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: Buck, Bethany A.

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

POSITION TITLE: Associate Professor of Chemistry

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 Wisconsin - Stevens Point, Stevens Point , Wisconsin	BS	05/2000	Chemistry
University of Minnesota - Twin Cities, Minneapolis, Minnesota	PhD	06/2005	Chemistry
The Scripps Research Institute, La Jolla, California	Postdoc	12/2010	Biophysics

A. Personal Statement

Throughout my career, I have taken on increasingly challenging problems requiring me to learn and utilize multidisciplinary approaches to achieve my research goals. This foundational experience has provided me with the ability to develop creative solutions to important large biological problems and has shaped the directions of my independent research program. The overarching focus of my research program is to utilize parallel *in vitro* biophysical/biochemical and cell-based methodologies to delineate the mechanisms by which the ZBTB family of methyl-CpG binding proteins (MBPs) mediate epigenetic-based transcriptional processes in disease. To date, I have firmly established myself as an expert in the structural and biophysical characterization of methylated DNA recognition mediated by zinc finger (ZF) proteins, particularly for the ZBTB family of MBPs (e.g. (a)). In addition, we have actively utilized and pursued development of cell-based genomics strategies to mechanistically investigate the transcriptional and phenotypic consequences of promoter occupation by these proteins in multiple cell line models (e.g. (b)). Few laboratories have this broad range of experimental capability, which undoubtedly makes my laboratory uniquely poised to fill a critical knowledge gap in the field of epigenetic-based transcription. Specifically, we are characterizing on the global genomic scale how target DNA recognition by the ZBTB MBPs leads to protein assembly recruitment, chromatin reorganization, and downstream transcriptional outcomes in disease. More generally, I have a broad expertise in the structural, biophysical and biochemical characterization of protein-nucleic acid interactions. This has led to a number of recent impactful collaborations (e.g. (c) and (d)).

After successfully building a research team, procuring funding, and making significant contributions to the field of epigenetic-based transcription, the momentum of my research program was significantly impacted by the COVID-19 pandemic. Most significant was the loss of trainees at the beginning of the pandemic. My two postdoctoral fellows left nearly simultaneously due to career opportunities in biotechnology they could not pass up just prior to the pandemic shutdown. A graduate student did not return from parental leave, and actually moved closer to family for support during the pandemic. Suddenly, I needed to rebuild my group from the ground up immediately after receiving tenure. The situation was further challenged by a limited ability to hire during the early part of the pandemic. In addition, many campus and National core facilities were closed or only accessible to COVID-19 related projects for many months, further impacting my group's ability to collect data to finish manuscripts and generate new preliminary results for grant applications. Compounding these issues was the challenge of being the primary caregiver and homeschooling an early elementary age child that did not thrive during the year while they were required to be home. Although I have rebuilt my group, these cumulative challenges led to an unavoidable productivity gap in my research program.

My current research group consists of 7 talented and dedicated research personnel; nearly all of which identify with diverse groups. I have additionally cultivated multiple productive collaborations, enhancing my publication and research funding opportunities. My demonstrated ingenuity, trajectory and strong track-record prior to the

COVID-19 pandemic in conjunction with my rapid re-establishment of research productivity and diversification efforts indicate that I will continue to lead in develop transformative solutions to broad disease-related problems. I have undoubtedly distinguished myself as a campus leader and champion for graduate student and DEIA interests, and will continue to pursue efforts to strengthen graduate education on the Univ. of Utah campus.

a. Hudson NO, Whitby FG, *Buck-Koehntop BA*. Structural insights into methylated DNA recognition by the C-terminal zinc fingers of the DNA reader protein ZBTB38. J. Biol. Chem. 2018 Dec 21;293(51):19835-19843. PubMed PMID: 30355731; PubMed Central PMCID: PMC6314133.

b. Pozner, A., Terooatea, T. W., *Buck-Koehntop BA*. Cell-specific Kaiso (ZBTB33) regulation of cell cycle through Cyclin D1 and Cyclin E1. J. Biol. Chem. 2016 Nov 18;291(47):24538-24550. PubMed PMID: 27694442; PubMed Central PMCID: PMC5114407.

