BIOGRAPHICAL SKETCH

NAME: Nicholas Gene Brown

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

POSITION TITLE: Associate Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date MM/YYYY	FIELD OF STUDY
Texas A&M University, College Station, TX	B.S.	12/2005	Biomedical Sciences
Baylor College of Medicine, Houston, TX	Ph.D.	07/2011	Biochemistry
St. Jude Children's Research Hospital, Memphis, TN	Postdoctoral Fellowship	08/2017	Structural Biology

A. Personal Statement

My long-term research interest is to understand the structural and biochemical mechanisms of molecular machines that are manipulated in disease processes. As a graduate student, I developed a strong background in biochemistry and biophysics while studying well-characterized bacterial enzyme/inhibitor interactions. For my postdoc, I sought to extend my experience in structural biology by using cryo-EM to investigate multi-protein eukaryotic complexes and ubiquitination. My postdoc project involved dissecting the mechanisms of ubiquitination by the massive 1.2 MDa ubiquitin ligase known as the Anaphase-Promoting Complex or Cyclosome (APC/C), which is regarded as a master regulator of the cell cycle and is composed of over a dozen subunits.

As an independent investigator, I aim to interrogate how other E3 ubiquitin ligases function and are dysregulated in disease. Together with our collaborators, we uncovered multiple mechanisms that control cell cycle transitions, identified chromatin regulators as E3 substrates, and demonstrated how mutations on E3s result in neurodevelopmental disorders. We also continue to push technological boundaries by applying new biophysical tools (e.g., time-resolved cryo-EM and mass photometry) to our fully recombinant systems. These tools allow us to interrogate complex mechanisms with unprecedented detail.

Ongoing Support:

08/01/2018 – 07/31/2028
R35 GM128855, Maximizing Investigators' Research Award
Brown, Nicholas G. (PI)
Spindle Assembly Checkpoint Silencing
The proposed research will address how the destruction of proteins by a complex set of large molecular machines drive the cell cycle and control cell division.

Selected Publications (out of 68)

1. Robertson KC, Amann SJ, Liu T, Funk AV, Wang X, Grishkovskaya I, Mehmood A, Tabor JR, Norris-Drouin JL, Arrowsmith CH, Collins JL, Miao Y, Emanuele MJ, Haselbach D[#], James LI[#], **Brown NG[#].** [#]Corresponding Author. Structural basis of NSD2 degradation via targeted recruitment of SCF-FBXO22. *Nat Commun*. 2026 Apr 23;. doi: 10.1038/s41467-026-72235-9. PubMed PMID: 42026065.

2. Skrajna A, Bodrug T, Martinez-Chacin RC, Fisher CB, Welsh KA, Simmons HC, Arteaga EC, Simmons JM, Nasr MA, LaPak KM, Nguyen A, Huynh MT, Fargo I, Welfare JG, Zhao Y, Lawrence DS, Goldfarb D, **Brown NG[#]**, McGinty RK[#]. [#]Corresponding Author. APC/C-mediated ubiquitylation of extranucleosomal histone complexes lacking canonical degrons. *Nat Commun*. 2025 Mar 15;16(1):2561. doi: 10.1038/s41467-025-57384-7. PubMed PMID: 40089476.

3. Bodrug T, Welsh KA, Bolhuis DL, Paulakonis E, Martinez-Chacin RC, Liu B, Pinkin N, Bonacci T, Cui L, Xu P, Roscow O, Amann SJ, Grishkovskaya I, Emanuele MJ, Harrison JS, Steimel JP, Hahn KM,

Zhang W, Zhong ED, Haselbach D[#], **Brown NG[#]**. [#]Corresponding Author. Time-resolved cryo-EM Analysis of Substrate Polyubiquitination by the Anaphase-Promoting Complex/Cyclosome. *Nat Struct Mol Biol.* 2023 Nov;30(11):1663-1674. doi: 10.1038/s41594-023-01105-5. Epub 2023 Sep 21. PubMed PMID: 37735619.

