

# Reading the message: A structural and functional comparison of the ATAD2/B bromodomain binding activity

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Bromodomains are conserved epigenetic reader domains known to recognize acetylated lysine residues on the N-terminal histone tails protruding from the nucleosome. The 61 human bromodomains are found in 41 different proteins and have been classified into eight different subfamilies based on sequence conservation. These subfamilies often share unique sequence variations that drive differences in histone ligand preference for specific patterns of post-translational modifications on core and variant histone proteins. The BET bromodomains, which are an attractive drug target in multiple disease states, have been the focus of many studies to elucidate the molecular mechanism of histone recognition. However, much less is known about non-BET bromodomain-containing proteins. In this study, we examined the ligand specificity of the ATAD2 bromodomain and compared it to its closely related paralog in ATAD2B. We show that the ATAD2/B bromodomains recognize mono- and di-acetyllysine modifications on histones H4 and H2A. A structure-function approach using X-ray crystallography, isothermal titration calorimetry experiments, and site-directed mutagenesis coupled to ligand binding assays identified key residues in the acetyllysine binding pocket that dictate the molecular recognition process. Furthermore, our results demonstrate how cross-talk between multiple modifications alters the binding activity of the ATAD2/B bromodomains. The structure of the ATAD2B bromodomain in complex with a small molecule inhibitor of the ATAD2 bromodomain revealed that critical contacts required for coordination are conserved between the ATAD2/B bromodomains, and many of these residues play a dual role in acetyllysine recognition. We further characterized an alternative splice variant of ATAD2B that results in a loss of bromodomain function. Our comparative analysis of the structural and functional features of the ATAD2 and ATAD2B bromodomains highlights features that contribute to their unique binding specificities driving histone recognition in the epigenetic landscape.