantaged backgrounds, and those with disabilities to promote diversity and inclusion in health-related research. My laboratory aims to increase the representation of minorities and women in academic research by recruiting and retaining students with diverse ethnic and economic backgrounds. Inclusion is an important element to my consideration of prospective students, and I encourage qualified applicants of all races and backgrounds to apply to work in my laboratory. As we all know that the scientific community benefits from the inclusion of a diverse workforce, I actively recruit students, research assistants, and postdocs who are underrepresented minorities, have disabilities, and/or have disadvantaged backgrounds to perform research in my laboratory.

Highlighted ongoing and recently completed projects:

1R01AI166363-01 09/01/21-08/31/26 (Role: PI)
NIH-NIAID $2,473,757 (DC+IC)
Biogenesis of the Trypanosoma brucei subpellicular microtubule array.
Major goal: to study the biogenesis of the subpellicular array by identifying essential proteins that participate in this process and by characterizing a kinesin that is necessary for assembling a new array.

1R21AI151490 12/01/20-11/30/22 (Role: PI)
NIH-NIAID $425,208 (DC+IC)
Revealing spatio-temporal dynamics with long-term trypanosomatid live-cell imaging.
Major goal: to develop a strategy for imaging live parasites using agarose microwells as a means to understand novel aspects of cell division and the emergence of cellular phenotypes.

CBHD COBRE Pilot Project Grant de Graffenried (PI) 11/1/22-10/31/23
$50,000
Morphogenesis and Virulence in Trypanosoma cruzi.
Major goal: The purpose of this grant is to study the cell cycle and life cycle transitions of *T. cruzi* using single-cell RNASeq and organelle markers identified in related trypanosomatid species

Citations:
1. de Graffenried CL, Laughlin ST, Kohler JJ, Bertozzi CR. A Small-Molecule Switch for Golgi Sulfotransferases, Proceedings of the National Academy of Sciences of the U.S.A., 2004, 101, 16715- 20. PMCID: PMC534710

2. de Graffenried CL*, Ho HH, Warren G. Polo-like kinase is required for Golgi and bilobe biogenesis in *Trypanosoma brucei*. Journal of Cell Biology, 2008; 181: 431-438. PMCID: PMC2364693.

3. Ikeda KN and **de Graffenried CL***. Polo-like kinase is necessary for flagellum inheritance in *Trypanosoma brucei*. Journal of Cell Science, 2012; 125: 3173-3184. PMID: 22427687.

4. McAllaster MR, Ikeda KN, Lozano-Núñez A, Anrather D, Unterwurzacher V, Gossenreiter T, Perry JA, Crickley R, Mercadante CJ, Vaughan S, and **de Graffenried CL***. Proteomic identification of novel cytoskeletal proteins associated with TbPLK, an essential regulator of cell morphogenesis in *T. brucei.* Molecular Biology of the Cell, 2015; 26: 3013-29. PMCID: PMC4551316

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2022-present	Associate Professor with tenure, Department of Molecular Microbiology and Immunology, Brown University, Providence, RI, USA
2021	Reviewer, Department of Scientific Programming and Incentive Actions, Institute Pasteur
2016-present	Member, British Society for Parasitology
2015-present	Member, American Society for Microbiology
2015-present	Assistant Professor, Department of Molecular Microbiology and Immunology, Brown University, Providence, RI, USA
2014-present	Member, American Society for Cell Biology
2013- 2015	Assistant Professor, Research, Department of Molecular Microbiology and Immunology, Brown University, Providence, RI, USA

Journal Reviewing Experience

Ad hoc Reviewer for: Molecular Biology of the Cell, Cytoskeleton, Nucleus, Journal of Cell Science, PLoS Pathogens, Trends in Parasitology, International Journal of Parasitology, Molecular and Biochemical Parasitology, Angewandte Chemie, mSphere, Nature Reviews Microbiology, Molecular and Cellular Biology, Molecular Microbiology, Memórias do Instituto Oswaldo Cruz, ACS Infectious Diseases, mBio, EMBO Molecular Medicine, PLoS Neglected Tropical Diseases, Frontiers Molecular Biosciences, Current Biology.

Honors and Awards

2017, 2018, 2019, 2022	Dean's Research Bonus Program Award
2016	Brown Research Seed Fund Award
2013	Salomon Faculty Research Award

2005	NIH NRSA Postdoctoral Fellowship
1998	National Science Foundation Pre-Doctoral Fellowship awardee
1998	Phi Beta Kappa Honor Society
1997	Sigma Xi

C. Contributions to Science

1) As a graduate student in the laboratory of Carolyn Bertozzi, I showed that the carbohydrate sulfotransferases have limited substrate specificity encoded in their catalytic domains when they are tested in vitro against a panel of different synthetic glycans. These sulfotransferases are resident in the Golgi apparatus and have domain architectures similar to glycosyltransferases, with an N-terminal single-pass alpha helical domain and C-terminal catalytic domain. When full-length sulfotransferases were expressed in mammalian cells, the three enzymes we studied localized to different compartments within the Golgi apparatus and had distinct preferences for either *N*-linked or *O*-linked glycans, which mirrored the biosynthetic process for each glycan type. Therefore, the selectivity of the sulfotransferases is primarily encoded in their Golgi localization and not their catalytic domains. This result showed why certain sulfotransferases were involved in making L-selectin ligands, while others were tasked with different carbohydrate sulfation events. We subsequently showed that generating chimeric proteins that contained different localization and catalytic domains led to enzymes whose glycan selectivity was dominated by their localization within the Golgi. This showed that the carbohydrate sulfotransferases were modular enzymes similar to glycosyltransferases. This mode of controlling carbohydrate sulfation was much more rapid than transcriptional methods and formally proved the modularity of the carbohydrate sulfotransferases.

