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Scaling the Druggability Landscape of Human Bromodomains, a New Class of Drug Targets

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Gene transcriptional activation of the human genome in response to physiological and environmental stimuli requires chromatin structure changes defined by enzymes that modify chromatin and directed by proteins that interact with chromatin in a modification-sensitive manner. This highly complex system operates with a large number of chemical modifications on chromatin (both DNA and histones) and transcription-associated proteins.[1] Of these, lysine acetylation functions to facilitate chromatin opening and productive transcriptional machinery assembly required for gene activation. These activities are directed by the acetyl-lysine binding activity of the bromodomain (BrD), a fundamental molecular mechanism for gene transcriptional activation that was discovered in the structural biology study of the histone acetyltransferase (HAT) transcriptional coactivator PCAF.[2] The human genome encodes a total of 61 bromodomains in 46 chromatin regulator proteins, some of which comprise multiple bromodomains.[2] As a key epigenome reader, the bromodomain is almost solely responsible for binding to acetylated lysine in histones and transcription-associated proteins, thereby orchestrating gene transcription in chromatin in an ordered fashion.[2] Recent studies show that pharmacological small molecule modulation of the acetyl-lysine binding activity of BrD proteins such as the BET (bromodomain and extra-terminal domain) family protein BRD4 and the HAT coactivator CBP/p300 dictates gene transcription outcome in disease models[3] such as multiple myeloma, lymphoma, acute myeloid leukemia, mixed lineage leukemia, HIV-associated kidney disease, and ischemia, indicating these bromodomains as attractive drug targets for diseases including cancer and inflammation.

All bromodomains share an evolutionarily conserved structural fold consisting of a left-handed four-helix bundle with two interhelix-connecting loops, termed ZA and BC loops, which together constitute the acetyl-lysine binding pocket.[2] Given high degree variations in the amino acid sequence and structural flexibility of the ZA and BC loops, the acetyl-lysine binding pocket poses a challenge to developing potent and selective small molecule inhibitors for bromodomains. In an effort to explore the physicochemical basis for small molecule inhibition of bromodomains, Vidler et al. conducted a family-wide survey of the druggability landscape of the acetyl-lysine binding site in human bromodomains.[4]

Generally speaking, a target protein is considered druggable if it can be modulated in vivo by a druglike molecule.[5] Structure-based target druggability assessment, however, uses a less restrictive definition of druggability, namely, the ability of a protein to bind to druglike molecules with high affinity. While related, the two definitions are not identical. A high-affinity druglike ligand may not be active in vivo for a number of reasons including bioavailability. Structure-based druggability assessment predicts a degree by which a ligand-binding site in a protein is able to bind to a druglike molecule with high affinity.[5]

The recent availability of several validation sets of druggable versus undruggable targets has led to the development of a number of computational methods.[5] Most methods combine geometrical and physicochemical properties of the protein surface to define mainly the size, shape, and hydrophobicity of binding pockets, although other properties may also be included.[5] Among these SiteMap is one of the most widely used[5] and is also the one used in the Vidler study of bromodomains. SiteMap combines binding site identification with druggability assessment. First, a binding site is identified as a set of reasonably enclosed points that are outside the protein; groups of these points define "sites" that are characterized their size, degree of enclosure by the protein, hydrophilicity, hydrophobicity, and other properties. A SiteScore is defined using a subset of these properties and used for binding-site identification. A different score, Dscore, which uses the same properties as the SiteScore but with different coefficients, is then used to assess the druggability of the predicted binding sites. Hydrophobicity plays a larger role in Dscore than in SiteScore because of the fact that "undruggable" sites typically are much more hydrophilic and much less hydrophobic than "druggable" sites.

Using SiteMap, Vidler and co-workers assessed the druggability of human bromodomains using 105 crystal structure entries available in Protein Data Bank, which encompass 33 of the 61 human bromodomains. Eight residues near the acetyl-lysine binding site of the BET protein BRD4 (i.e., W81, L92, L94, N140, D144, D145, I146, and M149) were utilized as references (Figure 1A,B). In addition to the key residues that interact with the bound acetyl-lysine, five water molecules are found at the conserved sites within the ligand-binding pocket in many different bromodomains, suggesting that they are an integrated part of the acetyl-lysine binding pocket (Figure 1C). As such, these bound water molecules were included in the SiteMap analysis of protein druggability. Superimpositions of these structures led to classification of the human bromodomain family into nine groups that show distinctive structural features. The Dscore analysis further predicted that the BET bromodomains, group 1, are highly druggable, which is in an agreement with the fact that several highly potent BET bromodomain-specific small molecule inhibitors such as JQ1, MS417, and iBET have been reported in the literature.[3,6] Group 2, which consists of the

