ICR The Institute of Cancer Research

UKRI Medical Research Council

# PhD Project Proposal

## Funder details

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| **Studentship funded by:** | MRC DTP iCASE, Merck Healthcare KGaA (“Merck”) |

## Project details

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| **Project title:** | Development of small molecules targeting the TRF1 subunit of the telomeric Shelterin complex |

## Supervisory team

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| **Primary Supervisor:** | Professor Sebastian Guettler (ICR) |
| **Associate Supervisor(s):** | Dr Astrid Zimmermann (Merck) |
| **Secondary Supervisor:** | Professor Swen Hoelder (ICR) |

## Divisional affiliation

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| **Primary Division:** | Cancer Therapeutics & Structural Biology |
| **Primary Team:** | Swen Hoelder & Sebastian Guettler |
| **Site:** | Sutton & Chelsea |

## Project background

Telomeres solve two fundamental challenges associated with linear chromosomes: the end protection problem and the end replication problem[1,2]. Shelterin is a six-subunit protein complex that binds to telomeres where it protects chromosome ends from an unwarranted activation of the DNA damage response and, in collaboration with telomerase, mediates homeostatic telomere length maintenance[2,3]. Restored telomere length homeostasis is a hallmark of cancer initiation and progression[4], which makes Shelterin a potential therapeutic target. The direct modulation of the protein:protein interactions within Shelterin has remained unexplored, in part due to a lack of a structural understanding of the overall Shelterin complex, and given the essential roles of Shelterin in telomere protection.

Among the core components of Shelterin is the telomeric repeat-binding factor 1, TRF1, which binds double-stranded telomeric repeats and contributes to telomere protection, replication and extension (Figure 1B). The inclusion of TRF1 into Shelterin is proposed to be transiently countered by the ADP-ribosyltransferase tankyrase, which is critical to telomerase-dependent telomere lengthening in human cells[5,6]. Brain-specific genetic ablation or pharmacological interference with Trf1 localisation in mouse models of glioblastoma multiforme (GMB), and TRF1 pharmacological inhibition in human xenografts, limit GMB growth[7]. Similarly, TRF1 loss or inhibition of its telomeric localisation were shown to block the growth of p53-null *K-Ras*$^{G12V}$-induced lung carcinomas, without affecting mouse survival or tissue function[8,9]. Whilst these observations suggest that TRF1 may be a suitable therapeutic target, they also raise questions regarding the function of TRF1 in telomere length maintenance and protection.

To answer this question, we have embarked on a programme to develop a chemical probe to directly modulate the incorporation of TRF1 into Shelterin (Casale *et al*., manuscript in preparation). We performed fragment screening against the TRF homology (TRFH) domain of TRF1, which mediates TRF1 recruitment to Shelterin via a domain:peptide interaction with the central TIN2 (TRF1-interacting nuclear factor 2) scaffolding component of Shelterin[10] (Figure 1C). Through ligand-observed NMR (LO-NMR) and X-ray crystallography-based fragment screening (XChem), we have discovered a series of fragments that engage the TIN2 binding site of TRF1 TRFH with

double-digit micromolar affinity. These LO-NMR affinity values are in line with a competitive fluorescence polarisation (FP) assay that demonstrated the micromolar potency of the NMR hit series through the displacement of a TIN2 peptide (Casale *et al*., manuscript in preparation). With a robust assay cascade in place, the fragment hits obtained from both the NMR and XChem screens will serve as a basis for further optimisation by structure-activity relationship (SAR) studies and structure-based design.

In this project, we propose to further develop potent TRF1-binding molecules into cell-permeable tool compounds to modulate Shelterin function at the levels of telomere protection and telomere length maintenance.

## Project aims

- To synthesise compounds based on existing fragment hit matter from NMR-based and XChem-based screens
- To perform iterative structure-activity relationship (SAR) studies and structure-based design to obtain compounds that inhibit the TRF1:TIN2 interaction with nanomolar potency
- To establish cell permeability of the above compounds
- To functionally characterise the compounds in biophysical, biochemical and cell-based functional studies

## Research proposal

Disrupting protein:protein interactions using small molecules holds substantial potential but remains challenging, for example due to the size and features of protein:protein interaction interfaces[11]. Setting out to identify molecules that disrupt the interface between TRF1 and TIN2, we performed one fragment screen by ligand-observed NMR spectroscopy and two fragment screens by X-ray crystallography (XChem), using two distinct crystal systems (Casale *et al*., manuscript in preparation). The screens identified extensive hit matter, with the most potent fragments binding and inhibiting the TRF1:TIN2 interaction with double-digit micromolar potency.