1. Bowman KG, Cook BN, **de Graffenried CL**, Bertozzi CR. Biosynthesis of L-selectin ligands: sulfation of sialyl Lewis x-related oligosaccharides by a family of GlcNAc-6-sulfotransferases. Biochemistry 2001, 40, 5382-5391.

2. de Graffenried CL and Bertozzi CR. Golgi localization of carbohydrate sulfotransferases is a determinant of L-selectin ligand biosynthesis. Journal of Biological Chemistry, 2003, 278, 40282- 40295.

3. de Graffenried CL and Bertozzi CR. The stem region of the sulfotransferase GlcNAc6ST-1 is a determinant of substrate specificity. Journal of Biological Chemistry. 2004, 279, 40035-43.

4. de Graffenried CL, Laughlin ST, Kohler JJ, Bertozzi CR. A Small-Molecule Switch for Golgi Sulfotransferases. Proceedings of the National Academy of Sciences of the U.S.A., 2004, 101, 16715- 20. PMCID: PMC534710

2) As a postdoctoral fellow in Graham Warren's lab, I studied the biogenesis of the Golgi apparatus in *Trypanosoma brucei*. This parasite has a single Golgi apparatus that is situated at a specific site in the cell body, which allowed us to easily assess the mode of duplication of the structure and describe defects in this process. We showed that the new Golgi is formed a specific distance away from the old structure and that its assembly occurs in an ordered fashion, with scaffolding and structural proteins added to the new structure before cargo was recruited. We also showed that the Golgi became competent for cargo transport while only containing the volume of 3-4 vesicles, which is remarkable for such a complex organelle. We identified a novel kinetoplastid-specific golgin, termed TbGolgin63, which bound the early secretory organizing protein TbRab1 and was important for maintaining the morphology of the Golgi. I also showed that the *T. brucei* homolog of polo-like kinase, termed TbPLK, was important for maintaining the correct number of Golgi in the parasite. We implicated a cytoskeletal structure that neighbored the ER exit site as a possible organizer of Golgi positioning and number whose component, TbCentrin2, was phosphorylated by TbPLK.

1. Ho HH, He CY, **de Graffenried CL**, Murrells LJ, Warren G. Ordered assembly of the duplicating Golgi in *Trypanosoma brucei*. Proceedings of the National Academy of Sciences of the U.S.A. 2006; 103(20):7676-81. PMCID: PMC1472504

2. Ramirez IB, **de Graffenried CL**, Ebersberger I, Yelinek J, He CY, Price A, Warren G. TbG63, a golgin involved in Golgi architecture in *Trypanosoma brucei*. Journal of Cell Science, 2008; 121: 1538-1546. PMID: 18411253

3. de Graffenried CL*, Ho HH, Warren G. Polo-like kinase is required for Golgi and bilobe biogenesis in *Trypanosoma brucei*. Journal of Cell Biology, 2008; 181: 431-438. PMCID: PMC2364693.

4. de Graffenried CL*, Anrather D, Von Raußendorf F, Warren G. Polo-like kinase phosphorylation of bilobe-resident TbCentrin2 facilitates flagellar inheritance in *Trypanosoma brucei*. Molecular Biology of the Cell, 2013; 24: 1947-63. PMCID: PMC3681699.

3) In my own laboratory, I studied the function of TbPLK during cytoskeletal biogenesis. I mapped the phosphosites generated by the kinase on TbCentrin2 and showed that one specific site was important for the replication of the bilobe structure. We showed that TbPLK was essential for the assembly of the flagellum attachment zone, which adheres the flagellum to the cell surface, and also for duplication of the flagellar pocket collar, which is essential for formation of the flagellar pocket. Blocking the duplication of these structures causes a near-total block in cytokinesis. We generated trypanosomes carrying an analog-sensitive variant of TbPLK, which allowed us to employ a modified kinase inhibitor to block TbPLK function. We used synchronized cells and electron microscopy to dissect specific cell cycle events that required TbPLK activity and showed that it was only essential early in the cell cycle, which influenced our understanding of kinase function.

1. de Graffenried CL*, Ho HH, Warren G. Polo-like kinase is required for Golgi and bilobe biogenesis in *Trypanosoma brucei*. Journal of Cell Biology, 2008; 181: 431-438. PMCID: PMC2364693.

