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Current Opinion in Chemical Biology

Chemical probes for methyl lysine reader domains

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The primary intent of a chemical probe is to establish the relationship between a molecular target, usually a protein whose function is modulated by the probe, and the biological consequences of that modulation. In order to fulfill this purpose, a chemical probe must be profiled for selectivity, mechanism of action, and cellular activity, as the cell is the minimal system in which 'biology' can be explored. This review provides a brief overview of progress towards chemical probes for methyl lysine reader domains with a focus on recent progress targeting chromodomains.

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Current Opinion in Chemical Biology 2016, **33**:135–141

This review comes from a themed issue on **Chemical genetics and epigenetics**

Edited by **Danica G Fujimori** and **Stuart Conway**

http://dx.doi.org/10.1016/j.cbpa.2016.06.004

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Introduction

Advances in understanding the regulation of chromatin accessibility via post-translational modifications (PTMs) of histones have rejuvenated drug discovery directed towards modulation of transcription as the opportunities for pharmacological intervention are significantly better than direct perturbation of transcription factors [1•,2,3]. Chemical biology is poised to play a central role in advancing scientific knowledge and assessing therapeutic opportunities in chromatin regulation. Specifically, cell penetrant, high-quality chemical probes that influence chromatin state are of great significance [4•,5•]. The advantages of a small molecule driven approach to modulating chromatin biology are numerous: temporal resolution; mechanistic flexibility (targeting a specific activity of a protein as opposed to ablating them all via DNA editing and RNA–interference techniques) [6]; ease of delivery; and most significantly, a small molecule tool has the potential to provide an immediate transition to a drug discovery effort, possibly cutting years off the time between target validation and therapeutic intervention [7,8].

While the enzymes that perform PTMs on histones are an important and precedented class of druggable targets [1•,9], the biological consequences of many PTMs result from their recruitment of regulatory machinery via protein–protein interactions (PPI) directly facilitated by the PTM [10]. The binding domains involved in PTM recognition on chromatin are referred to as 'readers'. We and others have been focused on exploration of the chemical biology of readers of methyl-lysine (Kme) as this PTM plays a central role in chromatin regulation and more than 200 Kme reader domains within several protein families occur within the human proteome, making this a large and relatively unexplored target-class for probe discovery [11–16,17••,18••,19••,20,21].

Probe validation

Characterization of selectivity and cellular target engagement are both essential and challenging aspects of probe validation [7,16]. In the case of the enzymes that regulate chromatin state, a knockdown of the target by siRNA, shRNA or gene editing directly perturbs a PTM that can be readily monitored at either a global level or at a specific gene locus [1•,9]. For Kme readers genetic manipulations tend to result in biochemical or phenotypic outcomes that are less easily attributed to specific biochemical changes at the level of chromatin. Additionally, since most Kme readers occur in the context of multi-domain and hence multi-functional proteins, there is no *a priori* basis to expect that pharmacologic antagonism of the Kme reader function will be equivalent to the removal of the whole protein in which it is embedded [6]. For this reason, initial assessments of chromatin reader antagonism have frequently relied upon the effect of the probe on the localization or mobility of a tagged version of its reader target expressed in a cell of interest. This approach has been applied to bromodomains [22,23] and Kme readers in our own work [19••]. While changes in target localization gives a readout that is both proximal to chromatin and logically attributable to the likely mechanism of action of the ligand, this phenomenology is difficult to relate to any specific biological function of the endogenous reader and does not directly establish a molecular pathway connection to phenotypic effects [15]. New technologies to assess cellular target engagement could have a significant impact on validation of Kme reader probes [24].

Selectivity assessment is perhaps the most important aspect of chemical probe characterization, and unfortunately, one that is often lacking in the literature [4•,7,25].

