The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

PROJECT DETAILS	
Project Title:	Developing inhibitors of tankyrase protein-protein interactions

SUPERVISORY TEAM	
Primary Supervisor(s):	Professor Ian Collins
Other supervisory team members:	Dr Sebastian Guettler (joint supervisor) Dr Swen Hoelder

DIVISIONAL AFFILIATION	
Primary Division:	Cancer Therapeutics
Primary Team:	Medicinal Chemistry Team 2 (Collins)
Other Division (if applicable):	Structural Biology
Other Team (if applicable)):	Structural Biology of Cell Signalling (Guettler)

PROJECT DETAILS

BACKGROUND TO THE PROJECT

The human Tankyrase proteins (TNKS & TNKS2) are members of the poly(ADP-ribose) polymerase (PARP) family that catalyse the addition of poly(ADP-ribose) (PAR) chains onto substrate proteins [1]. The PAR chains can either, directly regulate substrate function, recruit PAR-binding effector proteins, or, as a specific example of the latter, mark PARylated proteins for degradation. Tankyrase PARylates many proteins implicated in the development and maintenance of cancer [1]. For example, the activity of the tumour suppressor and Wnt/beta-catenin signalling pathway component AXIN (AXIN1/AXIN2) is controlled by Tankyrase-dependent PARylation [2], as is the regulator of telomerase function TRF1 [3] and angiomotin family proteins in the tumour-suppressive Hippo pathway [4]. Pharmacological tools for the inhibition of Tankyrase have so far been restricted to inhibitors of the catalytic PARP domain. Recently, the Guettler group have shown that the scaffolding effects of Tankyrase are critical, and that PARP domain inhibitors achieve only partial modulation of TNKS/TNKS2 function [5]. A means to address the scaffolding role of TNKS would be to inhibit the binding of Tankyrase to its substrate proteins, which interact through a well-defined peptide binding groove, whose mutation abolishes Tankyrase function in WNT signalling [2, 5, 6]. The feasibility of this approach has been demonstrated with a stapled peptide derived from the consensus Tankyrase-binding motif (TBM) [7], but potent, cell-active small molecule chemical tools are required to further investigate the consequences of inhibiting Tankyrase-substrate interactions and to provide potential starting points for drug discovery. To address this, we applied fragment-based screening to find small molecules that interact with the substrate binding groove in the Tankyrase ankyrin repeat clusters (ARCs). Using NMR and thermal shift assays with Tankyrase ARC constructs [8], we successfully identified low-molecular-weight fragment hits that bind Tankyrase ARCs, and confirmed their location in the substrate binding region. The aim of this project is to develop these chemical starting points into potent, cell-permeable small molecule inhibitors of Tankyrase substrate binding.

PROJECT AIMS

- **Determine the binding modes of validated fragment hits to TNKS/TNKS2 ARCs using NMR and X-ray crystallography**
- **Synthesize analogues of the fragment hits to optimise fragment binding and explore binding modes**
- **Use iterative structure-based design, chemical synthesis and biological assays to discover molecules with sub-micromolar affinity for Tankyrase ARCs that compete for binding with known peptide TBMs**
- **Optimise the physicochemical properties of potent compounds to give cell-permeable tool molecules**

RESEARCH PROPOSAL

The TNKS and TNKS2 proteins share a high sequence homology and have overlapping functions. The substrate binding region consists of five ankyrin repeat clusters (ARC1-5), where ARC1, ARC2, ARC4 and ARC5 can all bind to target molecules. Several Tankyrase substrates interact through multiple ARCs e.g. AXIN [3]. We are therefore seeking to develop inhibitors that can bind to all functional ARCs in the proteins. We have discovered low-molecular-weight fragments that bind to the Tankyrase ARCs, as shown by ligand- and protein-observed NMR, e.g. **1** (Figure 1). We will use structure-based fragment optimisation and fragment growing to generate larger molecules with additional specific interactions in the binding site and increased affinity.