c. Kumbhar, R., Sanchez A, Perren J, Gong F, Corujo D, Medina F, Devanathan SK, Xhemalce B, Matouschek A, Buschbeck M, *Buck-Koehntop BA*, Miller KM. Poly(ADP-ribose)-binding and macroH2A mediate recruitment and functions of KDM5A at DNA lesions. J. Cell. Biol. 2021 Jul 5;220 (7):e202006149. PubMed PMID: 34003252; PubMed Central PMCID: PMC8135068.

d. Fleming AM*, Guerra Castañaza Jenkins BL, *Buck BA**, Burrows CJ*. DNA damage accelerates G-quadruplex folding in a duplex-G-quadruplex-duplex context. 2024 Jan bioRxiv 2024.01.20.576387; doi: https://doi.org/10.1101/2024.01.20.576387.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2021 -	Director, Biological Chemistry Graduate Program, University of Utah, Salt Lake City, UT
2020 -	Adjunct Associate Professor of Biochemistry, University of Utah, Salt Lake City, UT
2019 -	Associate Professor of Chemistry, University of Utah, Salt Lake City, UT
2016 -	Associate Member Huntsman Cancer Institute Cancer Center
2019 - 2020	Adjunct Assistant Professor of Biochemistry, University of Utah, Salt Lake City, UT
2011 - 2019	Assistant Professor of Chemistry, University of Utah, Salt Lake City, UT
2007 - 2010	American Cancer Society Postdoctoral Fellow, The Scripps Research Institute, La Jolla, CA
2005 - 2007	Skaggs Institute for Chemical Biology Postdoctoral Fellow, The Scripps Research Institute, La Jolla, CA
2001 - 2003	NIH-CBITG Fellow, University of Minnesota, Minneapolis, MN
2000 - 2005	Graduate Research Assistant, University of Minnesota - Twin Cities, Minneapolis, MN

Other Experience and Professional Memberships

05/2023	NSF Grant Reviewer
01/2020	NSF Grant Reviewer
04/2019	Session Chair – Spotlight Session; Epigenetic Factors that Contribute to Gene Regulation; ASBMB
10/2017	NIH Early Career Reviewer (ad hoc), MSFC study section
07/2016	Session Chair – Interface Between Proteins and Nucleic Acids, Protein Society Symposium
2016 - 2023	Member, American Cancer Society, Peer Review Committee - DNA Mechanisms in Cancer
06/2015	Ad hoc reviewer, American Cancer Society, Review Committee - DNA Mechanisms in Cancer
2008 - 2010	Associate Faculty Member, F1000

Honors and Awards

2016	Extraordinary Faculty Achievement Award, University of Utah
2016	Sigma Chi Fraternity Outstanding Faculty Award
2016	Career Services Faculty Recognition Award, University of Utah
2014	American Cancer Society Research Scholar, University of Utah
2007	American Cancer Society Postdoctoral Fellow, The Scripps Research Institute
2005	Skaggs Institute for Chemical Biology Postdoctoral Fellowship, The Scripps Research Institute
2001	NIH Chemical-Biology Interface Training Grant, University of Minnesota - Twin Cities
2000	Chancellor's Leadership Award, University of Wisconsin - Stevens Point
2000	L&S Academy Award to a Graduating Senior in Chemistry, Univ. of Wisconsin-Stevens Point
1999	NSF-REU Fellowship, University of Virginia
1999	ACS-Central Wisconsin Section Outstanding Chemistry Student Award
1998	Faust Award in Chemistry, University of Wisconsin-Stevens Point