4. **Brown NG***, VanderLinden R*, Watson ER*, Weissmann F, Ordureau A, Wu KP, Zhang W, Yu S, Mercredi PY, Harrison JS, Davidson IF, Qiao R, Lu Y, Dube P, Brunner MR, Grace CR, Miller DJ, Haselbach D, Jarvis MA, Yamaguchi M, Yanishevski D, Petzold G, Sidhu SS, Kuhlman B, Kirschner MW, Harper JW, Peters JM, Stark H, Schulman BA. *Equal first author. Dual RING E3 Architectures Regulate Multiubiquitination and Ubiquitin Chain Elongation by APC/C. *Cell*. 2016 Jun 2;165(6):1440-53. doi: 10.1016/j.cell.2016.05.037. PubMed PMID: 27259151.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2025	Ad hoc reviewer for National Ataxia Foundation
2024	Ad hoc reviewer for American Heart Association Innovative Project Award Basic Sciences
2023 – Present	Editorial Board for the Journal of Biological Chemistry (JBC)
2023 – Present	Associate Professor with tenure, Dept. of Pharmacology, University of North Carolina – Chapel Hill, Lineberger Comprehensive Cancer Center
2023	Ad hoc reviewer for UK Research and Innovation (UKRI) Medical Research Council
2023	Ad hoc reviewer for Fondazione Cariplo and Fondazione Telethon (Italy)
2018	Ad hoc reviewer for NIH Membrane Biology and Protein Processing (MBPP) Study Section
2017 – Present	Member, Cancer Cell Biology Program, Lineberger Comprehensive Cancer Center
2017 – 2023	Assistant Professor, Dept. of Pharmacology, University of North Carolina – Chapel Hill, Lineberger Comprehensive Cancer Center
2011 – 2017	Postdoctoral Research Associate, Dept. of Structural Biology, St. Jude Children's Research Hospital, Memphis, TN (Dr. Brenda Schulman's Lab)

Honors/Awards

2025	BrightFocus Alzheimer's Disease Research Award
2024	UNC Institute for Convergent Science (ICS) AGILE Award
2023	American Heart Association Innovative Project Award
2023	UNC Creativity Hub Award
2023	UNC Jefferson Pilot Fellowship Award
2023	UNC Boost Award
2020	IBM Junior Faculty Development Award
2019-2020	UNC Lineberger Development Award
2018-2023	National Institute of General Medicine Sciences Maximizing Investigators' Research Award for New and Early-Stage Investigators
2017-2018	National Cancer Institute (NCI) Career Transition Award
2015-2017	Leukemia and Lymphoma Society Special Fellowship
2012-2015	Jane Coffin Childs Memorial Fund for Medical Research Fellowship
2010-2011	Gulf Coast Consortia/Keck Center Biomedical Discovery from Large Scale Data Sets Training Program Fellowship
2008-2010	Gulf Coast Consortia/Keck Center Pharmacoinformatics Training Program Fellowship
2006	Baylor College of Medicine Verna and Marrs McLean Award

C. Contributions to Science

1. Antibiotic resistance remains a global health threat. To understand the evolution of the antibiotic resistance family of enzymes known as β-lactamases, structural and functional studies revealed the amino acid requirements for protein stability and enzymatic function. Biophysical and pre-steady state kinetic approaches uncovered the binding forces and binding residues required for affinity and specificity of the highly potent β-lactamase Inhibitory protein-II (BLIP-II).

a. **Brown NG**, Shanker S, Prasad BV, Palzkill T. Structural and biochemical evidence that a TEM-1 beta-lactamase N170G active site mutant acts via substrate-assisted catalysis. *J Biol Chem.* 2009 Nov 27;284(48):33703-12. doi: 10.1074/jbc.M109.053819. PubMed PMID: 19812041.

b. **Brown NG**, Pennington JM, Huang W, Ayvaz T, Palzkill T. Multiple global suppressors of protein stability defects facilitate the evolution of extended-spectrum TEM β-lactamases. *J Mol Biol*. 2010 Dec 17;404(5):832-46. doi: 10.1016/j.jmb.2010.10.008. PubMed PMID: 20955714.

