ICR The Institute of Cancer Research

PhD Project Proposal

Funder details

Studentship funded by:	ICR

Project details

Project title:	Exploring the differences in the structural characterisation of inhibitor binding between cryo- and room-temperature using multicrystal X-ray crystallography

Supervisory team

Primary Supervisor:	Rob van Montfort
Associate Supervisor(s):	James Sandy Michael Hough Yann-Vai le Bihan Benjamin Bellenie
Secondary Supervisor:	Swen Hoelder

Divisional affiliation

Primary Division:	Cancer Therapeutics
Primary Team:	Hit Discovery and Structural Design
Site:	Chelsea

Project background

The ability to routinely determine experimental protein-ligand structures is essential in fragment-based and structure-based drug discovery campaigns. Currently, most fragment- and inhibitor-bound protein structures are determined using X-ray crystallography. However, protein targets of therapeutic interest are increasingly challenging to work with. They are often difficult to express and purify, and yields are typically low.1 Moreover, while it is often possible to obtain small and/or intergrown crystals, it remains challenging to routinely grow single protein crystals of a sufficient size and quantity to enable a multi-year FBLD/SBDD campaign. In addition, crystallographic data is generally collected at cryogenic temperatures of ~100K. However, for some crystals systems it is extremely difficult to find suitable conditions for cryo-cooling, either because they are very fragile and difficult to harvest, or because they are not tolerant to cryoprotection. Furthermore, one of the questions that has remained largely unanswered is if compounds bind to a protein target in the same conformation and in the same binding sites at cryogenic compared to room temperature and whether radiation damage to ligands alters binding.

The Diamond Light Source (DLS) has developed a high flux microfocus macromolecular crystallography beamline for automatic screening and data collection from protein crystals in situ.1 This Versatile Macromolecular X-ray crystallography in situ (VMXi) beamline addresses some of the current needs in protein crystallography. Firstly, to identify diffraction quality crystals of biological systems that are difficult to crystallise, while also guiding crystallisation optimisation. Secondly, to obtain complete multicrystal diffraction data sets for crystals which difficult to harvest and cryo-protect. Thirdly, to characterise protein-ligand interactions at room temperature by rapidly collecting a large number of datasets from crystals of a particular therapeutic target soaked with different compounds.

Project aims

- Solve BCL6 and HSP70 fragment/inhibitor structures from merged data collected from multiple crystals at room temperature.
- Compare quality of compound binding data collected at cryogenic temperatures versus that of VMXi data collected at room temperature.
- Compare compound binding mode from protein X-ray data collected at cryogenic temperatures with VMXi data collected at room temperature.
- Investigate X-ray induced radiation damage to bound ligands in VMXi room temperature data and compare with radiation damage to ligands in cryogenic data.
- Optimise data collection strategies for VMXi data to minimise radiation damage to bound ligands/inhibitors.

Research proposal

The Hit Discovery and Structural design team of Dr Rob van Montfort at the Cancer Therapeutics Unit (CTU) at the Institute of Cancer Research is highly experienced in structure-based drug discovery and in crystallographic fragment screening using the XChem facility at DLS. One of the targets under study in his team is the transcriptional repressor B-cell lymphoma 6 protein (BCL6). BCL6 plays a key role in the formation and maintenance of B-cell germinal centres, but in Diffuse Large B-cell Lymphoma (DLBCL) the aberrant persistence of BCL6 expression is critical in lymphomagenesis.2-3 This makes BCL6 an attractive cancer drug target. To repress transcription of its target genes, BCL6 interacts with various protein complexes and recruits corepressor proteins such as BCOR, NCOR, or SMRT to its N-terminal BTB domain dimer in a mutually exclusive manner.4 Disruption of the SMRT or BCOR corepressor interaction with the BCL6 BTB domain is sufficient to inhibit DLBCL cell growth.4 At CTU we have discovered potent and selective small-molecule inhibitors and degraders that disrupt these interactions as part of our aim to identify new treatments for BCL6-driven lymphomas.5-6 We based our inhibitor design on a broad hit identification campaign which included virtual screening, biochemical HTS and a variety of fragment screening approaches, including an XChem crystallographic fragment screen against the BCL6 BTB-domain. The XChem screen yielded ~20 hits in several different binding sites. Overall, we have determined many high-resolution protein-ligand structures to guide the design of BCL6 inhibitors (Figure 1).

