Addressing binding site specificities of bromodomains using an *in silico* drug discovery pipeline

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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. Bromodomains cluster into eight structural classes, all of which share the conserved bromodomain fold 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 in order to identify specificities of the binding sites within the different classes. Here we present the virtual screening results for the human bromodomain BRWD1 (Figure 1).

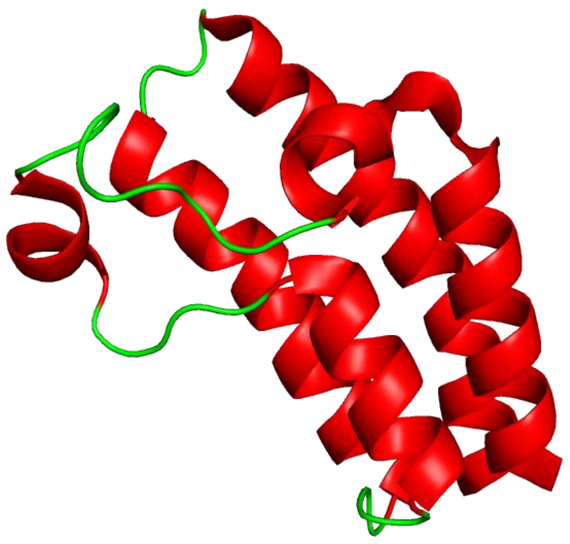


Figure 1. Ribbon diagram of the human bromodomain BRWD1 (PDB: 3Q2E)

Comparison of BRWD1 with the well-known bromodomain drug target BRD4(1)

