Investigating interactions between the BRCT Domain of BRCA1 & Affimers

Student: Tharushi Wijesiriwardena
Supervisor: Dr Qian Wu, Mr William Wilson

Hereditary breast and ovarian cancer (HBOC) occurs as a result of germline mutations to the tumour suppressor gene BRCA1 (Breast Cancer Suscpetibility Gene 1). In DNA damage response pathways, BRCA1 maintains genomic integrity through its interactions with mediators and effector proteins, thus facilitating the recognition and repair of DNA damage (Leung and Glover, 2011). Such interactions occur via the BRCT domains, present on the C-terminal of the protein. Characterising these interactions has become increasingly important in understanding overall BRCA1 function. Binding to BRCT may provide a novel therapeutic approach that can not only contribute towards modulating the physiological roles of BRCA1, and thereby improving patient outcomes, but also improving understanding of the protein's interactions and functions outside of HBOC. In this project, small, highly-specific stabilising molecules, called Affimers (~12 kDa) were used to target various regions of the BRCA1 BRCT domain, in order to characterise interactions. In doing so, this research will advance current understandings of the function of BRCA1.

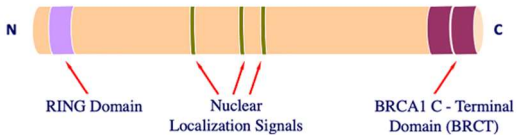

Fig.1 **Structure of BRCA1** The tandem C-terminal BRCT domain (the region of interest for this project) depicted has several tumour suppressive functions. Image obtained from Werner (2022).

AIMS

1) Express the target proteins, BRCA1-BRCT and selected Affimers from bacterial cells.
2) Purify samples using nickel affinity columns and size exclusion chromatography.
3) Characterise interactions of the BRCA1-BRCT and selected Affimer using a biophyscial assay (isothermal titration calorimetry, ITC)

METHODS

Previous research conducted by the lab confirmed the association of BRCA1-BRCT with 14 Affimers. One of these Affimers was selected for investigation due to its promising results in preliminary studies. Pet11 plasmid containing the selected Affimer and pHAT2 plasmid containg His-BRCT DNA was transformed into BL21(DE3) cells. IPTG (isopropyl β-d-1-thiogalactopyranoside) was used to induce expression. Cells then underwent lysis and nickel purification. BRCT was concentrated using a Vivaspin column and both samples then underwent S75 Size Exclusion Chromatography (SEC) and dialysis. ITC was conducted on a MicroCal ITC200, testing Affimer samples pre- and post-gel filtration. Alongside this, primers were designed for mutant BRCT and then used to transform pHAT2 mutated His-BRCT into DH5α.

RESULTS

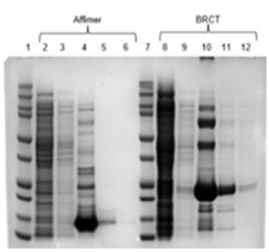

Fig.2 **SDS-PAGE of Flow through & Elutions post-Nickel purification.** (1) 1kB DNA ladder, (2) Affimer flow-through, (3) Affimer Wash, (4-6) Affimer Elutions 1,2,3, (7) ladder, (8) BRCT flow-through, (9) BRCT Wash, (10-12) Elutions 1-3.

Following Ni-affinity, an SDS-PAGE gel was conducted to confirm whether the proteins of interest had been eluted (Fig.2). Using SEC chromatograms, chosen samples were dialysed for ITC (Fig.3). The pre-Affimer+BRCT had aggregated, and thus could not confirm protein-protein interactions. Similarly, the post-Affimer+BRCT sample had an endothermic signal, suggesting multimerisation or a change in conformation. When mapping these results to the preliminary results conducted by a previous student, there was a distinct difference in binding interactions, suggesting that further repeats should be conducted to better characterise the interactions between BRCT and the selected Affimer.

The designed primers were used in a gradient PCR, with ranging annealing temperatures. Proteins showed multiple banding and low expression (Fig.4) resulting in no colony formation. Therefore primers were redesigned (elongated by several bases either side) for future use.

