PhD Project Proposal

Funder details

Studentship funded by:	MRC iCASE (AstraZeneca)

Project details

Project title:	Development of tankyrase-directed PROTACs as novel scaffolding inhibitors

Supervisory team

Primary Supervisor:	Sebastian Guettler
Associate Supervisor(s):	Thomas Hayhow (AstraZeneca)
Secondary Supervisor:	Swen Hoelder

Divisional affiliation

Primary Division:	Structural Biology
Primary Team:	Sebastian Guettler's Team
Site:	Chelsea

Project background

Tankyrase, with two paralogues in humans (TNKS and TNKS2), is a poly(ADP-ribosyl)transferase and as such catalyses the synthesis of poly(ADP-ribose) (PAR) chains on its target proteins and itself1. PARylation by tankyrase typically triggers the PAR-dependent ubiquitylation (PARdU) and subsequent proteasomal degradation of tankyrase substrates and tankyrase itself through the action of PAR-activated E3 ubiquitin ligases7. Tankyrase controls a wide range of cancer-relevant cellular processes, including Wnt/□-catenin signalling, telomere length homeostasis and Hippo signalling1. A range of inhibitors of tankyrase's catalytic function have recently been developed as molecular probes and explored as potential therapeutic agents2. All tankyrase inhibitors generated thus far act as antagonists of NAD+ binding by the catalytic PARP domain. Besides inhibiting tankyrase catalytic activity, these compounds typically give rise to a strong accumulation of tankyrase due to the blockage of PARdU. Recent research has revealed the contribution of non-catalytic scaffolding functions of tankyrase in various contexts3,4, pointing towards potential limitations of catalytic tankyrase inhibitors. We hypothesise that the accumulation of tankyrase upon its catalytic inhibition may even potentiate its scaffolding functions.

Tankyrase's catalysis-independent functions are highly dependent on its ability to engage effectors (binders and substrates) via its N-terminal ankyrin repeat clusters (ARCs), which recognise degenerate tankyrase-binding peptide motifs (TBMs)3,8. In collaboration with Professor Ian Collins (ICR), we have been developing effector binding antagonists of tankyrase as chemical tools to investigate catalytic vs. non-catalytic functions of tankyrase5. Elaborating a small-molecule fragment hit, we have developed compounds that can competitively displace a model TBM peptide from the ARC at double-digit micromolar concentrations (Black et al., unpublished).

In this project, we aim to further optimise effector binding antagonists of tankyrase and use the resulting molecules to develop novel PROteolysis TArgeting Chimeras (PROTACs)6 that engage tankyrase via its ARCs rather than its catalytic domain. Unlike catalytic inhibitors, we predict that ARC-binding molecules will not trigger the accumulation of tankyrase and may therefore be more suitable for PROTAC development. We envisage that tankyrase ARC-

directed PROTACs will serve as valuable molecular probes to dissect the functions of tankyrase as a key player in numerous processes critical to cancer and other pathologies such as diabetes and neurodegeneration.

Project aims

- Synthesize analogues of existing hit matter to optimise ligand binding and explore binding modes
- Determine the tankyrase binding modes of small molecules using NMR and X-ray crystallography
- Use iterative structure-based design, chemical synthesis and biophysical assays to develop molecules with sub-micromolar affinity for tankyrase that compete with known tankyrase binders
- Design and validate bifunctional PROTACs based on the identified tankyrase ARC binders
- Optimise the physicochemical properties of potent compounds to give cell-permeable tool molecules

Research proposal

The two tankyrase paralogues TNKS and TNKS2 display a high degree of sequence similarity and therefore functional redundancy, although there are indications of paralogue-specific functions8,9. The substrate/effector binding regions of tankyrases consists of five ankyrin repeat clusters (ARC1-5; Figure 1A). ARCs 1, 2, 4 and 5 are all capable of binding to target molecules, some of which display multiple tankyrase-binding motifs (TBMs)8. We are therefore seeking to develop inhibitors that can bind to all functional ARCs in the tankyrases. Through extensive fragment screening, we have discovered low-molecular-weight fragments that bind to all substrate/effector-binding tankyrase ARCs, as shown by ligand- and protein-observed NMR5. Subsequent SAR studies gave rise to molecules with increased affinity for the ARCs in the double- to triple-digit micromolar range (Black et al., unpublished; Figure 1C-E).

