A crystallographic-based fragment screen against human BRD4 bromodomain

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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 data 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, where a focused set of 1,376 fragment-like compounds containing an acetyl lysine 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 analyze 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 specificity.

Solution of the apo structure

Prior to selecting 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.

Results of the fragment 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-diaminopropanol-4-4quinozalinone-2-one (PQL). A protein crystal soaking route was developed using ethanol as the compound solvent, and a co-structure was solved at 2.2 Å resolution. The refined co-structure showed that the side chain of Lys99 adopts a new conformation, suggesting that this residue is unlikely to impede fragment binding.

Fragment pooling for soaking

Fragments were batched into groups of three based on shape diversity so as to allow 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 1. 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 spotted onto SWISSCI MRC Under Oil Crystalization 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

Materials and methods

Expression and purification
DNA corresponding to residues 144 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 2.

Crystalization, 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 ~5 x 8 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 μM. Crystals were added to the soaking solution 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 Rigaku Saturn 944 CCD detector, or a Rigaku RAXIS IV++ image plate.

Structure determination
Crystal structures were solved using replacement techniques as implemented in PHASER 3, with PDB ID: 2J0S 4 as the search model. Structures were automatically rebuilt using UCSF Chimera 5 and refined using REFMAC 6. Model adjustments and ligand building using maps calculated in REFMAC were performed in COOT 7, 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, compound-like lead fragment library
• The diversity of chemotypes found in the fragment bounds 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 8

References