TARGETING BINDING SITE SPECIFICITIES OF BROMODOMAIN BRWD1(2)

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Bromodomains (BRDs) are emerging epigenetic targets in various types of cancer [1]. They specifically recognize ε-N-acetylated lysine residues (K_{ac}) on the unstructured histone tails. The human bromodomain family comprises 61 BRDs, distributed across a wide range of functionally diverse proteins. BRDs cluster into eight structural classes, all of which share the conserved bromodomain fold (Fig. 1) with a largely hydrophobic binding pocket [2], making it difficult to achieve selectivity when looking for potential binders, particularly within a structural class. In this work we compared proteins from the human bromodomain family (Fig. 2 & 3) in order to identify specificities of binding sites within the different classes. Here we present the virtual screening results for the bromodomain BRWD1(2).

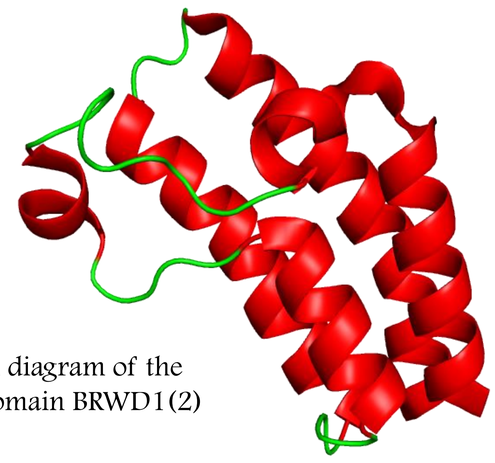


Figure 1. Ribbon diagram of the human bromodomain BRWD1(2) (PDB: 3Q2E).

COMPARISON OF BRWD1(2) WITH OTHER BROMODOMAINS

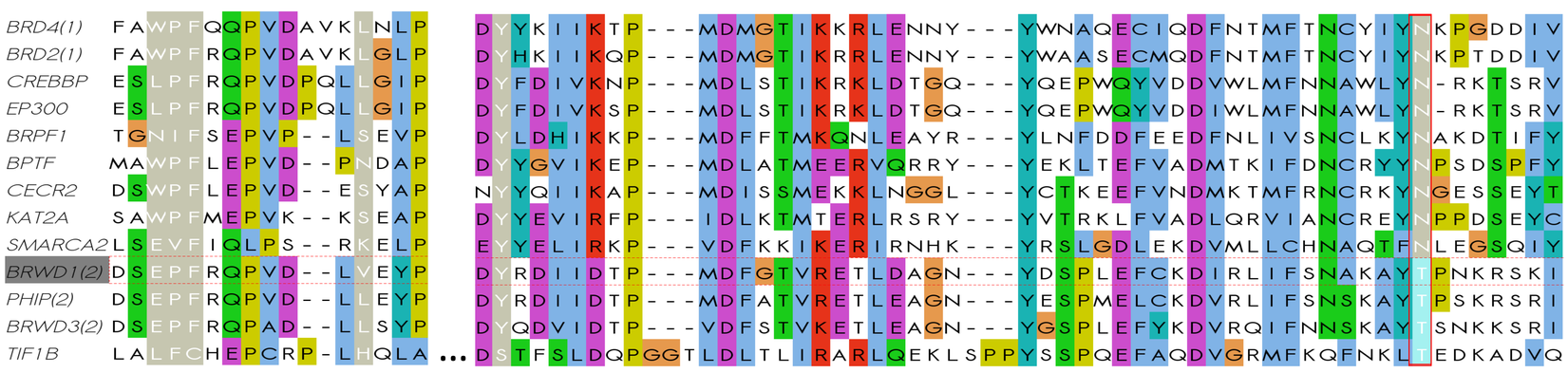


Figure 2. Multiple sequence alignment of selected BRDs from the human bromodomain family. Most BRDs feature a conserved asparagine residue in their binding site, which acts as a hydrogen-bond donor to the acetylated lysine. Four BRDs, from the human bromodomain family of 61, contain a threonine residue in the same position. This residue along with other features of these atypical binding sites are good starting points for designing selective inhibitors. The ligand interacting residues in the binding site of the well-known bromodomain BRD4(1) as well as their co-aligned residues from other BRDs are highlighted in gray. The threonine residues are shown in cyan inside a red vertical box. The sequence of target BRWD1(2) is highlighted with a horizontal dotted box.