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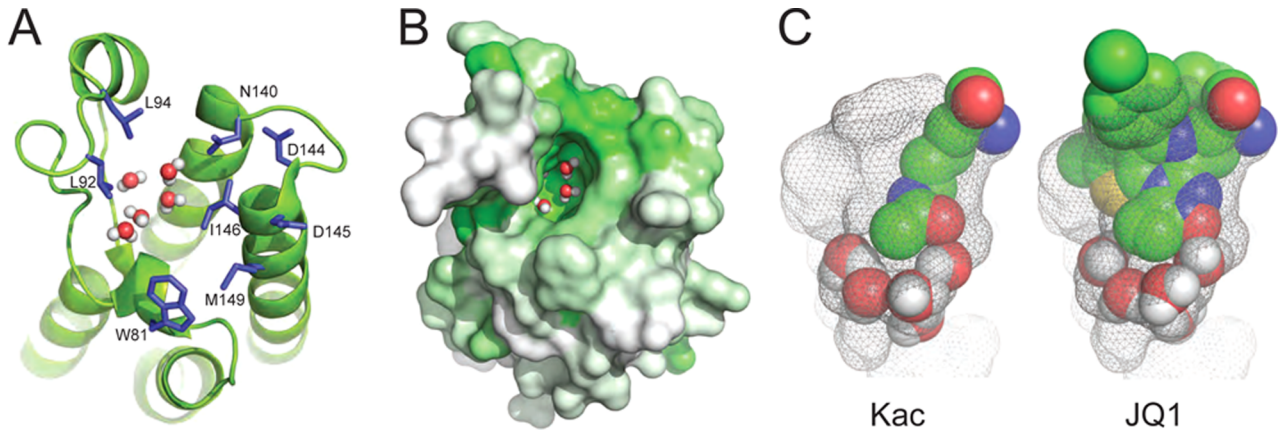

Figure 1. Structural features of the acetyl-lysine binding pocket of the bromodomain. (A) The three-dimensional structure of the first bromodomain of human BRD4 (BRD4-BD1) (PDB code 3mxf), illustrating key amino acid residues and five bound water molecules located at the acetyl-lysine binding pocket. The side chains of these residues are color-coded by atom type. (B) Surface representation of the BRD4-BD1 that is colored according to amino acid sequence conservation over the entire human bromodomain family (green is more conserved, whereas white is not conserved). (C) Acetyl-lysine (left) and a small molecule bromodomain inhibitor, JQ1 (right) shown when bound in the acetyl-lysine binding pocket (PDB codes 3uvx and 3mxf, respectively). The ligands and bound water molecules are depicted in colored spheres according to atom type (red, blue, green, yellow, and white for oxygen, nitrogen, carbon, sulfur, and hydrogen, respectively). The ligand binding site in the bromodomain protein is defined by mesh.

bromodomains from transcriptional cofactor proteins GCN5, PCAF, FALZ, and CECR2, shows a highly predicted druggability. Low micromolar affinity inhibitors for this group of bromodomains have also been reported,[6] which represent attractive drug targets for future drug design efforts. Note that although the CBP/p300 bromodomain shows marked variations in structural features compared to the other groups, it has intermediate druggability. This prediction is supported by the recent development of the CBP bromodomain inhibitors using target structure-guided methods.[3,6] The prediction further reveals that other groups of bromodomains appear less druggable than the BET family bromodomains. These groups and their structural correlations were depicted in a bromodomain phylogenetic tree.

One of the limitations of SiteMap and most druggability assessment methods is that the druggability measure corresponds to the predicted binding site, which may or may not be an accurate representation of the true binding site for a ligand. For instance, it is possible that a ligand will exploit multiple neighboring sites that are considered independent by SiteMap, which might result in greatly increased druggability.

While this study represents the first family-wide druggability analysis for bromodomains, some predictions shall be interpreted with caution. First, as reported previously, the interhelical ZA and BC loops of bromodomains are highly flexible.[2] Local conformational changes in these loops upon binding to a small-molecule ligand could result in changes in the pocket volume and enclosure, leading to a different Dscore value. Consequently, new predictions of druggability score could vary as more available bromodomain structures are included in calculations. Second, possible contribution of secondary cavities adjacent to the acetyl-lysine binding site was not fully explored in this study. Such neighboring cavities could provide new opportunities to improve affinity for small molecules. Lastly, as more druggability prediction programs such as fPocket, and MAP_{POD}[5] become capable of handling bound water molecules, it will be interesting to compare results obtained with different computational methods. This will enable one to validate and even further improve the fidelity of such predictions.