2. Ikeda KN and **de Graffenried CL***. Polo-like kinase is necessary for flagellum inheritance in *Trypanosoma brucei*. Journal of Cell Science, 2012; 125: 3173-3184. PMID: 22427687.

3. Lozano-Núñez A, Ikeda KN, Sauer T, **de Graffenried CL***. An analogue-sensitive approach identifies basal body rotation and flagellum attachment zone elongation as key functions of PLK in *Trypanosoma brucei*. Molecular Biology of the Cell, 2013; 24: 1321-33. PMCID: PMC3639044

4. de Graffenried CL*, Anrather D, Von Raußendorf F, Warren G. Polo-like kinase phosphorylation of bilobe-resident TbCentrin2 facilitates flagellar inheritance in *Trypanosoma brucei*. Molecular Biology of the Cell, 2013; 24: 1947-63. PMCID: PMC3681699

4) Using proximity-dependent biotin identification (BioID) and phosphoproteomics, we identified a large number of potential TbPLK binding partners and interactors, which has allowed us to go from merely describing the morphological defects that occur when the kinase is inhibited to dissecting the molecular mechanisms of kinase function. We identified the first known component of the flagella connector, which connects the tip of the new flagella to the old flagella and showed that this structure did not have its proposed function. We also identified the first unique component of the *T. brucei* cytokinetic complex, known as TOEFAZ1, and showed that it recruits TbPLK onto the tip of the new FAZ. We have subsequently mapped the functional domains of TOEFAZ1 to understand how this scaffold protein directs the positioning of the parasite cleavage furrow. Recent work has also focused on the hook complex protein TbSmee1, which is essential for maintaining the morphology of this cytoskeletal structure and for recruitment of TbPLK to the tip of the new FAZ.

1. McAllaster MR, Ikeda KN, Lozano-Núñez A, Anrather D, Unterwurzacher V, Gossenreiter T, Perry JA, Crickley R, Mercadante CJ, Vaughan S, and **de Graffenried CL***. Proteomic identification of novel cytoskeletal proteins associated with TbPLK, an essential regulator of cell morphogenesis in *T. brucei.* Molecular Biology of the Cell, 2015; 26: 3013-29. PMCID: PMC4551316

2. Identification of TOEFAZ1-interacting proteins reveals key regulators of Trypanosoma brucei cytokinesis. Hilton NA, Sladewski TE, Perry JA, Pataki Z, Sinclair-Davis AN, Muniz RS, Tran HL, Wurster JI, Seo J, **de Graffenried CL***. Molecular Microbiology. 2018; 109(3):306-326. PMCID: PMC6359937.

3. Sinclair-Davis AN, McAllaster MR, **de Graffenried CL***. Functional analysis of TOEFAZ1 uncovers protein domains essential for cytokinesis in *Trypanosoma brucei.* Journal of Cell Science, 2017; 130(22):3918-3932. PMID: 28993462. PMCID: PMC5702046.

4. Perry JA, Sinclair-Davis AN, McAllaster MR, **de Graffenried CL***. TbSmee1 regulates hook complex morphology and the rate of flagellar pocket uptake in Trypanosoma brucei. Molecular Microbiology, 2018; 107(3):344-362. PMCID: PMC5777864.

5) We have also now used proximity biotinylation to map TOEFAZ1-interacting partners, which has identified components necessary for biogenesis of the subpellicular array, including the kinesin KLIF and the microtubule-crosslink component PAVE1. Work on these proteins has now provided new insight into the function of the subpellicular microtubule array, which is essential for shaping the *T. brucei* cell body. We have shown that PAVE1 forms an obligate partner with PAVE2, which binds directly to microtubules. The PAVE complex is localized to the posterior portion of the subpellicular array and is essential for its formation and stability. We also identified additional proteins that localize to other regions of the array, which suggests that it contains subdomains that may be tuned to shape the cell body and stabilize the microtubules against the beat of the flagellum.

1. Hilton NA, Sladewski TE, Perry JA, Pataki Z, Sinclair-Davis AN, Muniz RS, Tran HL, Wurster JI, Seo J, **de Graffenried CL***. Identification of TOEFAZ1-interacting proteins reveals key regulators of Trypanosoma brucei cytokinesis. Molecular Microbiology. 2018; 109(3):306-326. PMCID: PMC6359937.

2. Sinclair AN**, de Graffenried CL***. More than Microtubules: The Structure and Function of the Subpellicular Array in Trypanosomatids. Trends in Parasitology. 2019; 35(10):760-777. PMCID: PMC6783356.

3. Campbell PCC**, de Graffenried CL***. Alternate histories of cytokinesis: lessons from the trypanosomatids. Molecular Biology of the Cell. 2020; Nov 15;31(24):2631-2639. PMCID: PMC7927182.

4. Sinclair AN, Huynh CT, Sladewski TE, Zuromski JL, Ruiz AE, **de Graffenried CL***. The *Trypanosoma brucei* subpellicular microtubule array is organized into functionally discrete subdomains defined by microtubule-associated proteins. PLoS Pathogens, May 19;17(5):e1009588. PMCID: PMC8168904.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1FWQagT6jMe/bibliography/public/