c. **Brown NG**, Chow DC, Sankaran B, Zwart P, Prasad BV, Palzkill T. Analysis of the binding forces driving the tight interactions between beta-lactamase inhibitory protein-II (BLIP-II) and class A beta-lactamases. *J Biol Chem.* 2011 Sep 16;286(37):32723-35. doi: 10.1074/jbc.M111.265058. PubMed PMID: 21775426.

d. **Brown NG**, Chow DC, Ruprecht KE, Palzkill T. Identification of the β-lactamase inhibitor protein-II (BLIP-II) interface residues essential for binding affinity and specificity for class A β-lactamases. *J Biol Chem*. 2013 Jun 14;288(24):17156-66. doi: 10.1074/jbc.M113.463521. PubMed PMID: 23625930.

2. Since 1998, it was believed that all RING-containing E3 ubiquitin ligases functioned the same way. During my postdoctoral work, we discovered that the Anaphase-Promoting Complex/Cyclosome (APC/C) uses multiple mechanisms of substrate ubiquitination by its E2s (UBE2C and UBE2S). These studies include the first structures of a RING E3 ubiquitin ligase (the largest family with >600 members) mimicking ubiquitin transfer to a disordered substrate or building a polyubiquitin chain. We also dissected how distinct APC/C inhibitors (EMI1 and the Mitotic Checkpoint Complex) differentially modulate APC/C activity during cell cycle transitions.

   a. Frye JJ*, **Brown NG***, Petzold G*, Watson ER, Grace CR, Nourse A, Jarvis MA, Kriwacki RW, Peters JM, Stark H, Schulman BA. Electron microscopy structure of human APC/C(CDH1)-EMI1 reveals multimodal mechanism of E3 ligase shutdown. *Equal first author. *Nat Struct Mol Biol*. 2013 Jul;20(7):827-35. doi: 10.1038/nsmb.2593. PubMed PMID: 23708605.

   b. **Brown NG**, Watson ER, Weissmann F, Jarvis MA, VanderLinden R, Grace CR, Frye JJ, Qiao R, Dube P, Petzold G, Cho SE, Alsharif O, Bao J, Davidson IF, Zheng JJ, Nourse A, Kurinov I, Peters JM, Stark H, Schulman BA. Mechanism of polyubiquitination by human anaphase-promoting complex: RING repurposing for ubiquitin chain assembly. *Mol Cell*. 2014 Oct 23;56(2):246-60. doi: 10.1016/j.molcel.2014.09.009. PubMed PMID: 25306923.

   c. **Brown NG**, VanderLinden R, Watson ER, Qiao R, Grace CR, Yamaguchi M, Weissmann F, Frye JJ, Dube P, Ei Cho S, Actis ML, Rodrigues P, Fujii N, Peters JM, Stark H, Schulman BA. RING E3 mechanism for ubiquitin ligation to a disordered substrate visualized for human anaphase-promoting complex. *Proc Natl Acad Sci U S A*. 2015 Apr 28;112(17):5272-9. doi: 10.1073/pnas.1504161112. PubMed PMID: 25825779.

   d. **Brown NG***, VanderLinden R*, Watson ER*, Weissmann F, Ordureau A, Wu KP, Zhang W, Yu S, Mercredi PY, Harrison JS, Davidson IF, Qiao R, Lu Y, Dube P, Brunner MR, Grace CR, Miller DJ, Haselbach D, Jarvis MA, Yamaguchi M, Yanishevski D, Petzold G, Sidhu SS, Kuhlman B, Kirschner MW, Harper JW, Peters JM, Stark H, Schulman BA. *Equal first author. Dual RING E3 Architectures Regulate Multiubiquitination and Ubiquitin Chain Elongation by APC/C. *Cell*. 2016 Jun 2;165(6):1440-53. doi: 10.1016/j.cell.2016.05.037. PubMed PMID: 27259151.

