A crystallographic-based fragment screen against human BRD4 bromodomain 1

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Introduction

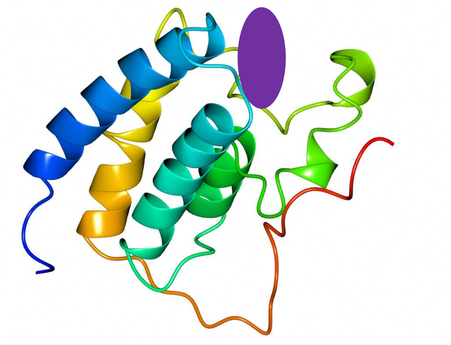
Fragment-based drug discovery has now become firmly established as an important method in the lead discovery process, and is an integral component of drug discovery at BioFocus. Using a selection of compounds from our fragment collection, we have performed a crystallographic-based screen against human BRD4 bromodomain 1. Given that DMSO is a well known competitor of compound binding in this family of proteins, we initially solved the crystal structure of this protein soaked in the presence of DMSO. Confirming that DMSO binds to the acetyllysine binding site, we commenced the fragment screen using ethanol as the compound solvent, allowing solvent to evaporate before solutions for protein crystal soaks were prepared. Fragments were batched into groups of three based on shape diversity and chemical compatibility, and datasets from soaked crystals that spanned the entire fragment library were collected using in-house X-ray diffraction equipment. A summary of the results are presented, and demonstrate how our structural biology capabilities complement the range of fragment screening services we offer.

Fragment screening of bromodomains

Bromodomains are emerging as potential epigenetic targets of high therapeutic value; however, their high degree of sequence similarity means that target selectivity poses a significant problem. In an effort to develop novel leads, fragment screening has recently been reported by GlaxoSmithKline on human BRD2 bromodomain 1 [1], where a focused set of 1,376 fragment-like compounds containing an acetyllysine mimetic were assembled and then screened using a fluorescence anisotropy assay. A total of 132 compound hits were identified by GlaxoSmithKline, with crystallography used to analyse 40 structures. In contrast to this, BioFocus has completed a fragment library screen against human BRD4 bromodomain 1. Due to the high sequence and structure similarity shared between these two proteins, and in an attempt to discover novel chemotypes, BioFocus has used crystallography to screen human BRD4 bromodomain 1 against an entire non-focused fragment library. These results, in conjunction with our "EpiRoadmap" approach to target analysis, aims to produce lead-like compounds with high selectivity.

Solution of the apo structure

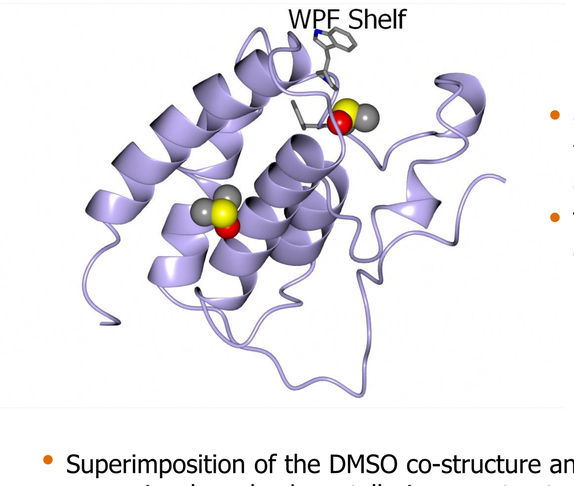
Prior to commencing protein crystal soaks, we assessed whether the acetyllysine binding site of the crystal form we had isolated was potentially accessible to fragments through crystal soaking. This was achieved by solution of the apo structure, which was refined to 1.72 Å resolution. The crystal structure and packing are much like previously solved structures of human BRD4 bromodomain 1, where the acetyllysine binding site is exposed to solvent, but is partially occluded by the side chain of Lys99 from an adjacent symmetry equivalent protein molecule.



- Ribbon diagram outlining the overall fold of human BRD4 bromodomain 1
- The diagram is colour-ramped from the N-terminus (red) to the C-terminus (blue)
- The acetyllysine binding site is highlighted by the purple oval shape

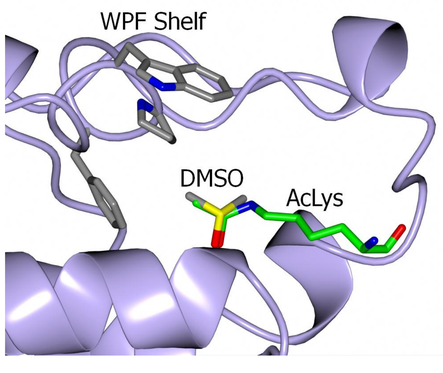
Results of the control soak

DMSO is often the preferred solvent for compounds used in protein crystal soaks as it can enhance compound solubility in the soak solution, and is usually well tolerated by protein crystals; however, DMSO is also a well known inhibitor of bromodomains. To confirm that DMSO could potentially interfere with fragment binding, we performed a control soak in the presence of 5% v/v DMSO. The structure was solved to 1.22 Å resolution, and confirmed that DMSO binds to the acetyllysine binding site, as well as to a second site on the surface of the protein molecule. Consequently, all subsequent compound soaks were performed with 100% v/v ethanol as the solvent, with sufficient time allowed during plate preparation so that ethanol could evaporate before formation of the soak solution.