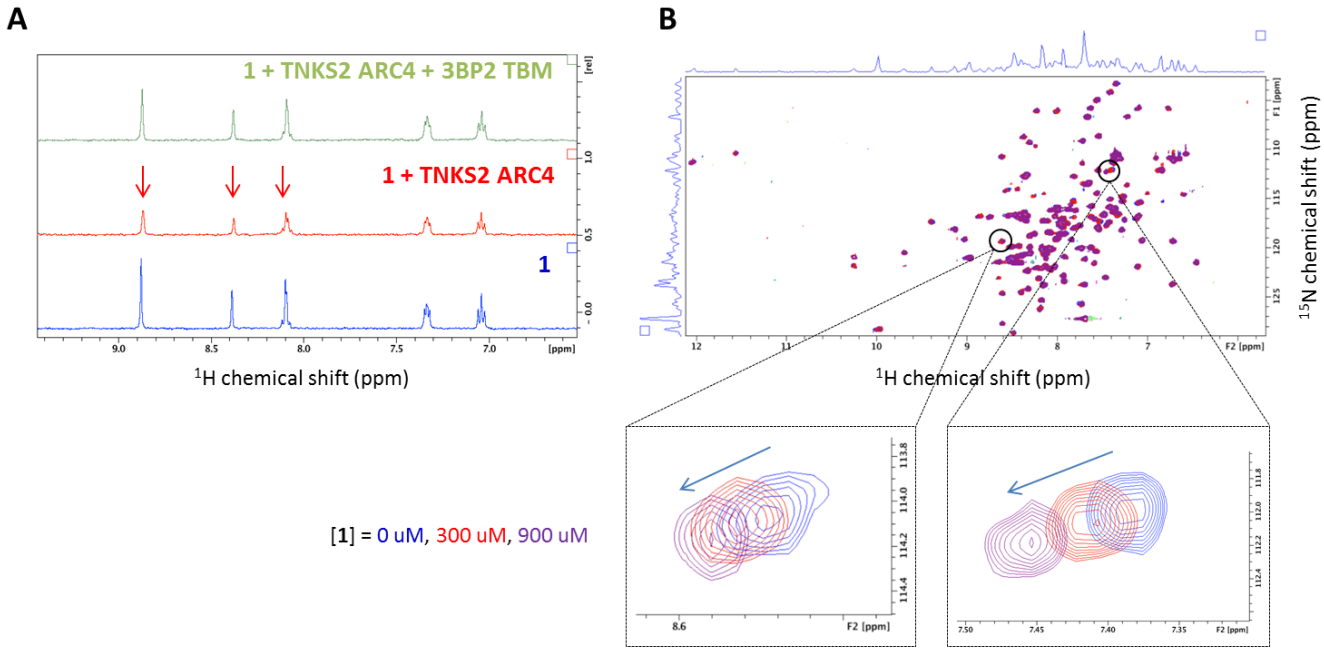

Figure 1. (**A**) Fragment **1** binds to TNKS2 ARC4 as shown by reduced signal intensities (arrowed) in the ligand-observed CPMG NMR assay in the presence of TNKS2 ARC4. Addition of the TBM peptide from 3BP2 reduces the binding of **1** and rescues signal intensity. (**B**) TNKS2 ARC4 ^{1}H, ^{15}N protein-observed NMR shows concentration-dependent chemical shift perturbations for specific protein residues in the presence of **1** that are also perturbed upon TBM binding.

The first aims of the project are to refine models of the location and binding mode of fragment hits such as **1** and its analogues within the Tankyrase ARCs, and to optimise the fragment scaffolds for potency and ligand efficiency. We have located the fragment binding site from the relative affinities of the fragments for ARC variants with amino acid substitutions in the substrate binding groove, and have shown that fragment binding is competed by TBM peptides (Figure 1A). Complementary approaches will be pursued to provide more detailed structural information to inform structure-based design. The student will use NMR-based techniques that have been demonstrated for fragment-based approaches to inhibit other protein-protein interactions and have been established for this project [9]. Following NMR assignment of key substrate-binding site residues using doubly-labelled (^{15}N, ^{13}C) TNKS2 ARC4 protein, epitope-mapping will be investigated using ^{1}H, ^{15}N HSQC NMR to identify the residues that have perturbed chemical shifts on binding of the fragments and interact directly (Figure 1B) [9]. The sparse distance constraints obtained from these experiments will be used to generate models of ligand binding from *in silico* docking programs. The student will next synthesise analogues of the fragments that systematically explore modification of the scaffolds, and are predicted to have improved binding affinity. Compounds will be screened against multiple Tankyrase ARCs using our established ligand- and protein-observed NMR assays (Figure 1).