As a distinct class of protein−protein interaction (PPI) domains that function to regulate gene transcription, bromodomains contrast with the classical highly druggable drug targets such as protein kinases, GPCRs, and proteases.[7] In GPCRs the ligand-binding pockets generally are deeply buried in the protein and often nearly fully enclosed, which gives them more contacts with the protein for the same pocket volume.[7] In kinases the pockets are large and very deep (ATP-binding site),[7] thereby providing many contacts with the protein for the size of the ligand. In comparison, the acetyl-lysine binding pockets in bromodomains are not as large, deep, and enclosed as those of these highly druggable targets. As such, bromodomains probably lie between these highly druggable targets and difficult targets such as protein−protein interaction surfaces. Nevertheless, it is difficult to perform a quantitative comparison because of the fact that druggability measures are merely trying to classify targets as "druggable", "difficult", and "undruggable" rather than correctly rank targets within one category.

It is worth noting that what one considers druggable now is biased by the type of molecules that have been used as starting points for drug discovery.[7] Discovery of new types of molecules may lead to redefinition of what is druggable. For example it has been shown that one can define libraries of small molecules that have an enhanced chance of inhibiting PPIs.[7] For this reason, it is important to emphasize that eventually one needs to attempt the targeting of all medically relevant bromodomains (and targets in general), not just those that are currently considered druggable. Accordingly, the classification of druggability of bromodomains is useful because it predicts which ones could be targeted using current approaches (i.e., low hanging fruit) and which ones would require new developments before one could successfully target them (e.g., different small-molecule libraries such as the case for PPIs). They certainly should not be simply abandoned because they seem intractable in light of current druggability estimates.

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As a parallel in drug discovery that is moving from highly druggable targets such as kinases and GPCRs to more challenging PPI targets, rapidly growing ligand design efforts for bromodomains are expected to progress from targeting a few druggable bromodomains to more difficult ones. The outcome of these studies will undoubtedly enable us to validate members of this new class of drug targets and develop more effective targeted epigenetic therapies for human diseases including cancer and inflammation.

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REFERENCES

(1) Kouzarides, T. Chromatin modifications and their function. *Cell* **2007**, *128*, 693−705.

(2) (a) Dhalluin, C.; Carlson, J. E.; Zeng, L.; He, C.; Aggarwal, A. K.; Zhou, M. M. Structure and ligand of a histone acetyltransferase bromodomain. *Nature* **1999**, *399*, 491−496. (b) Filippakopoulos, P.; Picaud, S.; Mangos, M.; Keates, T.; Lambert, J. P.; Barsyte-Lovejoy, D.; Felletar, I.; Volkmer, R.; Muller, S.; Pawson, T.; Gingras, A. C.; Arrowsmith, C. H.; Knapp, S. Histone recognition and large-scale structural analysis of the human bromodomain family. *Cell* **2012**, *149*, 214−231. (c) Jacobson, R. H.; Ladurner, A. G.; King, D. S.; Tjian, R. Structure and function of a human TAFII250 double bromodomain module. *Science* **2000**, *288*, 1422−1425. (d) Owen, D. J.; Ornaghi, P.; Yang, J. C.; Lowe, N.; Evans, P. R.; Ballario, P.; Neuhaus, D.; Filetici, P.; Travers, A. A. The structural basis for the recognition of acetylated histone H4 by the bromodomain of histone acetyltransferase Gcn5p. *EMBO J.* **2000**, *19*, 6141−6149. (e) Sanchez, R.; Zhou, M. M. The role of human bromodomains in chromatin biology and gene transcription. *Curr. Opin. Drug Discovery Dev.* **2009**, *12*, 659−665. (f) Zeng, L.; Zhou, M. M. Bromodomain: an acetyl-lysine binding domain. *FEBS Lett.* **2002**, *513*, 124−128.

