Evaluation of the Differential Binding Preferences of Histone Trimethyllysine Reader Proteins

Christopher R. Travis, Marcey L. Waters

Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Trimethyllysine (Kme3) is a post-translational modification on histones which regulates gene expression, often via binding of reader proteins. Many human Kme3 readers have been identified, often binding the same histone sequence but with different biological implications. Many readers are therapeutic targets, but selective inhibition of these conserved binding motifs remains challenging. Reader proteins universally bind Kme3 in an aromatic cage. Typically, this binding is attributed to cation-π interactions but recently exceptions have been suggested. We investigated the generality of this assumption with the goal of providing insight into novel approaches for selective inhibition. We utilized biophysical, mechanistic, and structural studies to discover that the ability of readers to bind the neutral isostere of Kme3 (tBuNle) is more widespread than previously thought: ~5% of human proteins in this class. This discovery establishes a new framework for selective inhibitor design by exploiting differences in charge-dependence among readers. We find that readers that bind both Kme3 and tBuNle do so through different mechanisms, utilizing cation-π interactions to recognize the former and solely the hydrophobic effect to bind the latter. This finding is significant, as these readers represent rare examples of proteins recognizing differently charged ligands in the same binding site via distinct mechanisms.