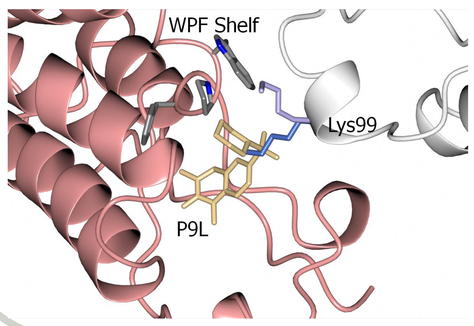
- Overall BRD4 bromodomain 1 fold with the two bound DMSO molecules highlighted as spheres
- The WPF shelf, with side chains highlighted as cylinders, is included for reference

- Superimposition of the DMSO co-structure and a previously solved acetyllysine co-structure (PDB ID: 3JVK [2])
- One of the DMSO molecules (grey carbon and yellow sulfur atoms) from the DMSO co-structure aligns with the acetyl moiety of the acetyllysine-bound structure (green carbon atoms)



Results of the reference compound soak

Before commencing the fragment screen, we assessed whether the side chain of Lys99 could interfere with small molecule binding. To do this, all publicly available structures of compound-bound BRD4 bromodomain 1 were superimposed on to our apo structure, and the compound that gave one of the largest number of clashes with Lys99 of our apo structure was used as the reference compound; 1,3-dimethyl-6-morpholinosulfonyl-4H-quinazolin-2-one (P9L). A protein crystal soaking route was developed using ethanol as the compound solvent, and a co-structure was solved to 1.22 Å resolution. The refined co-structure showed that the side chain of Lys99 adopts a new conformer, suggesting that this residue is unlikely to impede fragment binding.



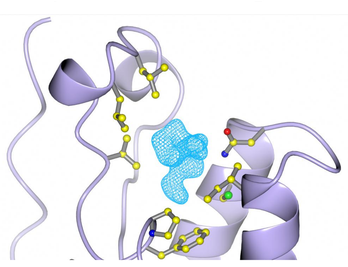
- Superimposition of the apo and P9L co-structures; the two structures are isomorphous so, for simplicity, ribbons corresponding only to the P9L co-structure are included (pink for the asymmetric unit and white for the nearest symmetry equivalent)
- The side chain of Lys99 in the apo structure (blue cylinders) adopts a new conformer (purple cylinders) when P9L, highlighted in yellow, is soaked in to the crystal lattice

Fragment pooling for soaking

Compounds were initially batched into groups of three based on shape diversity so as to aid interpretation of subsequent electron density maps derived from X-ray diffraction of soaked crystals. This was performed using our proprietary shape fingerprint and a maximum dissimilarity algorithm [3]. The results were examined and modified to account for chemical compatibility, such that acids were not mixed with bases, and electrophiles kept separate from nucleophiles. Depending on solubility, compounds were dissolved or suspended to 50 mM in 100% v/v ethanol, with stocks stored at -20 °C. For crystal soaking, compounds were stamped onto SWISSCI MRC Under Oil Crystallization plates, with pooling performed during plate preparation.

Results of the fragment library screen

- Nearly 23% of all soaked crystals produced electron density maps that allowed unambiguous fitting of fragments
- A further 14% of soaked crystals produced electron density maps that showed strong fragment-like density in the acetyllysine binding site, but too ambiguous to allow fitting
- Only 11% of crystals were damaged during soaking to the extent that data could not be collected



- Refined electron density map encompassing one of the successfully bound fragments
- Residues that are in close contact with the compound, and that also line the acetyllysine binding site, are highlighted in balls and sticks

Materials and methods

Expression and purification

DNA corresponding to residues 44 to 168 of human BRD4 was sub-cloned into an expression vector that introduced a cleavable N-terminal 6xHis-tag. Expression and purification followed methods published by the Structural Genomics Consortium [4].

Crystallization, crystal soaking, and data collection

Protein was crystallized using the hanging drop technique, with plates incubated at 20 °C. Crystals took approximately three days to reach their maximum dimensions of ~50 x 80 x 150 µm. Fragment cocktails were dissolved/suspended in the same precipitant solution used to grow BRD4 bromodomain 1 protein crystals so that each compound in the cocktail was at a final concentration of 50 mM. Crystals were added to the soaking solutions and incubated overnight at 20 °C, after which they were mounted in cryo-loops, plunged in liquid nitrogen, and diffraction data collected in-house on a Rigaku MicroMax-007 X-ray generator with either a Riguaku Saturn 944 CCD detector, or a Rigaku R-AXIS IV++ image plate.

Structure determination

Crystal structures were solved using the molecular replacement technique as implemented in PHASER [5], with PDB ID: 2OSS [4] as the search model. Structures were automatically rebuilt using BUCCANNEER [5] and refined using REFMAC5 [5]. Model adjustments and ligand building using maps calculated in REFMAC5 were performed in COOT [6], with subsequent rounds of alternating reciprocal space refinement and manual adjustments until convergence was achieved.

Conclusions

- Human BRD4 bromodomain 1 has been successfully screened against a non-focused, chemotype-diverse fragment library
- The diversity of chemotypes found in the bound fragments has allowed us to begin developing novel lead-like compounds targeting human BRD4 bromodomain 1
- These results complement a range of fragment screening services we offer [7]

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