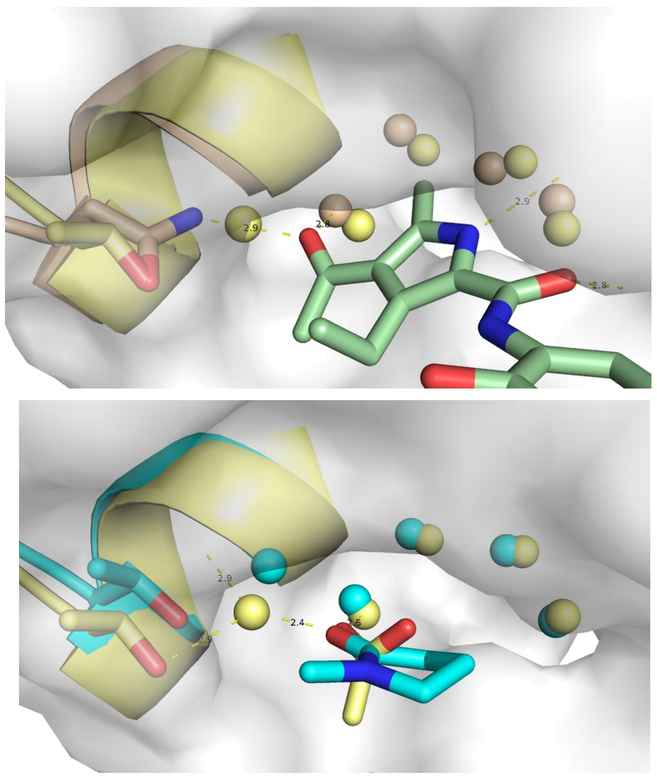


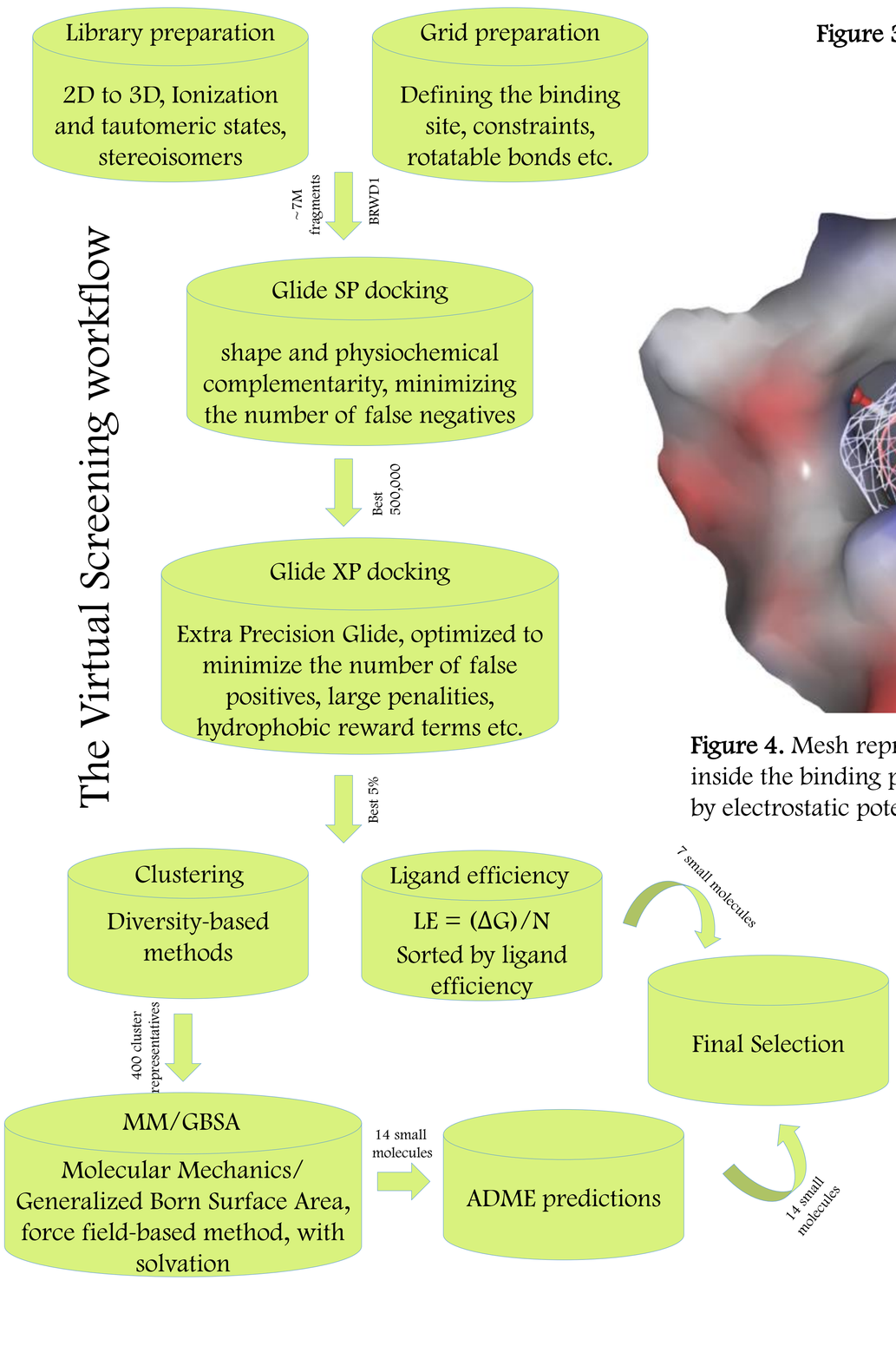
Figure 2. Top: An acetylated lysine peptide (shown in pale green) in the binding pocket of bromodomain BRD4(1) (PDB: 4LYW). Like most bromodomains, the binding pocket of BRD4(1) features a conserved asparagine residue (shown in wheat) which acts as a hydrogen-bond donor to the acetylated lysine. In BRWD1 however, this asparagine is substituted by a threonine (shown in yellow). Note the presence of conserved waters in both structures. Bottom: Superposition of bromodomains BRWD1 (PDB: 3Q2E) and PHIP (PDB: 3MB3). Both feature a threonine residue in their binding pockets instead of the well-known, conserved asparagine. A comparison of these two structures reveals the presence of an additional water molecule located in front of the threonine. The binding mode of co-crystallized small molecules (an acetate ion in BRWD1 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 (as shown in the figure above).

Methods

Target: BRWD1

Library: Commercially available compounds were collected and filtered using an automated workflow designed within the ChemicalToolBoX [3]. Fragments were selected using the Rule of Three [4]. The resulting 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 figure 2 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 [5].



Results

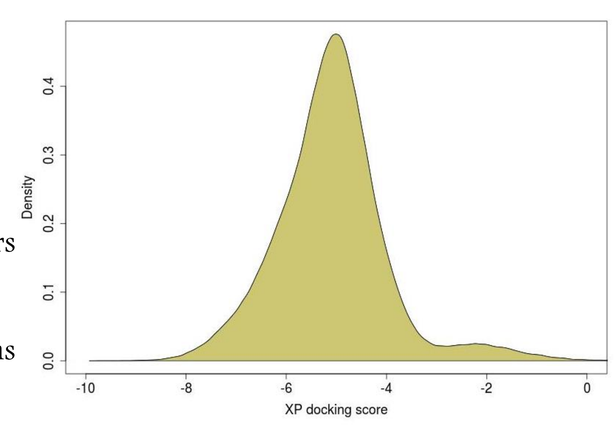


Figure 3. XP docking score distribution

Table 1. The table shows relevant ADME predictions for a select few candidate molecules along with docking scores and MM/GBSA $\Delta G_{binding}$. The range of these properties for 95% of the drugs is also shown for comparison.