Elaborated compounds will be used to generate more distance constraints and iteratively refine the *in silico* models of compound binding. As an alternative approach, we will explore chemo-proteomic epitope mapping through photoaffinity capture of diazirine-tagged fragments followed by MS sequencing and identification of labelled Tankyrase peptides [10]. In parallel with the NMR studies, we will also investigate crystallisation of the fragment hits and analogues bound to Tankyrase ARCs using protein constructs and crystallisation conditions already established in our laboratories for apo- and peptide-bound ARC constructs. This will enable X-ray crystallography in Dr Guettler's laboratory to provide high-resolution structures of the bound fragments. Where necessary, we will investigate new ARC protein constructs to obtain crystals of Tankyrase ARC-ligand complexes. The output of this part of the project will be models of fragment binding supported by structure-affinity data.

Based on the models of fragment binding, the student will use iterative *in silico* design, chemical synthesis and assay cycles to grow the optimised fragments to more efficiently occupy and contact known, tractable 'hot spots' for TBM peptide binding in the substrate binding groove (Figure 2). These include the hydrophobic central portion of the binding groove, a further hydrophobic extension of the groove beyond residue 5 of the TBM that is not occupied by the TBM peptides, the glycine-binding motif and the C-terminal sub-site [6]. Compound affinities will be assessed using biophysical assays (DSF or NMR) for weakly binding fragments (K_d > 100 micromolar). As affinity for TNKS ARCs is increased, we will use a competitive fluorescence polarisation binding assay we have developed to measure compound binding [8] or isothermal titration calorimetry. Compounds with increased affinity will be prioritised for crystallisation and X-ray crystallographic studies. If an alternative approach to give more potent inhibitors is required, the student will investigate opportunities for hybridisation of the optimised fragments with peptide TBMs, e.g. the octamer derived from 3BP2 (Figure 2) [6]. Positions for incorporation of the fragments will be proposed from the models of fragment binding, and the hybrid molecules can be prepared using solid phase peptide synthesis protocols we have used previously to make modified TBMs. The output of this part of the project will be ligands with sub-micromolar affinity for the Tankyrase ARC domains that compete the binding of TBM peptides.