3. As an independent investigator, we have uncovered new mechanisms of APC/C activation in mitosis and revealed how the APC/C remains active during G1. For example, we were able to discover a paradigm-shifting mechanism by which the E2 UBE2S stimulates substrate priming by the E3 APC/C and E2 UBE2C through positive allosteric feedback during mitosis. Additionally, our recent paper was the first time-resolved cryo-EM study published of a RING E3 Ub ligase actively ubiquitinating a target, which revealed unexpected architectures of the APC/C during polyubiquitination.

   a. Martinez-Chacin RC, Bodrug T, Bolhuis DL, Kedziora KM, Bonacci T, Ordureau A, Gibbs ME, Weissmann F, Qiao R, Grant GD, Cook JG, Peters JM, Wade Harper J, Emanuele MJ[#], **Brown NG[#].** [#]Corresponding Author. Ubiquitin chain-elongating enzyme UBE2S activates the RING E3 ligase APC/C for substrate priming. *Nat Struct Mol Biol.* 2020 Jun;27(6):550-560. doi: 10.1038/s41594-020-0424-6. PubMed PMID: 32393902.

b. Welsh KA, Bolhuis DL, Nederstigt AE, Boyer J, Temple BRS, Bonacci T, Gu L, Ordureau A, Harper JW, Steimel JP, Zhang Q, Emanuele MJ, Harrison JS[#], **Brown NG[#]**. [#]Corresponding Author. Functional conservation and divergence of the helix-turn-helix motif of E2 ubiquitin-conjugating enzymes. *EMBO J*. 2022 Feb 1;41(3):e108823. doi: 10.15252/embj.2021108823. PubMed PMID: 34942047.

c. Bolhuis DL, Martinez-Chacin RC, Welsh KA, Bodrug T, Cui L, Emanuele MJ, **Brown NG[#].** [#]Corresponding Author. Examining the mechanistic relationship of APC/C^{CDH1} and its interphase inhibitor EMI1. *Protein Sci.* 2022. doi: 10.1002/pro.4324. 2022 Jun;31(6):e4324. doi: 10.1002/pro.4324. PubMed PMID: 35634770.

d. Bodrug T, Welsh KA, Bolhuis DL, Paulakonis E, Martinez-Chacin RC, Liu B, Pinkin N, Bonacci T, Cui L, Xu P, Roscow O, Amann SJ, Grishkovskaya I, Emanuele MJ, Harrison JS, Steimel JP, Hahn KM, Zhang W, Zhong ED, Haselbach D[#], **Brown NG[#]**. [#]Corresponding Author. Time-resolved cryo-EM Analysis of Substrate Polyubiquitination by the Anaphase-Promoting Complex/Cyclosome. *Nat Struct Mol Biol.* 2023 Nov;30(11):1663-1674. doi: 10.1038/s41594-023-01105-5. Epub 2023 Sep 21. PubMed PMID: 37735619.

4. My lab is highly collaborative to support the development of new techniques (e.g., Mass Photometry) and the identification of new APC/C-dependent biological processes. In the papers below, we identified multiple chromatin regulators as substrates of the APC/C and found the APC/C interacts with the repeating unit of chromatin, the nucleosome. These collaborations allow our work to span from biochemistry and structural studies to cell-based assays and mouse studies.

   a. Sonn-Segev A, Belacic K, Bodrug T, Young G, VanderLinden RT, Schulman BA, Schimpf J, Friedrich T, Dip PV, Schwartz TU, Bauer B, Peters JM, Struwe WB, Benesch JLP, **Brown NG[#],** Haselbach D[#], Kukura P[#]. [#]Corresponding Author. Quantifying the heterogeneity of macromolecular machines by mass photometry. *Nat Commun*. 2020 Apr 14;11(1):1772. doi: 10.1038/s41467-020-15642-w. PubMed PMID: 32286308.

   b. Franks JL, Martinez-Chacin RC, Wang X, Tiedemann RL, Bonacci T, Choudhury R, Bolhuis DL, Enrico TP, Mouery RD, Damrauer JS, Yan F, Harrison JS, Major MB, Hoadley KA, Suzuki A, Rothbart SB, **Brown NG[#]**, Emanuele MJ[#]. [#]Corresponding Author. In silico APC/C substrate discovery reveals cell cycle-dependent degradation of UHRF1 and other chromatin regulators. *PLoS Biol.* 2020 Dec;18(12):e3000975. doi: 10.1371/journal.pbio.3000975. PubMed PMID: 33306668.

