XChem Proposal

Section 1: Science Case *

Introduction with details of the target protein, in particular evidence of its relevance to human health and as a target for chemical inhibition. How has the target been validated? Are there existing inhibitors (and if yes, why we need more)?

Molecular details of the target, its function at molecular level, known ligands and activities.

Description of the target structure. Are the structures with natural ligands (substrates, co-factors or similar) or are the binding sites available for soaking? What is the site that is being targeted and why (ie what is the mechanism of inhibition)? What are the affinities of natural ligands you are hoping to compete with? Is there data already on the druggability? Computational or experimental? Have the crystals been used successfully already for soaking (see also below the section on details of the actual crystal form)?

What is the long term outcome of this project? Why is XChem needed at this stage? If this is a re-application, how was feedback from previous application taken into account?

Describe the data you have already and what expertise the team has to carry out the whole project, not just the XChem part.

References (up to 4-5):

Section 2: Technical Evaluation

Crystals

- *Crystal Resolution:* * 4 - better than 2.2 A
- *Crystal reproducibility:* * 4 - ready for experiment
- *Readiness of Crystals* *

We have determined the crystal structure of MyProtein at 2.0 several years ago (PDB code: 1ABC) and have recently revisitied this and reproduced the crystals. We have tested the effect of DMSO on crystals and know that they can tolerate up to 20% DMSO in soaking, and up to 5% in crystallization. We have (what we interpret as PEG) bound to the one of the target sites (type I receptor site), but we would be surprised if these affect fragment binding. As a homodimer, there are always two sites for binding of ligands. The most interesting pocket is unoccupied and free for ligand binding.

Assay strategy

- *Self-score the assay status:* * 4 - Robust assay in place, can be performed quickly and routinely
- *Assay Details – elaborate on the assay, or outline the strategy for developing one* *

We have number of assays for these proteins available in the lab and experience in different biophysical analysis, depending on what kind of fragments we find. We have access to NMR facility in the Dept and can evaluate all the ligands by ligand-based NMR methods as a primary validation assay. We can use competition assays to evaluate the ability of the fragments to disrupt the protein-protein interaction receptor. Other standard biophysical techniques such as ITC are routinely used in the lab. We have also very reproducible cellular luciferase assay for evaluation of bioactivity of inhibitors in cell culture quantitatively, and we have used these for determining IC50 values for known inhibitors of the pathway.

Chemistry strategy

- *Self-score your medchem capability:* * 2 - no personal medchem experience, but have access to synthesis (e.g. collaborators)
- *Medchem strategy – elaborate; include access to in vivo assays* *

We collaborate with experienced chemists, in the group of Prof Phenol at the our neighbouring Department of Chemistry. We have worked together for a number of years and this project is part of the wider collaboration to develop inhibitors against clinically relevant protein-protein interfaces. We have decicated resource to carry out follow-up synthesis and structure-guided drug discovery once first fragments have been identified.

Project Status

- *Project focus – describe how project fits within the lab's overall research goals* *

This proposal fits two main themes in my lab. We have been working on fragment-based drug discovery for several years and as part of a multidisciplinary team developed inhibitors against challenging protein-protein interaction sites. Other half of my lab has been studying the target protein and we are actively pursuing ways to inhibit these proteins using antibodies, peptides and small molecules. We have significant experience in different biophysical and cellular assays for these proteins and several collaborators who can assist in downstream work with more biological assays.

- *Designated experimenter(s) – describe crystallographic or other relevant lab experience* *

The work will be done mainly by PhD student John Crystal whose PhD project this is. He has reproduced the crystals , tested their DMSO tolerance and just solved first of this own structures. He will be supervised and guided by an experienced postdoctoral fellow Dr Paul Structure, who has determined hundreds of co-crystal structures in his own structure-guided and fragment-based drug discovery projects.

iNEXT ARIA proposal number (PID): Click here to enter text. *(for European users; see notes)*