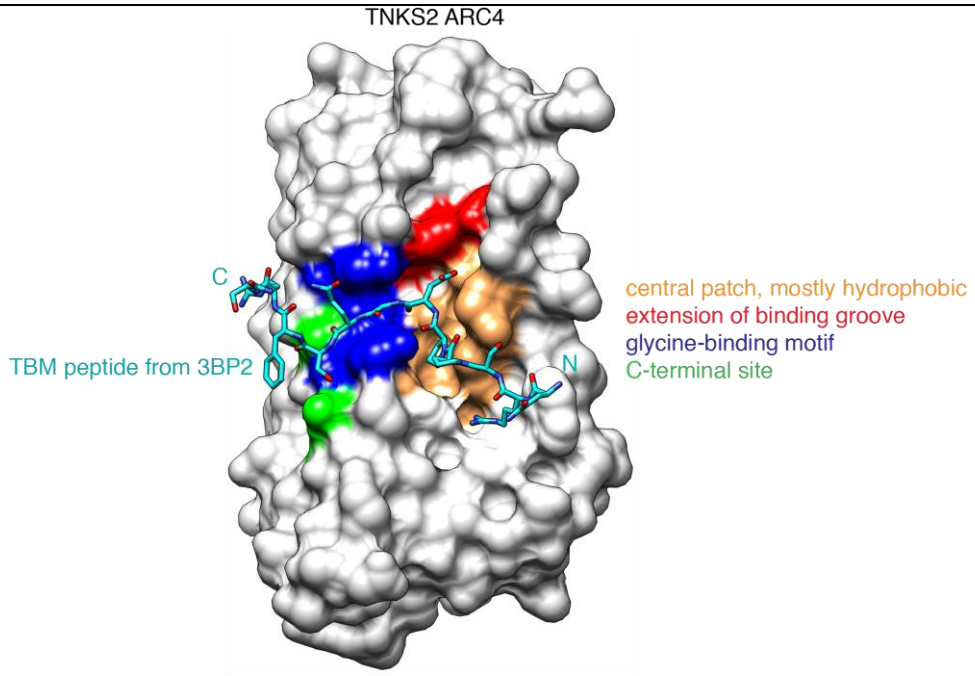

Figure 2. Binding of the 3BP2 8mer TBM peptide (cyan sticks) to TNKS2 ARC4 (white surface) [6]. TBM-ARC contacts are colour-coded.

When sufficiently potent compounds are identified, the student will refine these to make chemical tool molecules useful for exploring the biological consequences of inhibiting Tankyrase scaffolding functions. We will optimise the physicochemical properties of the compounds to give high solubility and membrane permeability, guided by relevant assays available in our laboratories. The selectivity of optimised compounds against non-Tankyrase targets will be assessed by broad biochemical screening through commercial services. Ultimately, the ability of compounds to modulate Tankyrase function in cancer cells will be assessed in assays established in Dr Guettler's laboratory, including for example Tankyrase-responsive reporter assays for Wnt/beta-catenin pathway activation [5].

LITERATURE REFERENCES

[1] Lehtiö L, Chi NW, Krauss S (2013) Tankyrases as drug targets. FEBS J 280:3576-93.
[2] Morrone S, Cheng Z, Moon RT et al (2012) Crystal structure of a tankyrase-axin complex and its implications for axin turnover and tankyrase substrate recruitment. *Proc Natl Acad Sci USA* 109:1500-5
[3] Yang L, Sun L, Teng Y et al (2017) Tankyrase1-mediated poly(ADP-ribosyl)ation of TRF1 maintains cell survival after telomeric DNA damage. *Nucleic Acids Res* 45:3906-21
[4] Wang W, Li N, Li X, Tran MK, Han X, Chen J (2015) Tankyrase Inhibitors Target YAP by Stabilizing Angiomotin Family Proteins. Cell Reports 13:524–532.
[5] Mariotti L, Templeton CM, Ranes M et al (2016) Tankyrase requires SAM domain-dependent polymerization to support Wnt--catenin signaling. *Mol Cell* 63:498-513
[6] Guettler S, LaRose J, Petsalaki E et al (2011) Structural basis and sequence rules for substrate recognition by Tankyrase explain the basis for cherubism disease. 147: 1340-54
[7] Xu W, Lau YH, Fischer G et al (2017) Macrocyclized extended peptides: inhibiting the substrate-recognition domain of tankyrase. *J Am Chem Soc* doi: jacs.6b10234
[8] Pollock K, Ranes M, Collins I, Guettler S (2017) Identifying and validating tankyrase binders and substrates: a candidate approach. *Methods Mol Biol* 1608:445-73

[9] Ma R, Wang P, Wu J, Ruan K (2016) Process of fragment-based lead discovery - a perspective from NMR. *Molecules* 21:854
[10] Smith E, Collins I (2015) Photoaffinity labeling in target- and binding-site identification. *Future Med Chem* 7:159-83

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:	MSc or MChem in Chemistry or Medicinal Chemistry, incorporating laboratory-based training in synthetic chemistry

Intended learning outcomes:

- Expert in the theory and practice of fragment-based ligand discovery
- Expert in synthetic and medicinal chemistry related to heteroaromatic compounds
- Experience and skills in the application of NMR techniques to measure ligand binding
- Experience and skills in the application of *in silico* methods for structure-based design
- Knowledge of protein crystallisation and X-ray crystallography