While single-target specificity is not an absolute requirement, sufficient profiling data to confidently attribute *in vivo* effects to the *in vitro* profile of a probe are essential. We have attempted to address this for Kme readers (in collaboration with the Bedford lab at MD Anderson) by evaluating the binding of biotinylated versions of Kme reader probes to a nitro-cellulose membrane upon which hundreds of potential chromatin-associated effector domains have been spotted [26]. Binding is then observed with a streptavidin-dye conjugate and positive results are followed up via quantitative measurements in solution by isothermal titration calorimetry (ITC) [13,18••]. In addition to assessing selectivity versus Kme reader proteins, probes must be profiled versus the enzyme families that modify lysine (PKMTs, lysine demethylases), as activity here would be likely to confound interpretation of both chromatin biochemical readouts and phenotypic outcomes. There is also a chemical logic for screening against these targets since Kme reader probes may mimic the substrates of these enzymes. Profiling versus general pharmacology panels is also performed in order to create a more complete assessment of potential off-target activities. While this data cannot rule out contributions from unexamined or unknown protein off-targets to a probe's activity, it does support the case for specificity when cellular target-engagement has also been proven [7]. Additionally, in the absence of comprehensive profiling data against all possible cellular targets, the use of a close structural analogue as an inactive control compound that lacks biochemical target activity is critical in order to establish a correlation between on-target *in vitro* activity and cellular effects [25].

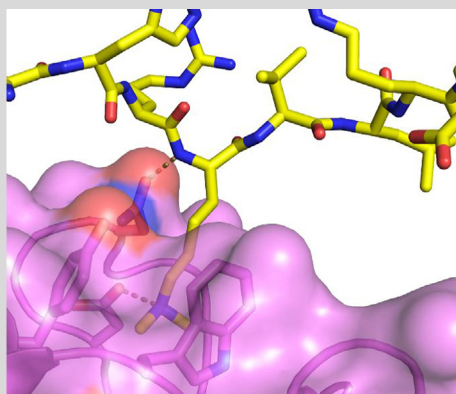
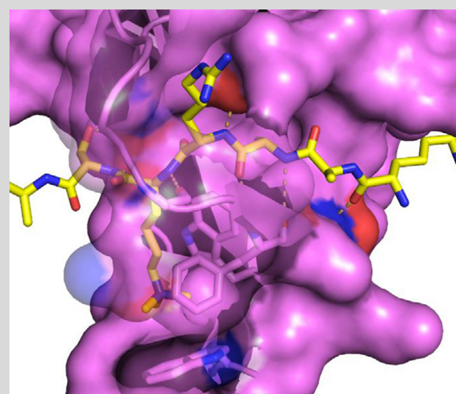
Chemical strategies

Kme binding sites are generally made up of an aromatic cage involving 3–4 aromatic amino acids, and often an acidic residue to hydrogen bond to the Kme cation in the case of monomethyllysine and dimethyllysine (Kme1,2) recognition, or simply to balance the charge in the case of Kme3 [27,28]. The Patel lab introduced a useful division of Kme readers into 'cavity insertion' versus 'surface groove' binders [29], and subsequent work towards chemical probes has been informed by this ontology and confirmed its relevance to ligand design. Table 1 illustrates chemical strategies and principles applied thus far to the discovery of Kme reader antagonists.

Our initial work focused on ligands for the MBT domains which utilize a cavity insertion recognition mode and led to the successful design of the first chemical probe for Kme readers, UNC1215 [19••]. However, the relatively

Table 1

Chemical strategies and principles applied to the discovery of antagonists for cavity insertion methyllysine binders and surface groove methyllysine binders

	Cavity insertion binders	Surface groove binders
Hit discovery & screening strategies	Screen focused small molecule or fragment libraries Employ target class cross screening Utilize structure-based design for hit optimization	Apply structure-based design Screen peptide or peptoid libraries
Design principles	Exploit cation-π and H-bonding interactions in aromatic cage Utilize conformational constrained alkyl amines	Use available Kme peptide SAR Target binding sites adjacent to aromatic cage Introduce unnatural amino acids, Kme mimics, and non-peptidic features
Major challenge	Fragment-like size may result in low affinity	Low cell permeability may decrease overall utility
Example	L3MBTL1 + H4K20me2 (pdb 2PQW) 	CBX7 + H3K27me3 (pdb 4X3K) 

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