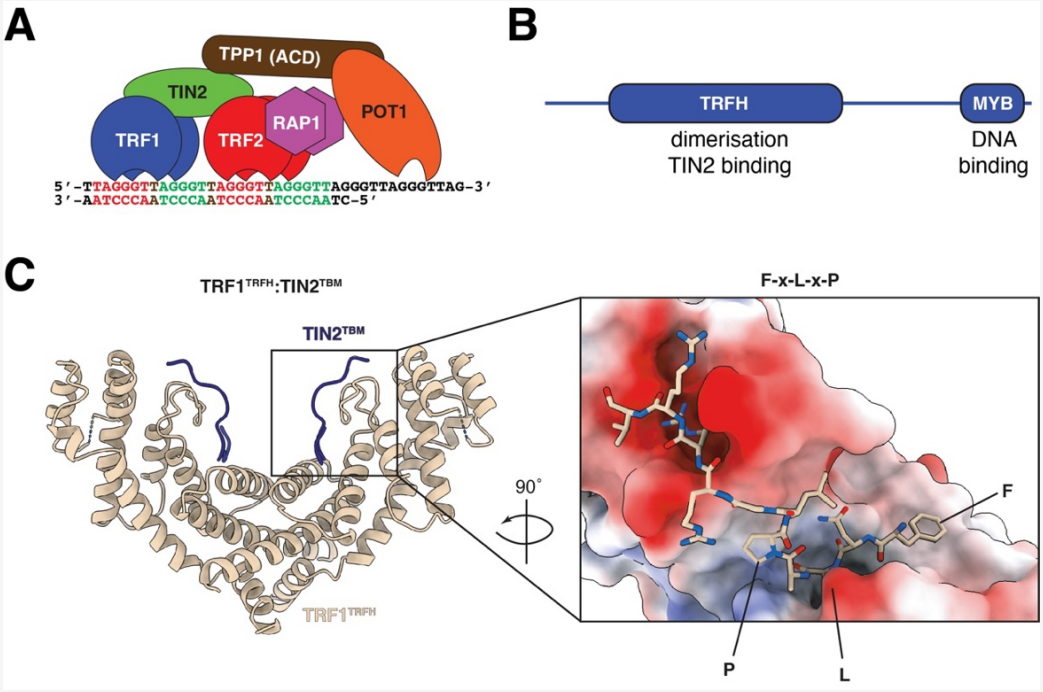


**Figure 1: The TRF1:TIN2 interaction in the telomeric Shelterin complex.**

**(A)** Schematic representation of the telomeric Shelterin complex. **(B)** Domain organisation and functions of TRF1. **(C)** Structural representation of the $TRF1^{TRFH}$:$TIN2^{TBM}$ complex[10] (PDB 3BQO). Left, the dimeric TRFH domain with TIN2's TRFH-binding motif (TBM) is shown in cartoon representation. Right, surface representation of the TRFH domain, coloured by electrostatic potential, with $TIN2^{TBM}$ shown is stick representation and coloured by heteroatom.

**1. SAR studies and structure-based design**

XChem screening illustrates that the TIN2 binding site on TRF1 is well-populated with fragment hits, which offers clear opportunities for developing fragments into drug-like molecules with higher potency. The candidate will use a combination of commercially sourced derivatives of selected fragments as well as chemical synthesis to perform extensive structure-activity relationship (SAR) studies as a foundation for optimising the potency of fragments. Fragment binding will be assessed by LO-NMR, using the Carr-Purcell-Meiboom-Gill (CPMG)[12] and/or R2KD[13] methods to quantitatively characterise binding. Subsequent strategies for obtaining more potent compounds will include fragment growth, linking and merging. Using a TRF1 TRFH domain crystal system in which the TIN2 binding site is accessible (Casale *et al*., manuscript in preparation), selected molecules will be soaked into crystals for subsequent structural analysis by X-ray crystallography. Structural data will in turn inform subsequent compound optimisation by structure-based design. The candidate will assess molecule binding by (1) isothermal titration calorimetry (ITC) and (2) the ability of compounds to displace TIN2 from TRF1 by a competitive fluorescence polarisation (FP) assay. In an iterative process involving chemical synthesis, biophysical characterisation and structural analysis, the potency of the compounds will be increased. The aim of this part of the project will be to obtain binders with ideally nanomolar potency to disrupt the TRF1:TIN2 complex.

