# The fragment based screening against the pH domain of Burton’s tyrosine kinase

Investigators: Paul Brear, Beata Blaszczyk, Marko Hyvonen

The aim of this project is to develop small molecule inhibitors that bind to a regulatory domain on Btk, a key regulator of B-cell development and driver of many B-cell malignancies, autoimmune diseases and inflammation. We will approach this from a non-conventional direction and instead of developing inhibitors against the active site of the kinase, we will target an essential regulatory interaction that the membrane binding PH domain has with phosphatidylinositol lipids. We believe that targeting the PH domain will result in increased specificity against Btk given that PH domains are much less conserved than kinase domains. Many of the key residues in the Btk PH domain are found mutated in X-linked agammaglobulinemia (XLA), disrupting the function of Btk suggesting that it will be difficult for cancer cells to develop resistance to PH domain inhibitors without at the same time compromising the integrity and function of the domain and of Btk as a whole. We already know that some of our early hits are dependent on the key residue (Arg28) in the inositol phosphate binding site and by ensuring this dependency is retained in the later molecules, we will minimize the likelihood of resistance.

A series of fragments were identified as binding to Btk pH domain. ID23-1 was used to attempt to produce crystal structures of these fragments bound to Btk pH domain. The current crystal forms were not suitable to produce these cocrystal complexes as the inositol phosphate binding site on the pH domain was blocked. Therefore attempts were made via the deletion of flexible loops and various crystallisation strategies, to develop suitable crystal forms for fragment screening. A crystal structure of the WT protein was eventually obtained with an unliganded binding site by seeding using a construct with a mutated binding site. This gave an accessible binding site without inositol phosphate bound. The crystal form was further optimised to give a robust crystal form that could be used for the drug discovery project. We have now successfully used this crystal form to confirm the binding mode of several of our fragments, identified with from a thermal shift screen, to the WT crystal form

Data sets of wild type Btk pH domain: 35
Structures:8 New ligand structures:0

## Parasitic Rad51 inhibitor discovery

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Rad51 is a recombinase protein involved in repairing double strand breaks by homologous recombination and its homologues are found in all domains of life. Rad51 is a putative oncology target that could be used as a co-treatment with chemo- and radiotherapies to sensitise tumors to these damaging agents.

A collaborative programme between Hyvönen lab and the groups of Prof Abell and Venkitaraman developed small-molecule inhibitors of human Rad51 by using a combination of fragment-based, biophysical, structural and biochemical approaches. The project for the development of human Rad51 inhibitors used fragment-based drug discovery (FBDD) methods to identify small-molecule hits to act as starting points for the structure-guided drug design process. Because fragment hits tend to bind with much lower affinity than standard small-molecule hits, fragment screening requires a non-oligomerising Rad51 protein with an open FxxA site that is not hindered by self-association. Furthermore, successful structural investigation of small-molecule binding to Rad51 requires a stable, rigid

protein that yields well-diffracting crystals. To meet these requirements, truncated Rad51 mutants lacking the NTD and the oligomerisation epitope were designed. However, such mutants of human Rad51 were not stable in solution and could not be utilised.

Instead, a highly stable archaeal RadA ortholog was used as a surrogate platform to engineer humanised mutants that would mimic the human Rad51 surface. Several fragment libraries were screened to identify initial fragment hits. A mutant with optimal crystallisability and HsRad51-likeness was also used in a crystallographic screen with a commercially available 352 fragment library.

This project aims to find potent and selective inhibitors of parasitic Rad51 using the *Pf*RadA surrogate platform previously successfully applied to the human ortholog. Rad51 has been shown to be important for the pathology and survival of several protozoan parasitic species important in human health. Here, we plan to “parasitise” the archaeal homolog and perform crystallographic fragment screening on it to identify novel chemical starting points for structure-based design of inhibitors. In addition, we are employing biophysical methods to re-screen existing human inhibitors to find binders that have specificity for the parasitised mutants. All chemical hits from biophysical screens are also soaked into parasitised RadA crystals and their complex structures solved by x-ray diffraction.

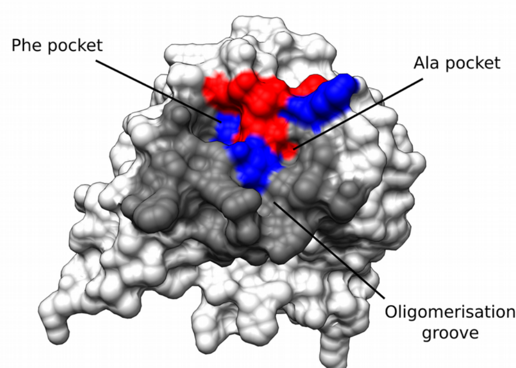


**Figure 6**. Mutations introduced in the wild-type RadA in the parasitisation approach. Red residues are identical between human and parasite, blue are unique to the parasitic ortholog.

## Beamline usage

ID23-1 was used to screen 23 crystals soaked with fragments however no new structures were derived from this data.