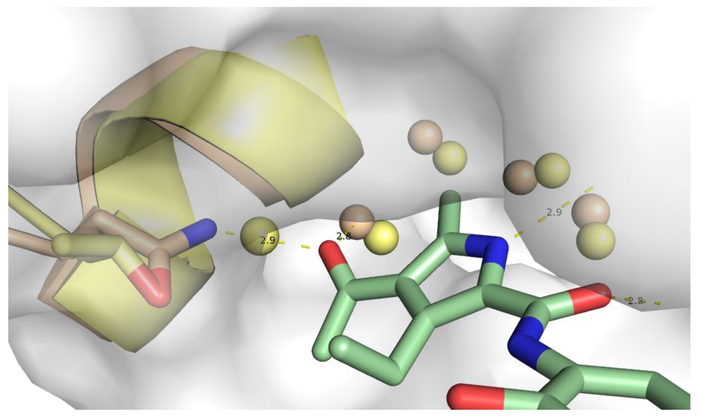


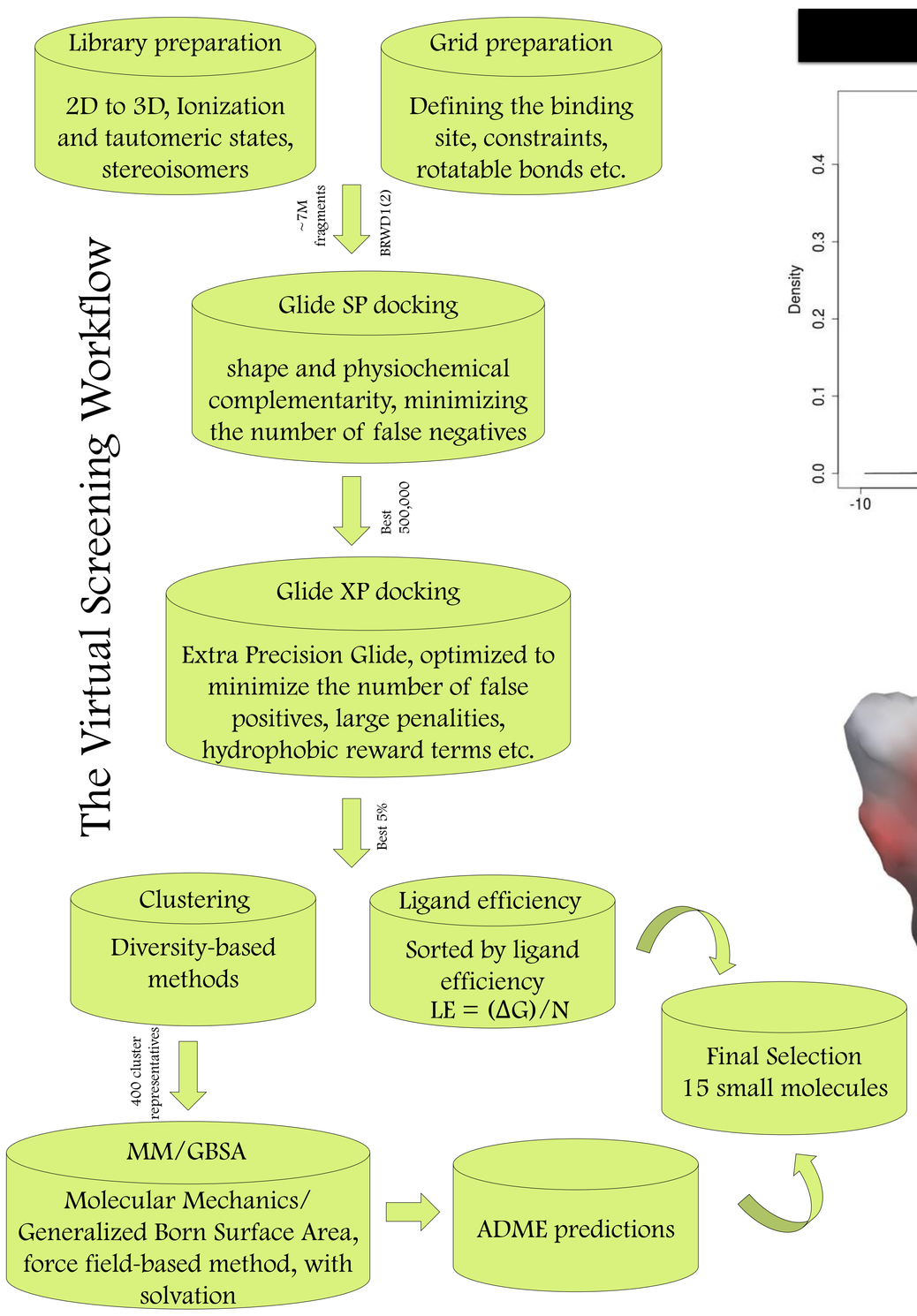
Figure 3a. Superposition of BRD4(1) (PDB:4LYW, shown in wheat) onto BRWD1(2) (PDB: 3Q2E, shown in pale green). The conserved asparagine residue in BRD4(1) which acts as a hydrogen-bond donor to the acetylated lysine is substituted by a threonine in BRWD1(2). Note the presence of conserved water molecules in the binding sites.

METHODS

Target: BRWD1(2)

Library: The fragment library used for screening was taken from the Purchasable Chemical Space [3] in which fragments were filtered according to the Rule of Three. The library contained a total of ~1.4M fragments with molecular weight up to 300 Daltons.