**2. Assessment of compound impact on Shelterin assembly and Shelterin-mediated telomerase processivity *in vitro***

The candidate will perform analytical size exclusion chromatography (SEC), electrophoretic mobility shift assays (EMSAs) and mass photometry to assess whether the compounds are able to expel TRF1 from the Shelterin complex. It remains possible that TRF1 remains in the complex, tethered via its DNA binding domains. The candidate will therefore use appropriate mutant variants of TRF1 which are either defective in TIN2 or DNA binding. Without DNA tethering of TRF1, the compounds are predicted to release TRF1 from Shelterin. Shelterin acts both as a negative and positive regulator of telomerase function[14]. The candidate will, in collaboration with members of the Guettler Lab, perform direct *in vitro* telomerase assays[15] to evaluate any potential effect of compounds on telomerase function.

**3. Generation of tool compounds**

The candidate will next use chemical synthesis to optimise the physicochemical properties of compounds to attain high solubility and membrane permeability. For the latter, passive or combined passive + active cell permeability will be evaluated using the parallel artificial membrane permeability assay (PAMPA) and Caco-2 cell monolayer assays, respectively[16,17]. Throughout this optimisation process, potency of compounds will be assessed by R2KD, ITC and FP, and where necessary compounds will be studied structurally by X-ray crystallography. Final compounds will be evaluated for their ability to disrupt the Shelterin complex, as above.

**4. Evaluation of the cellular impact of TIN2 antagonists**

The candidate will next collaborate with colleagues in the Guettler Lab and/or at Merck to assess the activity the optimised tool compounds in human cells. This part of the project will offer the candidate the opportunity to perform these assays him/herself, as desired and practically possible. To evaluate on-target vs. potential off-target activities, cellular thermal shift assays (CETSAs)[18] and affinity chromatography methods coupled to mass spectrometry[19] will be employed. Using immunoprecipitation, the ability of the compounds to disrupt the TRF1:TIN2 interaction will be evaluated. This will be complemented by fluorescence microscopy to assess the ability of compounds to mislocalise TRF1 from telomeres[6].

Long-term removal of TRF1 from Shelterin is predicted to deprotect telomeres and affect telomerase-dependent telomere lengthening. Telomere deprotection will be evaluated by monitoring the emergence of telomere dysfunction-induced foci (TIFs, which mark telomeric DNA damage)[20] upon inhibitor treatment of cells, using immunofluorescence microscopy. Furthermore, the ability of compounds to modulate homeostatic telomere length control will be evaluated by passaging cells over extended periods of time and analysing telomere length by telomeric restriction fragment analysis / Southern blotting as a function of population doublings[6].

**Perspective**

The proposed work aims to develop and characterise tool compounds that impair the function of TRF1 in the telomeric Shelterin complex. The compounds will enable the exploration of mechanisms underlying telomere length maintenance and telomere protection, and will allow us to evaluate whether the acute loss of TRF1 from Shelterin selectively impairs the fitness of cancer cells.

## Literature references


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## Candidate profile

**Note:** the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1).

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| **Pre-requisite qualifications of applicants:** | MSc or MChem in Chemistry or Medicinal Chemistry, incorporating laboratory-based training in synthetic chemistry, paired with an active interest in the drug discovery and development process throughout various stages |
| **Intended learning outcomes:** | • Expert in synthetic and medicinal chemistry<br>• Expert in the application of biophysical and biochemical methods to measure ligand binding and the impact of small-molecule compounds on protein:protein interactions<br>• Experience and skills in the application of *in silico* methods for structure-based design<br>• Knowledge of protein crystallisation and X-ray crystallography<br>• Knowledge of cellular methods to assess genome integrity |

## Advertising details

**Project suitable for a student with a background in:**

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