C. Contributions to Science

1. **Molecular basis for zinc finger recognition of methylated DNA.** ZBTB33 (Kaiso), ZBTB4 and ZBTB38 constitute a family of zinc finger (ZF)-containing methyl-CpG binding proteins (MBPs), two members of which (ZBTB33 and ZBTB4) exhibit bimodal DNA recognition by binding both mCpG and sequence-specific TpG-containing DNA sites with the same structural domain. We have discovered that the N-terminal ZF domain of ZBTB38 is unique in that it requires all five N-term ZFs for high-affinity mCpG DNA binding, and uniquely harbors an additional arginine residue in the core three ZF domain that allows it to preferentially discriminate against TpG DNA. There is mounting evidence that ZBTB MBP transcriptional activities are associated with the maintenance and progression of disease conditions, however, an extensive analysis for the mechanisms of bimolecular target recognition and subsequent transcriptional responses regulated by each protein had been elusive. Further, while structures of the other MBP families in complex with methylated DNA were determined, the structural basis for mCpG recognition by ZF MBPs remained unknown until I solved the structure of ZBTB33 in complex with its methylated and sequence-specific TpG-containing DNA targets (d). These structures provided the first molecular details for ZF recognition of this epigenetic mark and highlighted key residues involved in the bimodal DNA recognition exhibited by this protein. I have consequently firmly established myself as an expert in ZF recognition of methylated DNA (c). In particular, our laboratory has focused on biochemically and structurally characterizing ZBTB4 and ZBTB38 as there is sufficient evidence to demonstrate these proteins also have a role in disease, and that their modes of biomolecular target recognition are distinct from each other and ZBTB33. Further, each of these proteins has additional ZF domains, the functions of which we have determined to also be nucleic acid binding. Importantly, we recently determined that the C-terminal ZFs of ZBTB38 also participate in methyl-selective DNA binding *in vitro* and within cells utilizing a novel mode of methyl-CpG recognition (a and b), which expands our understanding for how ZFs can recognize this essential epigenetic mark.
    a. Hudson NO, Whitby FG, *Buck-Koehntop BA*. Structural insights into methylated DNA recognition by the C-terminal zinc fingers of the DNA reader protein ZBTB38. J. Biol. Chem. 2018 Dec 21;293(51):19835-19843. PubMed PMID: 30355731; PubMed Central PMCID: PMC6314133.
    b. Pozner A*, Hudson NO*, Trewhella J, Terooatea TW, Miller SA, *Buck-Koehntop BA*. The C-Terminal zinc fingers of ZBTB38 are novel selective readers of DNA methylation. J. Mol. Biol. 2018 Feb 2;430(3):258-271. PubMed PMID: 29287967. *Co-first authorship
    c. Hudson NO, *Buck-Koehntop BA*. Zinc finger readers of methylated DNA. Molecules. 2018 Oct;23(10):2555. PubMed PMID: 30301273; PubMed Central PMCID PMC6222495.
    d. *Buck-Koehntop BA*, Stanfield RL, Ekiert DC, Martinez-Yamout MA, Dyson HJ, Wilson IA, Wright PE. Molecular basis for recognition of methylated and specific DNA sequences by the zinc finger protein Kaiso. Proc Natl Acad Sci U S A. 2012 Sep 18;109(38):15229-34. PubMed PMID: 22949637; PubMed Central PMCID: PMC3458336.
2. **Cell based methodologies to delineate disease relevant transcriptional mechanisms mediated by the ZBTB MBPs.** My group has also acquired extensive experience in cell based methodologies, including several high-throughput sequencing strategies and bioinformatics, by examining the biological activities of the ZBTB MBPs in various human cell line models. Specifically, our strategy has been to combine ChIP-based methods to map gene occupancies for these proteins with methylome analyses to determine the DNA methylation status of occupied sites and RNA-seq coupled with phenotypic assays to discern the transcriptional consequence of promoter occupation by these proteins. Using this strategy, we have recently determined a novel molecular mechanism by which ZBTB33 regulates cellular proliferation and cell cycle progression in a cell-dependent manner (a). We have also determined that all three ZBTB MBPs are up-regulated in cancer and provide the first evidence that in prostate cancer, these three proteins may work in parallel to maintain the disease state (manuscript in revision). Further, we identified an issue that has prevented advanced ChIP-based methods (e.g. ChIP-exo) from becoming mainstream and have found a solution that has not only advanced our own research program, but has impacted research capabilities within the broader scientific community (b). In recent years, I have also become recognized as an expert in the cell based functions of the ZBTB MBPs (c). Thus, our laboratory is uniquely poised to combine *in vitro* biophysical and cell based genomic approaches to delineate the mechanisms by which this MBP family recognize biomolecular targets and regulate transcription in normal as well as disease state cellular functions.