(3) (a) Borah, J. C.; Mujtaba, S.; Karakikes, I.; Zeng, L.; Muller, M.; Patel, J.; Moshkina, N.; Morohashi, K.; Zhang, W.; Gerona-Navarro, G.; Hajjar, R. J.; Zhou, M. M. A small molecule binding to the coactivator CREB-binding protein blocks apoptosis in cardiomyocytes. *Chem. Biol.* **2011**, *18*, 531−541. (b) Dawson, M. A.; Prinjha, R. K.; Dittmann, A.; Giotopoulos, G.; Bantscheff, M.; Chan, W. I.; Robson, S. C.; Chung, C. W.; Hopf, C.; Savitski, M. M.; Huthmacher, C.; Gudgin, E.; Lugo, D.; Beinke, S.; Chapman, T. D.; Roberts, E. J.; Soden, P. E.; Auger, K. R.; Mirguet, O.; Doehner, K.; Delwel, R.; Burnett, A. K.; Jeffrey, P.; Drewes, G.; Lee, K.; Huntly, B. J.; Kouzarides, T. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. *Nature* **2011**, *478*, 529−533. (c) Delmore, J. E.; Issa, G. C.; Lemieux, M. E.; Rahl, P. B.; Shi, J.; Jacobs, H. M.; Kastritis, E.; Gilpatrick, T.; Paranal, R. M.; Qi, J.; Chesi, M.; Schinzel, A. C.; McKeown, M. R.; Heffernan, T. P.; Vakoc, C. R.; Bergsagel, P. L.; Ghobrial, I. M.; Richardson, P. G.; Young, R. A.; Hahn, W. C.; Anderson, K. C.; Kung, A. L.; Bradner, J. E.; Mitsiades, C. S. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell* **2011**, *146*, 904−917. (d) Mertz, J. A.; Conery, A. R.; Bryant, B. M.; Sandy, P.; Balasubramanian, S.; Mele, D. A.; Bergeron, L.; Sims, R. J. Targeting MYC dependence in cancer by inhibiting BET bromodomains. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 16669−16674. (e) Zhang, G.; Liu, R.; Zhong, Y.; Plotnikov, A. N.; Zhang, W.; Zeng, L.; Rusinova, E.; Gerona-Nevarro, G.; Moshkina, N.; Joshua, J.; Chuang, P. Y.; Ohlmeyer, M.; He, J. C.; Zhou, M. M. Down-regulation of NF-kappaB transcriptional activity in HIV-associated kidney disease by BRD4 inhibition. *J. Biol. Chem.* **2012**, *287*, 28840−28851. (f) Zuber, J.; Shi, J.; Wang, E.; Rappaport, A. R.; Herrmann, H.; Sison, E. A.; Magoon, D.; Qi, J.; Blatt, K.; Wunderlich, M.; Taylor, M. J.; Johns, C.; Chicas, A.; Mulloy, J. C.; Kogan, S. C.; Brown, P.; Valent, P.; Bradner, J. E.; Lowe, S. W.; Vakoc, C. R. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. *Nature* **2011**, *478*, 524−528.

(4) Vidler, L. R.; Brown, N.; Knapp, S.; Hoelder, S. Druggability analysis and structural classification of bromodomain acetyl-lysine binding sites. *J. Med. Chem.* **2012**, DOI: 10.1021/jm300346w.

(5) (a) Cheng, A. C.; Coleman, R. G.; Smyth, K. T.; Cao, Q.; Soulard, P.; Caffrey, D. R.; Salzberg, A. C.; Huang, E. S. Structure-based maximal affinity model predicts small-molecule druggability. *Nat. Biotechnol.* **2007**, *25*, 71−75. (b) Hajduk, P. J.; Huth, J. R.; Tse, C. Predicting protein druggability. *Drug Discovery Today* **2005**, *10*, 1675−1682. (c) Halgren, T. A. Identifying and characterizing binding sites and assessing druggability. *J. Chem. Inf. Model.* **2009**, *49*, 377−389. (d) Krasowski, A.; Muthas, D.; Sarkar, A.; Schmitt, S.; Brenk, R. DrugPred: a structure-based approach to predict protein druggability developed using an extensive nonredundant data set. *J. Chem. Inf. Model.* **2011**, *51*, 2829−2842. (e) Le Guilloux, V.; Schmidtke, P.; Tuffery, P. Fpocket: an open source platform for ligand pocket detection. *BMC Bioinf.* **2009**, *10*, 168. (f) Nisius, B.; Sha, F.; Gohlke, H. Structure-based computational analysis of protein binding sites for function and druggability prediction. *J. Biotechnol.* **2012**, *159*, 123−134. (g) Schmidtke, P.; Barril, X. Understanding and predicting druggability. A high-throughput method for detection of drug binding sites. *J. Med. Chem.* **2010**, *53*, 5858−5867. (h) Seco, J.; Luque, F. J.; Barril, X. Binding site detection and druggability index from first principles. *J. Med. Chem.* **2009**, *52*, 2363−2371. (i) Sheridan, R. P.; Maiorov, V. N.; Holloway, M. K.; Cornell, W. D.; Gao, Y. D. Drug-like density: a method of quantifying the "bindability" of a protein target based on a very large set of pockets and drug-like ligands from the Protein Data Bank. *J. Chem. Inf. Model.* **2010**, *50*, 2029−2040. (j) Sugaya, N.; Ikeda, K. Assessing the druggability of protein−protein interactions by a supervised machine-learning method. *BMC Bioinf.* **2009**, *10*, 263. (k) Volkamer, A.; Kuhn, D.; Grombacher, T.; Rippmann, F.; Rarey, M. Combining global and local measures for structure-based druggability predictions. *J. Chem. Inf. Model.* **2012**, *52*, 360−372.