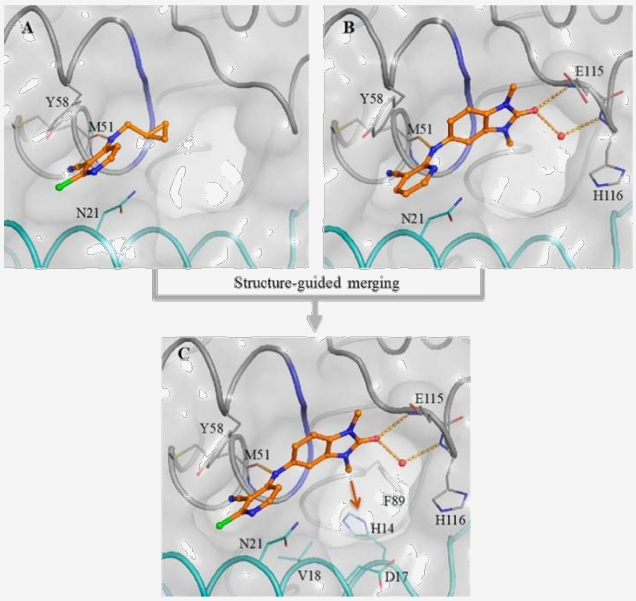

Figure 1. Example of obtained BCL6 crystal structures: Structure-guided merging of 5 (A, PDB code 6TOG) and 4 (B, PDB code 6TOF) yielded the more potent 6 (CCT365386) (C, PDB code 6TOH. The surface of the BCL6 dimer is shown as a grey transparent surface. The two individual monomers are highlighted in grey and cyan ribbons except for residues 53–55 which are indicated in blue. Key protein residues are shown in line representation. The compounds are shown as orange ball and sticks, a selected water molecule is shown as a red sphere, and H-bonds

are shown as yellow dashed lines. In panel C the orange arrow indicates an exit vector towards a large accessible pocket close to our compounds.

A second protein target of interest at CTU is Heat Shock Protein 70 (HSP70). HSP70s are an abundant family of ATP-dependent molecular chaperones, involved in many cellular processes including protein folding, prevention of protein aggregation, and protein transport between cellular compartments.7 Because of their central role in cellular homeostasis, they have been implicated in several diseases, including cancer, Alzheimer's and Parkinson's disease. The human HSP70 family has at least 17 members. The two main cytosolic HSP70s are the ubiquitously expressed HSC70 (HSPA8), and the stress-induced HSP72 (HSPA1A). HSP72 is overexpressed in many tumours including breast, skin and oesophageal cancers, and haematological malignancies with overexpression often correlating with metastasis and poor outcome in cancer patients. We have shown that dual siRNA silencing of HSC70 and HSP72 induced extensive apoptosis in human colon and ovarian cancer cell lines, but not in non-tumorigenic cell lines, demonstrating a therapeutic differential7. As part of our attempts to discover potent HSP70 inhibitors we carried out a comprehensive hit identification campaign including biochemical HTS, and biophysical fragment screening followed by structural characterisation compound binding to HSC70 in complex with its nucleotide exchange factor BAG1 and in HSP72. 8-9

For both BCL6 and HSP70 all high-resolution crystallographic data had been collected from single crystals cooled at cryogenic temperatures, which provides an extensive resource that can be used as a basis to explore the usability of multicrystal room temperature X-ray crystallography in structure-based drug discovery and to compare ligand- and inhibitor binding from data collected at cryogenic temperatures and room temperature. The available data for BCL6 and HSP70 will be complemented by data collected at the VMXi beamline at the Diamond light source.