a. Pozner A, Terooatea TW, *Buck-Koehntop BA*. Cell-specific Kaiso (ZBTB33) regulation of cell cycle through Cyclin D1 and Cyclin E1. J. Biol. Chem. 2016 Nov 18;291(47):24538-24550. PubMed PMID: 27694442; PubMed Central PMCID PMC5114407.

b. Terooatea TW, Pozner A, *Buck-Koehntop BA*. PAtCh-Cap: input strategy for improving analysis of ChIP-exo data sets and beyond. Nucleic Acids Res. 2016 Dec 1;44(21):e159. PubMed PMID: 27550178; PubMed Central PMCID: PMC5137431.

c. Hodges AJ, Hudson, NO, *Buck-Koehntop BA*. Cys_2His_2 zinc finger methyl-CpG binding proteins: Getting a handle on methylated DNA. J. Mol. Biol. 2019 Oct 16;doi:10.1016/j.jmb.2019.09.012. PubMed PMID: 31628952.

3. **Interrogation of novel PAR chain recognition by KDM5A.** In collaboration with Dr. Kyle M. Miller (UT Austin), we have been mechanistically interrogating novel cellular functions for the lysine demethylase KDM5A. While KDM5A is well-known to function as a transcriptional regulator, recent evidence from the Miller lab demonstrated that KDM5A collaborates with PARP1 and the histone variant macroH2A1.2 to modulate chromatin, promoting DNA repair mechanisms. In investigating the role of KDM5A in this process, it was determined that relative to the other KDM5 family members, KDM5A uniquely harbors a putative C-terminally localized coiled-coil (CC) domain that was predicted through domain mapping to directly engage poly-ADP-ribose (PAR) chains. This domain was determined to be essential for recruitment in cells to DNA damage sites. We demonstrated through a combination of circular dichroism and EMSA analyses, that the putative CC domain is helical in nature, and preferentially recognizes mid- to long-chained PAR polymers. This is the first evidence of a CC domain participating in direct PAR readout and has formed the basis for an on-going collaboration to mechanistically interrogate KDM5A PAR recognition, as well as investigate the broader scope of CC domains in direct PAR chain binding.

   a. Kumbhar, R., Sanchez A, Perren J, Gong F, Corujo D, Medina F, Devanathan SK, Xhemalce B, Matouschek A, Buschbeck M, *Buck-Koehntop BA*, Miller KM. Poly(ADP-ribose)-binding and macroH2A mediate recruitment and functions of KDM5A at DNA lesions. J. Cell. Biol. 2021 Jul 5;220 (7):e202006149. PubMed PMID: 34003252; PubMed Central PMCID: PMC8135068.

   b. Sanchez A, *Buck-Koehntop BA**, Miller KM*. Joining the PARty: PARP Regulation of KDM5A during DNA Repair (and Transcription?). Bioessays. 2022 Jul;44(7):e2200015. doi: 10.1002/bies.202200015. Epub 2022 May 9. Review. PubMed PMID: 35532219; PubMed Central PMCID: PMC9233061. *co-corresponding authors.

4. **Mechanistic insight into dealkylation of organotin compounds by biological dithiols.** As a graduate student I focused on investigating how the mitochondrial membrane protein stannin (SNN) mediates neuronal cell apoptosis in response to the environmental toxin trimethyltin chloride (TMT). Utilizing solution NMR, the structure of SNN in detergent micelles was elucidated and found to be composed of a single transmembrane helix and an amphipathic cytoplasmic helix separated by a long unstructured linker. Using a combination of solution NMR experiments and solid-state NMR analysis of the protein reconstituted in synthetic lipid bilayers we were able to determine that the long unstructured linker between the helices is highly dynamic and is likely involved in protein/protein interactions, while the cytoplasmic helix interacts with the micelle surface. This structure also highlighted that the predicted CXC organotin binding site resides at the membrane surface. To determine the mechanism of SNN induced organotin toxicity, a nine residue peptide (SNN-PEP) incorporating the CXC metal binding motif was designed and its binding interactions with various organotin compounds were investigated. Using a combination of spectroscopic techniques, it was determined that SNN-PEP dealkylates trialkyltin compounds, up to three carbons in length, to their dialkyltin counterparts through a pseudoenzymatic reaction mechanism. The high-resolution structure in conjunction with density functional theory analysis (collaboration with Dr. C. Cramer, U of MN) revealed that SNN-PEP forms a type I β-turn upon binding DMT and adopts a distorted tetrahedral coordination geometry around the tin atom. Peptide variants confirmed that both cysteine residues are necessary and sufficient to bind and dealkylate trialkyltin compounds. Together these findings support the mechanism that dealkylation of TMT to DMT by the CXC motif induces substantial structural and/or dynamical changes in SNN that recruits other protein binding partners, initiating the apoptotic cascade.