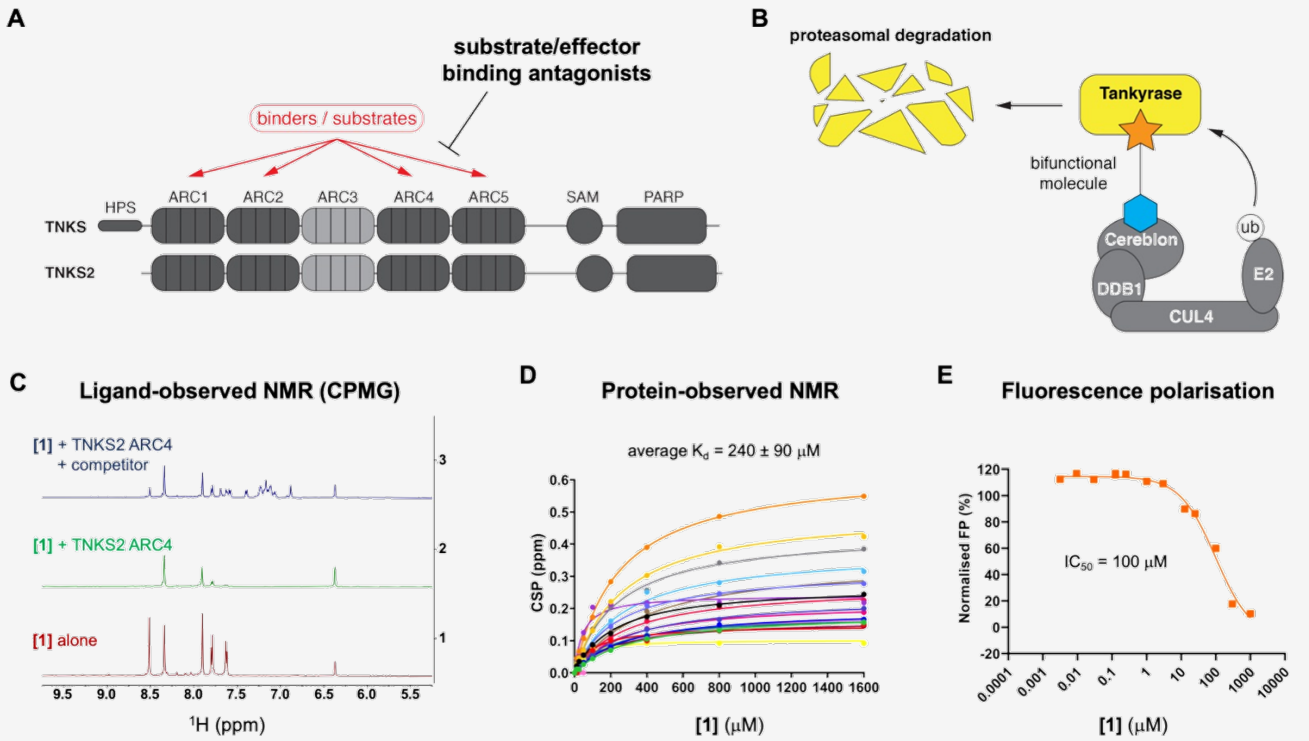

Figure 1. (A) Schematic representation of the two human tankyrase paralogues with substrate/effector binding antagonists targeting ARCs. (B) Schematic illustrating the principle of tankyrase-directed PROTACs. (C) Ligand-observed NMR of compound [1]. A TBM peptide competes off compound [1]. (D) Protein-observed NMR showing binding of compound [1]. (E) Competitive FP assay, showing displacement of a fluorescently labelled TBM peptide from TNKS2 ARC4 by compound [1].

1. Structural characterisation of ARC:small molecule interactions

In a first aim, the candidate will perform extensive co-crystallisation and crystal soaking experiments to gain direct structural insights into the binding mode of the most potent ARC-binding molecules available so far by X-ray crystallography. Protein expression (in E. coli) and purification of ARCs is established, and hands-on crystallisation and X-ray crystallography support is available through the Biophysics and Crystallisation Facility in the Division of Structural Biology at the ICR and Dr Guettler's team. Structural insights from this work and other lines of investigation will form a foundation for the subsequent rational design of optimised compounds to generate larger molecules with additional specific interactions in the binding site.

2. Compound optimisation through rational design, synthesis, biophysical and structural evaluation

Guided by structural insights, the student will next use iterative in silico design, chemical synthesis, biophysical and biochemical assays to grow and modify the optimised fragments to more efficiently occupy known, tractable 'hotspots' for TBM peptide binding in the TBM-binding groove of the ARC (Figure 2). These include the hydrophobic central portion of the binding pocket, 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 a polar C-terminal sub-site. Weak binders will be evaluated by ligand-observed NMR (CPMG, WaterLOGSY) and protein-observed NMR. Fragments and compounds with dissociation constants < 100 □M will be evaluated for ARC binding by isothermal titration calorimetry (ITC) and a competitive fluorescence polarisation (FP) assay, which measures displacement of a labelled TBM peptide from an ARC5 (Figure 1E). Compounds with increased affinity will be prioritised for crystal soaking and/or co-crystallisation for structural studies by X-ray crystallography and iterative optimisation cycles. The output of this part of the project will be ligands with nanomolar affinity for the tankyrase ARC modules that efficiently out-compete TBM peptides at the ARCs of both tankyrase paralogues.