The virtual screening workflow used for selection of potential binders is shown below. Information from the comparison shown in figures 2 and 3 was incorporated into the workflow. Small molecules with many stereoisomers were discarded from the final selection. All calculations were performed using the Schrödinger Suite 2014-2 [4].



RESULTS

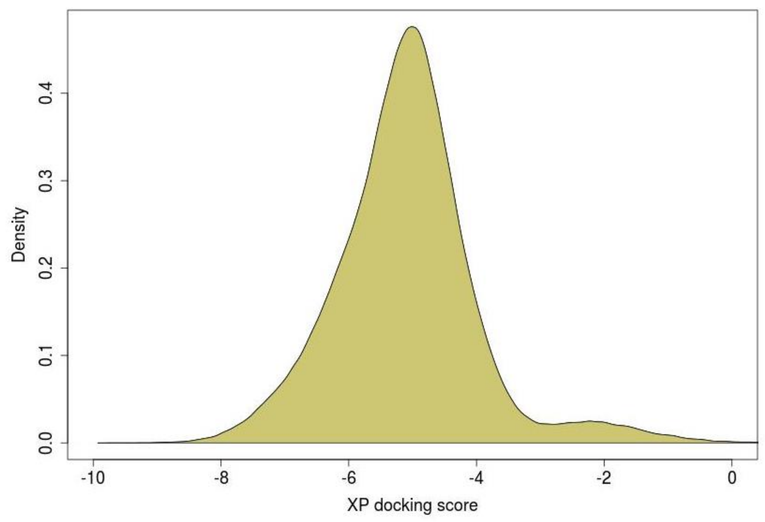


Figure 4. XP docking score distribution.

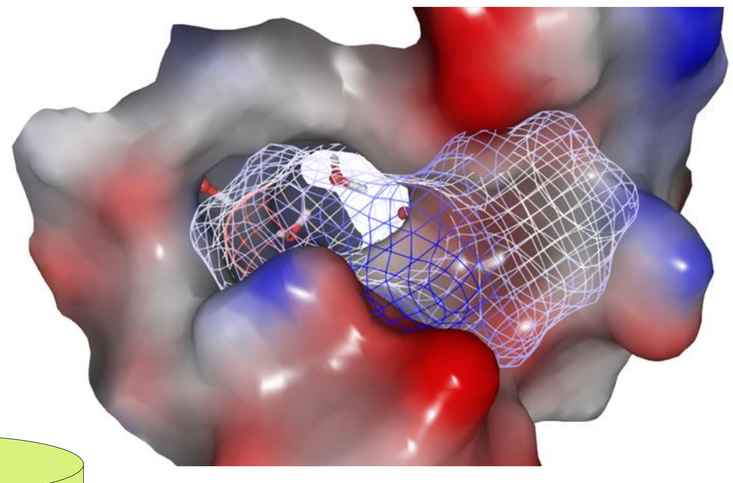


Figure 5. Mesh representation of one of the selected small molecules inside the binding pocket of BRWD1(2). The receptor surface is colored by electrostatic potential.

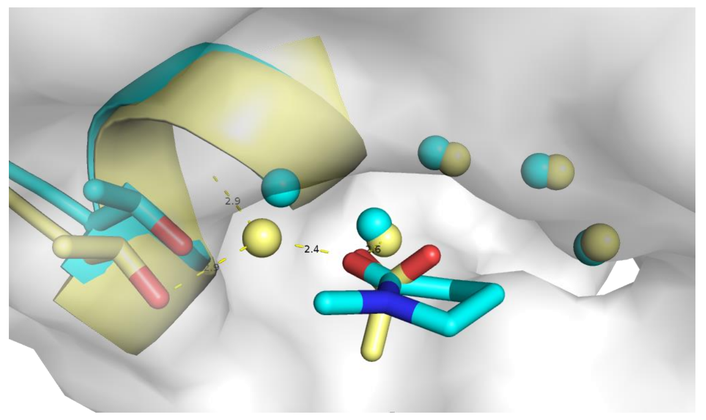


Figure 3b. Superposition of BRWD1(2) (PDB: 3Q2E, shown in pale green) onto PHIP (PDB: 3MB3, shown in cyan). Both feature a threonine residue in their binding sites, in front of which a water molecule was observed. The binding mode of co-crystallized small molecules (an acetate ion in BRWD1(2) and 1-methylpyrrolidin-2-one in PHIP) mimics that of the acetylated lysine by using the water molecule as a bridge. Note that this water is not present in the asparagine-containing structures (See Fig. 2a).

FUTURE PROSPECTS

Potential ligands are being verified experimentally. High affinity binders will be obtained by performing iterative steps of modeling including fragment growing and linking, lead optimization, and experimental validation.

COLLABORATIONS

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Institute of Organic Chemistry, Albert-Ludwigs University of Freiburg (organic synthesis): Dr. Dmytro Ostrovskyi, Prof. Dr. Bernhard Breit

Institute of Pharmaceutical Sciences, Albert-Ludwigs University of Freiburg (cellular assays): Prof. Dr. Manfred Jung, Karin Schmidtkunz

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