   a. *Buck B*, Mascioni A, Que L Jr, Veglia G. Dealkylation of organotin compounds by biological dithiols: toward the chemistry of organotin toxicity. J Am Chem Soc. 2003 Nov 5;125(44):13316-7. PubMed PMID: 14583001.

b. *Buck BA*, Mascioni A, Cramer CJ, Veglia G. Interactions of alkyltin salts with biological dithiols: dealkylation and induction of a regular beta-turn structure in peptides. J Am Chem Soc. 2004 Nov 10;126(44):14400-10. PubMed PMID: 15521759.

c. *Buck-Koehntop BA*, Mascioni A, Buffy JJ, Veglia G. Structure, dynamics, and membrane topology of stannin: a mediator of neuronal cell apoptosis induced by trimethyltin chloride. J Mol Biol. 2005 Dec 2;354(3):652-65. PubMed PMID: 16246365.

d. *Buck-Koehntop, BA*, Porcelli, F, Lewin, JL, Cramer, CJ, Veglia, G. Biological chemistry of organotin compounds: Interactions and dealkylations by dithiols. J Organomet Chem. 2006; 691:1748-55.

5. **Contributions to structure-function correlations of membrane proteins and membrane associating peptides.** In addition to the contributions outlined above, during my graduate studies I made contributions to several other structure-function investigations of transmembrane proteins and membrane associating peptides. In particular, I helped develop a protein expression and purification procedure for the NMR-scale production of sarcolipin (SLN) and phospholamban (PLN); membrane proteins involved in regulating skeletal and heart muscle contractility through direct interactions with the Ca-ATPase, respectively. Utilizing this protocol, I was able to then elucidate the solution NMR structure and backbone dynamics of SLN in detergent micelles. The dynamics show that the single transmembrane helix can be divided into four sub-domains necessary for molding onto the surface of the Ca-ATPase. These interactions with Ca-ATPase were similar to those observed for PLN confirming the hypothesis that both proteins bind in the same major groove. Furthermore, in collaboration with Dr. A. Ramamoorthy (University of Michigan) and Dr. F. Porcelli (University of Tuscia, Viterbo, Italy), I probed the topological orientation and insertion depths of a toxic amphipathic pardaxin peptide and two membrane disrupting antibacterial magainin peptides in detergent micelles by solution NMR utilizing paramagnetic quenching experiments. These findings were used in conjunction with solid-state NMR to determine the membrane topology/membrane insertion mechanisms of these peptides.

   a. *Buck B*, Zamoon J, Kirby TL, DeSilva TM, Karim C, Thomas D, Veglia G. Overexpression, purification, and characterization of recombinant Ca-ATPase regulators for high-resolution solution and solid-state NMR studies. Protein Expr Purif. 2003 Aug;30(2):253-61. PubMed PMID: 12880775.

   b. Porcelli F, *Buck B*, Lee DK, Hallock KJ, Ramamoorthy A, Veglia G. Structure and orientation of pardaxin determined by NMR experiments in model membranes. J Biol Chem. 2004 Oct 29;279(44):45815-23. PubMed PMID: 15292173; PubMed Central PMCID: PMC1513189.

   c. Buffy JJ, *Buck-Koehntop BA*, Porcelli F, Traaseth NJ, Thomas DD, Veglia G. Defining the intramembrane binding mechanism of sarcolipin to calcium ATPase using solution NMR spectroscopy. J Mol Biol. 2006 Apr 28;358(2):420-9. PubMed PMID: 16519897.

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