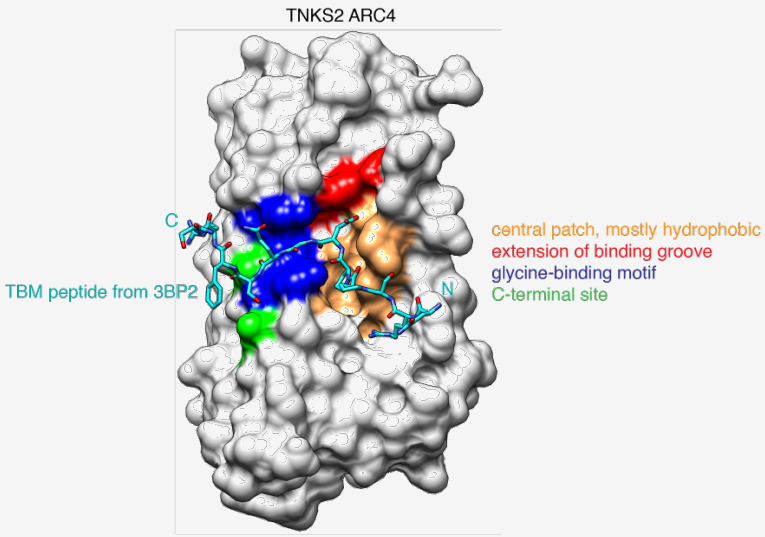

Figure 2. Binding of an 8-mer TBM peptide (cyan sticks) from the model substrate 3BP2 to TNKS2 ARC4 (white surface; PDB code 3TWR)8. The most readily targetable key features of the ARC are colour-coded.

3. Development of PROTACs

With expertise available at the Centre for Protein Degradation (ICR, including Professor Swen Hoelder's team) and AstraZeneca (including Dr Thomas Hayhow's team), the student will next develop the most potent ARC binders into bifunctional molecules with an E3-ligase binding group conjugated via suitable linkers (Figure 1B)6,10. Structural and SAR data from part (2) will guide the choice of modification sites on the ARC binder to ensure tankyrase target engagement is maintained. Linkers of different length and different E3 ligase warheads targeting various E3 ligases (e.g., cereblon, VHL) will be explored. Compounds will be synthesised in collaboration with AstraZeneca using an identified ARC binder terminating in a group capable of reacting with amines (e.g., carboxylic acid). AstraZeneca has a large toolbox of various linkers attached to different E3 ligases, and experience of generating extensive arrays of bivalent compounds. The resulting molecules will be evaluated for E3 ligase target engagement in an FP-based assay. This assay will be modified to evaluate simultaneous engagement of the ARC and the E3 ligase.

4. Generation of tool compounds

Once sufficiently potent compounds are identified in stages (2) and (3), the student will refine these to make chemical tool molecules useful for exploring the biological consequences of universally inhibiting the tankyrases, i.e., its catalytic and non-catalytic (scaffolding) functions, through competition of client proteins or the additional degradation of tankyrase. The candidate will optimise the physicochemical properties of the compounds to give high solubility and high membrane permeability, guided by relevant assays available in our laboratories. The selectivity of optimised compounds against potential targets other than tankyrases 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 tankyrase-responsive gene expression assays for Wnt/□catenin pathway activation3.

Literature references

[1] Haikarainen, T., Krauss, S. & Lehtiö, L. Tankyrases: structure, function and therapeutic implications in cancer. Current Pharmaceutical Design 20, 6472–6488 (2014).
[2] Yu, M., Yang, Y., Sykes, M. & Wang, S. Small-Molecule Inhibitors of Tankyrases as Prospective Therapeutics for Cancer. J Med Chem 65, 5244–5273 (2022).
[3] Mariotti, L. et al. Tankyrase Requires SAM Domain-Dependent Polymerization to Support Wnt-β-Catenin Signaling. Molecular Cell 63, 498–513 (2016).
[4] Li, X. et al. Proteomic Analysis of the Human Tankyrase Protein Interaction Network Reveals Its Role in Pexophagy. Cell Reports 20, 737–749 (2017).
[5] Pollock, K. et al. Fragment-based screening identifies molecules targeting the substrate-binding ankyrin repeat domains of tankyrase. Sci. Rep. 9, 1–20 (2019).
[6] Collins, I., Wang, H., Caldwell, J. J. & Chopra, R. Chemical approaches to targeted protein degradation through modulation of the ubiquitin-proteasome pathway. Biochem. J. 474, 1127–1147 (2017).
[7] DaRosa, P. A. et al. Allosteric activation of the RNF146 ubiquitin ligase by a poly(ADP-ribosyl)ation signal. Nature 517, 223–226 (2015).
[8] Guettler, S. et al. Structural basis and sequence rules for substrate recognition by Tankyrase explain the basis for cherubism disease. Cell 147, 1340–1354 (2011).
[9] Bhardwaj, A., Yang, Y., Ueberheide, B. & Smith, S. Whole proteome analysis of human tankyrase knockout cells reveals targets of tankyrase-mediated degradation. Nat Comms 8, 2214 (2017).
[10] Hayhow, T. G. et al. A Buchwald–Hartwig Protocol to Enable Rapid Linker Exploration of Cereblon E3-Ligase PROTACs. Chem European J 26, 16818–16823 (2020).

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 synthetic and medicinal chemistry related to heteroaromatic compounds, including PROTACs
- Experience and skills in the application of biophysical and biochemical methods 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

Advertising details

Project suitable for a student with a background in:

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

Computer Science