(6) (a) Dekker, F. J.; Ghizzoni, M.; van der Meer, N.; Wisastra, R.; Haisma, H. J. Inhibition of the PCAF histone acetyl transferase and cell proliferation by isothiazolones. *Bioorg. Med. Chem.* **2009**, *17*, 460−466. (b) Filippakopoulos, P.; Qi, J.; Picaud, S.; Shen, Y.; Smith, W. B.; Fedorov, O.; Morse, E. M.; Keates, T.; Hickman, T. T.; Felletar, I.; Philpott, M.; Munro, S.; McKeown, M. R.; Wang, Y.; Christie, A. L.; West, N.; Cameron, M. J.; Schwartz, B.; Heightman, T. D.; La Thangue, N.; French, C. A.; Wiest, O.; Kung, A. L.; Knapp, S.; Bradner, J. E. Selective inhibition of BET bromodomains. *Nature* **2010**, *468*, 1067−1073. (c) Hewings, D. S.; Wang, M.; Philpott, M.; Fedorov, O.; Uttarkar, S.; Filippakopoulos, P.; Picaud, S.; Vuppusetty, C.; Marsden, B.; Knapp, S.; Conway, S. J.; Heightman, T. D. 3,5-Dimethylisoxazoles act as acetyl-lysine-mimetic bromodomain ligands. *J. Med. Chem.* **2011**, *54*, 6761−6770. (d) Nicodeme, E.; Jeffrey, K. L.; Schaefer, U.; Beinke, S.; Dewell, S.; Chung, C. W.; Chandwani, R.; Marazzi, I.; Wilson, P.; Coste, H.; White, J.; Kirilovsky, J.; Rice, C. M.; Lora, J. M.; Prinjha, R. K.; Lee, K.; Tarakhovsky, A. Suppression of inflammation by a synthetic histone mimic. *Nature* **2010**, *468*, 1119−1123. (e) Sachchidanand; Resnick-Silverman, L.; Yan, S.; Mutjaba, S.; Liu, W. J.; Zeng, L.; Manfredi, J. J.; Zhou, M. M. Target structure-based discovery of small molecules that block human p53 and CREB binding protein association. *Chem Biol* **2006**, *13*, 81−90. (f) Zeng, L.; Li, J.; Muller, M.; Yan, S.; Mujtaba, S.; Pan, C.; Wang, Z.; Zhou, M. M. Selective small molecules blocking HIV-1 Tat and coactivator PCAF association. *J. Am. Chem. Soc.* **2005**, *127*, 2376−2377.

(7) (a) Billingsley, M. L. Druggable targets and targeted drugs: enhancing the development of new therapeutics. *Pharmacology* **2008**, *82*, 239−244. (b) Hopkins, A. L.; Groom, C. R. The druggable genome. *Nat. Rev. Drug Discovery* **2002**, *1*, 727−730. (c) Mason, J. S.; Bortolato, A.; Congreve, M.; Marshall, F. H. New insights from

structural biology into the druggability of G protein-coupled receptors. *Trends Pharmacol. Sci.* **2012**, *33*, 249−260. (d) Metz, A.; Ciglia, E.; Gohlke, H. Modulating protein−protein interactions: from structural determinants of binding to druggability prediction to application. *Curr. Pharm. Des.* [Online early access]. Published Online: May 29, **2012**. (e) Reynes, C.; Host, H.; Camproux, A. C.; Laconde, G.; Leroux, F.; Mazars, A.; Deprez, B.; Fahraeus, R.; Villoutreix, B. O.; Sperandio, O. Designing focused chemical libraries enriched in protein−protein interaction inhibitors using machine-learning methods. *PLoS Comput. Biol.* **2010**, 6. (f) Russ, A. P.; Lampel, S. The druggable genome: an update. *Drug Discovery Today* **2005**, *10*, 1607−1610. (g) Villoutreix, B. O.; Labbé, C. M.; Lagorce, D.; Laconde, G.; Sperandio, O. A leap into the chemical space of protein−protein interaction inhibitors. *Curr. Pharm. Des.* [Online early access]. Published Online: May 29, **2012**