Ligand	Mol. Wt. (130 – 725)	MM/GBSA ΔG binding	Docking score	LogPo/w (-2 - 6.5)	LogS (-6.5 - -0.5)	LogHERG (concern below -5)	Caco permeability (<25 poor >500 great)	Blood-brain parition coefficient (-3-1.2)	MDCK permeability (<25 poor >500 great)	Human Oral Absorption (1: low, 2: medium, 3: high)	CNS activity (-2 to +2)
L1	289.3	-75.8	-7.1	1.782	-2.875	-4.676	519.604	0.319	269.715	3	1
L2	196.2	-75.6	-7.2	0.651	-0.373	-2.521	172.775	-0.007	152.883	2	1
L3	282.3	-72.4	-7.0	1.790	-2.259	-5.637	217.905	-0.186	105.432	3	1
L4	260.3	-70.7	-7.0	1.631	-2.597	-4.785	301.700	0.047	149.869	3	1
L5	274.3	-70.7	-7.0	1.834	-2.770	-4.709	312.330	0.067	155.585	3	1
L6	246.3	-69.5	-7.5	1.352	-1.993	-4.763	224.253	-0.180	108.757	3	1
L7	294.3	-68.0	-7.2	-1.314	1.334	-2.361	31.475	0.408	38.913	2	1
L8	294.3	-65.9	-7.2	2.870	-3.319	-5.534	363.796	-0.010	183.472	3	1
L9	286.3	-64.2	-6.9	2.599	-3.379	-5.872	305.025	-0.004	276.153	3	1
L10	266.3	-62.1	-7.1	2.333	-2.676	-5.785	269.807	-0.200	132.821	3	1
L11	196.2	-58.2	-7.3	0.043	-0.592	-3.841	204.430	-0.093	98.404	2	1
L12	238.2	-57.7	-7.5	1.204	-1.785	-4.578	252.612	-0.006	223.288	3	1
L13	234.2	-57.7	-7.5	1.261	-1.955	-4.663	252.524	-0.143	123.649	3	1
L14	275.3	-53.3	-6.9	0.766	-1.838	-4.709	265.484	0.003	130.522	3	1

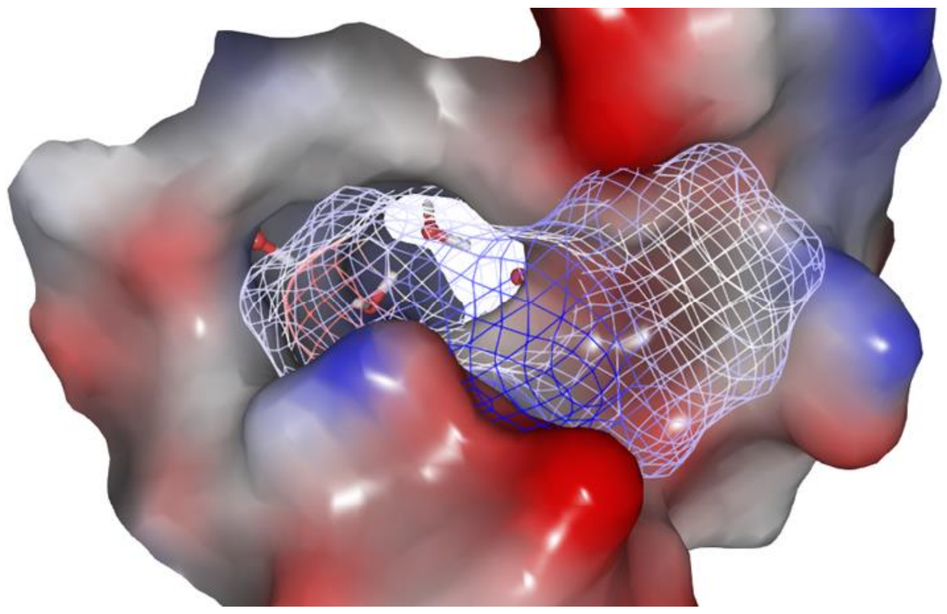


Figure 4. Mesh representation of one of the selected small molecules inside the binding pocket of BRWD1. The receptor surface is colored by electrostatic potential.

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

Institute of Biochemistry, Albert-Ludwigs University of Freiburg (experimental validation, X-ray crystallography): Martin Hügle, Dr. Daniel Wohlwend, Prof. Dr. Oliver Einsle

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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JUNIOR RESEARCH GROUP PROGRAMME

C-factors RTG1976

Black Forest Grid

DFG Deutsche Forschungsgemeinschaft

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