VMXi is a dedicated room temperature X-Ray diffraction beamline with an intense Micro Focus beam. Up to 60 degrees rotation datasets are collected from crystals within crystallisation plates with highly automated data processing and merging of data from multiple crystals into high quality, complete datasets at high resolution. VMXi has developed approaches with the XChem team to soak fragments or larger ligands into in situ plates for data collection. The on-site Research Complex at Harwell provides local crystallisation expertise and facilities to feed into VMXi or alternatively users may bring their own plates for data collection.

To enable this the project will involve recombinant protein production of the BCL6 BTB domain and of HSP72 and the HSC70/BAG1 complex, subsequent biochemical and biophysical characterisation, crystallisation, crystallographic data collection, processing, refinement and analysis.

A specific project aim is to investigate if we can routinely obtain protein-ligand structures of a quality sufficient for structure-based drug discovery from merged data obtained at room temperature from multiple small crystals. We will explore if it is possible to identify weakly binding fragments and larger ligands from merged data collected at room temperature as reliably as is possible from single crystal data at cryogenic temperatures. We will also investigate differences in inhibitor and protein conformations between data collected at room and cryogenic temperatures identify fragment binding to the same sites at cryogenic compared to room temperature. Finally, the project will build on research from the van Montfort lab on radiation damage to halogenated inhibitors in protein-ligand crystal structures obtained from data collected at cryogenic temperatures. Our studies revealed X-ray induced cleavage of halogen-carbon bonds in halogenated inhibitors and consequent loss of anomalous signal on brominated ligands. 10 Radiation damage to ligands in room temperature crystals is not well characterised but has clear implications for structure-based drug design. We will expand our studies to protein-inhibitors structures from VMXi data collected at room temperature with the aim to improve data collection strategies to minimise radiation damage to bound protein-inhibitor structures used for structure-based drug discovery.

Literature references

[1] Sanchez Weatherby J. et al. (2019). VMXi: a fully automated, fully remote, high flux in situ macromolecular crystallography beamline. J. Synchrotron Rad. 26, 291-301.

[2] Huang, C. X. & Melnick, A. (2015). Mechanisms of action of BCL6 during germinal center B cell development. Sci China Life Sci 58, 1226-1232.

[3] Cardenas, M. G. et al. (2017). The Expanding Role of the BCL6 Oncoprotein as a Cancer Therapeutic Target. Clin Cancer Res 23, 885-893.

[4] Ghetu, A. F. et al. (2008) Structure of a BCOR corepressor peptide in complex with the BCL6 BTB domain dimer. Mol Cell 29, 384-391.

[5] Bellenie B.R. et al. (2020). Achieving in vivo target depletion through the discovery and optimisation of benzimidazolone BCL6 degraders. J. Med. Chem. 63, 4047-4068.

[6] Davis O.A. et al. (2022). Optimising shape complementarity enables the discovery of potent tricyclic BCL6 inhibitors. J. Med. Chem. 65, 8169-8190.

[7] Powers M.V. et al. (2010). Targeting HSP70: The second potentially druggable heat shock protein and molecular chaperone? Cell Cycle 9, 1541-1550.

[8] Jones A.M., et al. (2016). A Fragment-based approach applied to a highly flexible target: Insights and challenges towards the inhibition of HSP70 isoforms. Sci. Rep. 6, 34701.
[9] Cheeseman MD, et al. (2016). Exploiting Protein Conformational Change to Optimize Adenosine-Derived Inhibitors of HSP70. J Med Chem. 59, 4625-4636.
[10] Rodrigues et al. Specific radiation damage to inhibitors and ligands in protein-ligand crystal structures. In preparation.

Candidate profile

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1).

Pre-requisite qualifications of applicants: BSc or MSc in Life Sciences

Intended learning outcomes:

- Knowledge in cloning and protein biochemistry
- Expert in advanced X-ray crystallography methodologies
- Knowledge in synchrotron radiation
- Knowledge of the application of structural biology in structure-function relationships, chemical biology, and drug discovery
- Ability to present results in a coherent and analytical fashion both in person and in writing

Advertising details

Project suitable for a student with a background in:

- ☒ Biological Sciences
- ☒ Physics or Engineering
- ☐ Chemistry
- ☐ Maths, Statistics or Epidemiology
- ☐ Computer Science