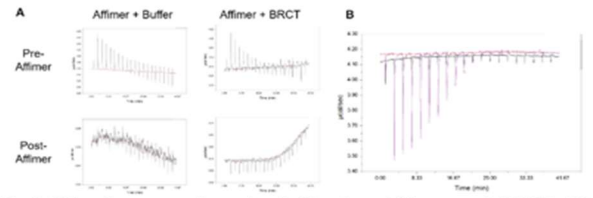

Fig.3 **ITC of pre-, and post-gel filtration Affimer and BRCT.** (A) Interactions between the Affimer and buffer, and the Affimer with BRCT. (B) Overlay of results with the preliminary study between the same proteins.

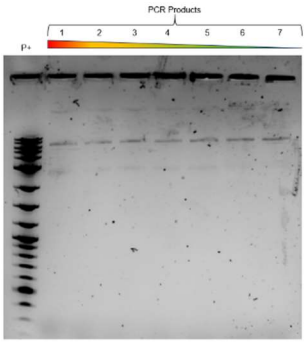

Fig.4 Agarose Gel of PCR products. From left to right: P+ ladder, (1-7) PCR products with varying annealing

OUTCOMES OF THE STUDENTSHIP

These findings contradict the previously demonstrated interaction between the selected Affimer and the BRCT-BRCA1 domain. Due to low sample size, the previous experiment was conducted only once, and the additional data gained during this project will enable future researchers working on BRCT-BRCA1 and Affimer interactions to gain a better awareness of the range of possible interactions. Due to these unexpected outcomes, I have developed my ability to troubleshoot experiments, learing how to critically analyse methodologies, identifiy potential isses and systematically explore alternative approaches.

DEPARTURES FROM ORIGINAL PROPOSAL

Due to the unexpected outcomes observed during this project, many experiments were conducted to repeat and confirm unexpected results and to investigate why such results occurred. As we were unable to characterise interactions between the BRCA1-BRCT and selected Affimer through the ITC, I did not continue the project to characterise the interactions of BRCA1-BRCT with the 13 other Affimers. Furthermore, I was not able to learn X-Ray crystallography due to insufficient quantities of protein sample.

VALUE OF THE STUDENTSHIP TO THE STUDENT AND THE LAB

The studentship significantly contributed to the expansion of my technical and transferable skills. I became proficient in various wet lab techniques, such as protein expression and purification, PCR, and training on how to use an AKTA and ITC. Furthermore, I gained a comprehensive understanding of experimental design and execution, teaching me the importance of planning and understanding protocols. The challenging nature of the project also taught me perseverance and adaptability, both skills that I wish to develop further during a PhD. I was well-integrated into both the Wu lab and The Astbury Centre for Structural Molecular Biology, which the Wu lab is part of, enabling me to form meaningful working relationships with both members within and outside of the lab. Through this, I was able to shadow cryo-EM sample preparation and given the opportunity to attend weekly electron-microscopy based seminars delivered by PhD students. Also, I attended the 2023 Astbury Away Day, where I listened to numerous talks and poster presentations related to structural biology, furthering my interest in the research area, and diversifying my understanding of the range of projects available to pursue through a PhD.

FUTURE DIRECTIONS OF THE PROJECT

This project will be taken over by the new PhD student in the lab, who will have a much longer period with which to characterise interactions of BRCA1-BRCT with a range of Affimers, using multiple biophysical techniques. Additionally, the newly designed primers will aid them to produce mutant protiens, to compare against interactions of the wildtype. Ultimately, such research will further the understanding of BRCA1 and its role in cancer proliferation.

Fig.5 **Lab Work and Members** The first panel is the student conduct transformations, the second panel shows the student operating the AKTA during size exclusion chromatography and the third panel shows the members of the lab (from left to right: Rouyi Guo (the previous masters student), Tharushi Wijesiriwardena (summer student), and William Wilson (PhD Supervisor).

REFERENCES

Leung, C.C. and Glover, J.N. 2011. BRCT domains: easy as one, two, three. *Cell Cycle.* **10**(15), pp.2461-2470.
Werner, H. 2022. BRCA1: An Endocrine and Metabolic Regulator. *Frontiers in Endocrinology.* **13**.