An article in *Time Magazine* titled "Why Your DNA Isn't Your Destiny" discusses how even though the DNA in all of our cells is nearly the same, it is epigenetic factors, including modifications to DNA, RNA and chromatin, that control the gene expression patterns in different cells, under various environmental conditions, and at specific times[1]. Bromodomains (BRDs) play a crucial role in regulating many cellular and physiological processes, and disruption of their function contributes to the etiology of multiple diseases. Specifically, the BRDs in the ATPase family AAA domain-containing protein 2 (ATAD2 and ATAD2B), the bromodomain and PHD finger (BRPF1) protein, and the *Plasmodium falciparum* bromodomain protein 1 (BDP1) are associated with the development of several cancers[2-4], acute myeloid leukemia[5], and malaria pathogenesis[6], respectively. BRDs are chromatin reader domains that recognize acetylated lysine[7], and function by linking their associated protein/enzyme complex to chromatin. We are characterizing the structure and function of these BRD-containing proteins to outline the molecular mechanisms targeting them to specific histone modifications. For example, we recently identified the histone binding partners for the ATAD2B BRD and showed it specifically selects for mono- and di-acetylated histone ligands[8]. Our structural studies showed a disulfide bridge in the ATAD2 BRD contributes to ligand recognition (PDBID: 6CPS)[9], and that a small molecule ATAD2 inhibitor has overlapping specificity with the ATAD2B BRD (PDBID: 6VEO)[8]. We also established the molecular basis of histone acetyllysine recognition by the BRPF1 BRD (PDBID: 4QYD, 4QYL)[10], and discovered that it interacts with multiple acetylated histone peptides[11]. Our recent data on the PfBDP1 BRD indicate that it preferentially recognizes multiply acetylated histones, and we are using a combination of solution NMR, isothermal titration calorimetry (ITC) and X-ray crystallographic studies to characterize its binding specificity.

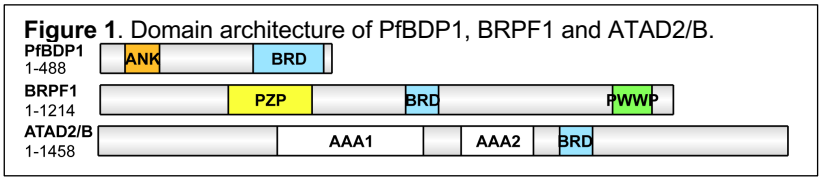

Figure 1. Domain architecture of PfBDP1, BRPF1 and ATAD2/B.

Inside the cell, multivalent interactions contribute to chromatin recognition, including a combination of histone and/or DNA interactions. However, there is limited information available on how histone modifications regulate BRD-protein binding, especially in the context of the whole nucleosome assembly. Thus, we are expanding our investigations to characterize the interactions of BRD-containing proteins with modified nucleosome core particles. As shown in **Figure 1**, BRD-containing proteins often possess multiple domains. PfBDP1 is a 56 kDa protein that contains ankyrin repeats in addition to the BRD. Interestingly, PfBDP1 forms a complex with the 125 kDa PfBDP2 protein to bind modified histones[12]. The BRPF1 protein contains three chromatin reader domains including the double PHD finger zinc knuckle region (PZP domain), which interacts with histone H3 as well as the nucleosomal DNA[13,14], a BRD that binds acetylated lysine[11,15], and a PWWP domain that recognizes histone H3K36me3[16]. Lastly, the ATAD2/B proteins contain two AAA ATPase domains in addition to the BRD. A recent study on the ATAD2 protein demonstrated that an ATPase mutant bound to di-acetylated histone marks inefficiently, indicating that the ATPase domain also plays a role in histone recognition[17]. We hypothesize that multiple domains are likely important for targeting these proteins to nucleosomes, where they function to regulate higher order chromatin structure and gene expression. Since these complexes are too large for solution NMR spectroscopy, and have proven difficult crystallize in X-ray studies, we aim to visualize these large multimeric complexes with the help of cryo-EM. We have cloned and expressed the GST-tagged BRDs of ATAD2/B, BRPF1, and the full-length PfBDP1 protein (**Figure 2**), and are characterizing their binding with specifically modified nucleosomes (210 kDa). We are assembling the protein-nucleosome complexes using 'designer' nucleosomes (dNucs) from EpiCypher, and also creating modified nucleosomes ourselves using well established protocols[18] along with the histone acetyltransferase HAT1p to add acetyllysine modifications to histone H4[19]. We aim to use Cryo-EM to solve the structures of BRD-containing proteins in complex with specifically modified nucleosomes, either using the BRD module alone, and/or with larger regions of the proteins containing multiple domains. The proposed research is expected to outline how the combinatorial activity of multiple chromatin reader domains recruits the ATAD2/B, BRPF1, and PfBDP1 proteins to acetylated histones in a chromatin relevant context.

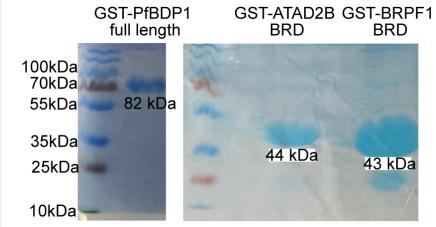

Figure 2. SDS-PAGE gel of purified of GST-tagged proteins.

University of Vermont Cryo-EM equipment:
FEI Tecnai T12 TEM
Gatan 914 high tilt liquid nitrogen cryo-transfer tomography holder
Gatan Cp3 – Cryoplunge 3 (and a homemade plunger)
Gatan dry pumping station
Edwards Auto 306 vacuum coater
Gatan Advanced Plasma System – Solarus 950
Harrick Plasma cleaner/sterilizer PDC-3XG
Various LN dewars and ancillary equipment.

Other local PI's interested in TEM/Cryo-EM:
Sylvie Doublie, Frances Carr, Dave Maughn, Keith Mintz, and David Warshaw.

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