REPORT

Proposal Title: Structural biology of multiprotein systems in signalling and metabolism

Final No: MX-1727

Main Proposer: BLUNDELL Tom L.

1. Structure-guided discovery of new anti-tuberculosis agents

Dr. Vitor Mendes and Prof. Tom L. Blundell

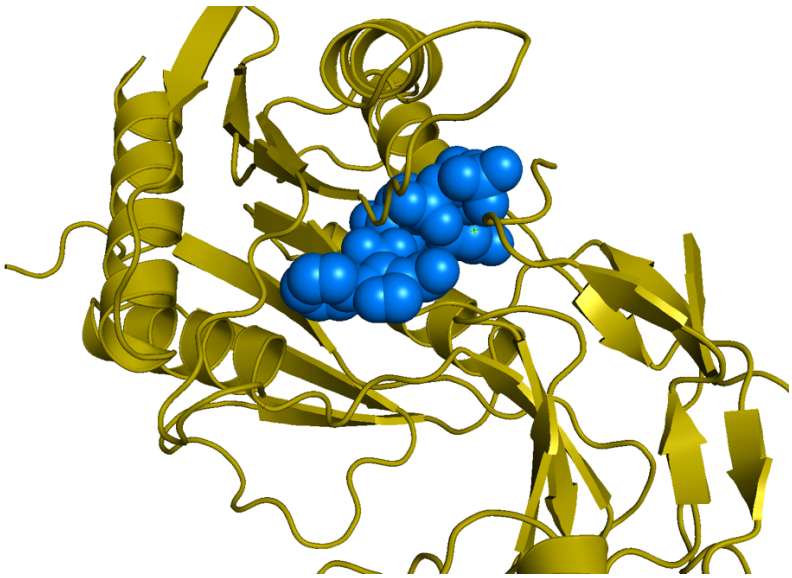
The development of new anti-tuberculosis agents is essential, with emphasis on previously unexploited, newly discovered drug targets. The overall goal of our drug discovery effort is to discover new anti-bacterial agents for newly identified drug targets of tuberculosis as well as to obtain new crystal structure of tuberculosis proteins in order to be able to apply fragment-based approaches on those new targets as a part of an academic drug discovery program supported by the European Union and the Bill and Melinda Gates Foundation. These crystal structures form the basis of further chemical elaboration of fragments leading to very high affinity lead compounds, which can be turned into new therapeutic agents.

At ESRF facility we collect X-ray diffraction data of our tuberculosis drug target crystals, with or without new small molecules. We have focused in enzymes involved in Coenzyme A biosynthesis.

We have tested 24 crystals and collected 12 datasets. Out of these datasets 8 were processable yielding 2 novel protein structures (*i.e.* either a new structure or a known structure bound to a novel chemical agent). All of these structures will eventually be reported in public domain and submitted to PDB.

Pathway/ drug target	**Number of crystals tested**	**Number of datasets collected**	**Resolution of datasets**	**Number of datasets processed**	**Number of novel structures obtained**
Coenzyme A biosynthesis	20	10	1.8–4.0 Å	6	2
Trehalose biosynthesis	4	2	1.7-2.5 Å	2	0
TOTAL	**24**	**12**		**8**	**2**

Crystal structure of an enzyme involved in coenzyme A biosynthesis in complex with a novel ligand at 2.0 Å resolution



Comments on beamline performance: In last year, our data collection was done remotely. The beam lines used was extremely stable. Along with fast data collection, data processing and reliable data hosting systems, very friendly, helping beam line scientists and support staff have made all of our experiments more productive.

Justification of the beamline time requests: Our approach of discovering new medicines for tuberculosis is guided by high-resolution crystal structures of new drug targets. Structures of a few of these drug targets are reported in literature while the quest for obtaining crystal structures of some of the challenging targets is still ongoing. In order to continue making progress towards discovering new therapeutics we must obtain crystal structures of new drug targets or/and in complex with novel ligands. Our approach is heavily dependent on the structural information and consequently there is a need for regular access to a reliable synchrotron X-ray source that would facilitate collection of high-resolution data of several protein-fragment complexes. ESRF facilities for X-ray data collection are extremely efficient for data collection and has excellent infrastructure for data hosting and processing. For all practical purpose of data collection for drug discovery purpose, ESRF facility is an ideal location.

2. Structural studies of human pro-mature myostatin

Dr. Thomas Cotton and Dr Marko Hyvonen

Myostatin is a member of the transforming growth factor-β (TGF-β) family of cell signalling proteins and plays critical regulatory roles in muscle development. Like other TGF-β family members, myostatin is biosynthesised as a precursor form, with a large N-terminal pro-

domain which is cleaved prior to secretion, but remains associated with the mature growth factor domain as a non-covalent complex. It is becoming increasingly clear that the pro-domains of TGF-β family proteins play critical roles in regulating aspects of signalling, including localisation of growth factors to the ECM and modulation of signalling range and activity. As it stands, there is a distinct lack of structural information pertaining to TGF-β family pro-domains, or the precursor forms as a whole.

The ID30A-3 beamline at ESRF was used to screen diffraction of 50 optimised native and seleno-methionine derivative pro-myostatin crystals, produced under a range of conditions, with and without small molecule additives. Despite extensive screening and crystal optimisation, diffraction was poor overall.

Ten full datasets (Six SeMet and four native) were collected, however these were auto-processed to a resolution of only 4 Å at best. This resolution limit is likely due to the inherent conformational flexibility of the protein. SAD datasets obtained from ID30A-3 were used to attempt to solve the structure at low resolution, without success. We are now attempting to improve diffraction by modifying the protein construct and identifying appropriate binding partners for co-crystallisation. Analysis of the data collected at ESRF has provided the information needed to guide the next phase of this challenging crystallographic project.

3. Structural studies of human Aurora A kinase

Dr. Gerhard Fischer and Dr Marko Hyvonen

Background:

Human Aurora A kinase is involved in cell cycle control with several active site inhibitors currently in clinical trials as anticancer drugs. Due to high similarities of the ATP-sites in kinases and the resulting low specificity and side effects of active site binders, we target Aurora A's protein-protein interaction with TPX2, which recruits Aurora A to the spindle during mitosis.

We have developed several crystallography models of Aurora A for structure and fragment based drug design. We are determining the structures of newly synthesised small molecule compounds bound to these constructs in order to develop chemical tools against Aurora A and ultimately an anticancer drug.

Results/Experiments:

We collected 42 datasets for 16 novel compounds in two crystal forms, typically to 2.0-2.5Å resolution. The new structures determined using these data gave us new insights into the compounds with respect to their binding modes and interactions with the target, as well as information on the impact of crystal packing effects onto the binding site. The information learned from these structures is being used to guide the development of a new set of improved inhibitors as part of the iterative process of developing specific, high affinity inhibitors against the target.

4. Structural studies of CK2alpha kinase

Dr. Paul Brear and Dr Marko Hyvonen

Background:

We are developing novel and selective inhibitors of protein-protein interaction between CK2alpha and it regulatory protein CK2Beta, and inhibitors of the kinase activity of CK2alpha using a novel allosteric site. Both these approaches are using fragment based drug discovery methods. This requires tens, if not hundreds of structures to be solved of fragments and synthetic inhibitors in complex of the target protein. The inhibitors have potential to be developed into novel anti-cancer agents.

Results:

10 crystals of the kinase CK2alpha soaked with various different ligands which were elaborated from a fragment hit and from a Virtual screening hit were screened. This resulted in 5 novel co-crystal structures which have allowed the design of more potent inhibitors.

Total number of PDB submissions: 5CVF