   c. Ferguson CJ, Urso O, Bodrug T, Gassaway BM, Watson ER, Prabu JR, Lara-Gonzalez P, Martinez-Chacin RC, Wu DY, Brigatti KW, Puffenberger EG, Taylor CM, Haas-Givler B, Jinks RN, Strauss KA, Desai A, Gabel HW, Gygi SP, Schulman BA, **Brown NG,** Bonni A. APC7 mediates ubiquitin signaling in constitutive heterochromatin in the developing mammalian brain. *Mol Cell*. 2022 Jan 6;82(1):90-105.e13. doi: 10.1016/j.molcel.2021.11.031. PubMed PMID: 34942119.

   d. Skrajna A, Bodrug T, Martinez-Chacin RC, Fisher CB, Welsh KA, Simmons HC, Arteaga EC, Simmons JM, Nasr MA, LaPak KM, Nguyen A, Huynh MT, Fargo I, Welfare JG, Zhao Y, Lawrence DS, Goldfarb D, **Brown NG[#]**, McGinty RK[#]. [#]Corresponding Author. APC/C-mediated ubiquitylation of extranucleosomal histone complexes lacking canonical degrons. *Nat Commun*. 2025 Mar 15;16(1):2561. doi: 10.1038/s41467-025-57384-7. PubMed PMID: 40089476.

5. Ubiquitination is tightly controlled by many ubiquitin ligases and deubiquitinase enzymes (DUBs). While ligases add ubiquitin to substrates, DUBs antagonize this activity by removing ubiquitin marks to the stabilize their targets. These complex enzymatic processes require new tools and a deep understanding of both systems. To this end, we have interrogated the mechanisms of several ligases and DUBs involved in different biological processes.

   a. Wick ET, Treadway CJ, Li Z, Nicely NI, Ren Z, Baldwin AS, Xiong Y, Harrison JS[#], **Brown NG[#]**. [#]Corresponding Author. Insight into Viral Hijacking of CRL4 Ubiquitin Ligase through Structural Analysis of the pUL145-DDB1 Complex. *Journal of Virology*. 2022 Sep 14;96(17):e0082622. doi: 10.1128/jvi.00826-22. Epub 2022 Aug 8. PubMed PMID: 35938868.

   b. Hodáková Z[#], Grishkovskaya I, Brunner HL, Bolhuis DL, Belačić K, Schleiffer A, Kotisch H, **Brown NG[#]**, Haselbach D[#]. [#]Corresponding Author. Cryo-EM structure of the chain-elongating E3 ligase UBR5. *EMBO J*. 2023 Aug 15;42(16):e113348. doi: 10.15252/embj.2022113348. Epub 2023 Jul 6. PubMed PMID: 37409633.

c. Bolhuis DL, Fleifel D, Bonacci T, Wang X, Mouery BL, Cook JG#, **Brown NG[#]**, Emanuele MJ[#]. [#]Corresponding Author. USP37 prevents unscheduled replisome unloading through MCM complex deubiquitination. *Nat Commun.* 2025 May 16;16(1):4575. doi: 10.1038/s41467-025-59770-7. PubMed PMID: 40379725.

d. Stewart M, Paththamperuma C, McCann C, Cottingim K, Zhang H, DelVecchio R, Peng I, Fennimore E, Nix JC, Saeed MN, George K, Makaroff K, Colie M, Paulakonis E, Almeida MF, Afolayan AJ, **Brown NG[#]**, Page RC[#], Schisler JC[#]. Crystal structures reveal phosphorylation-dependent disruption of the heat shock protein 70-CHIP interface: A compensatory G132N variant restores binding affinity. *Cell Stress Chaperones*. 2026 May;31(3):100166. doi: 10.1016/j.cstres.2026.100166. Epub 2026 Mar 13. PubMed PMID: 41833837; PubMed Central PMCID: PMC13090976.

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