香 港 大 學
THE UNIVERSITY OF HONG KONG

Laidlaw Scholars Programme 2020-21

Research Proposal

Guidelines on how to prepare a research proposal:

- Identify a project of your choice and discuss the topic with your academic supervisor at HKU.
- Prepare a research proposal including the following details:

1. Title of research project:

Activity-based protein profiling (ABPP) to investigate anticancer properties of cysteine-reactive compounds

2. Research question:

How to target undruggable protein to further advance targeted cancer therapy

3. Summary of the work to be undertaken by the applicant (e.g., background information, location, history, context, limitation, methodology, and timeline)

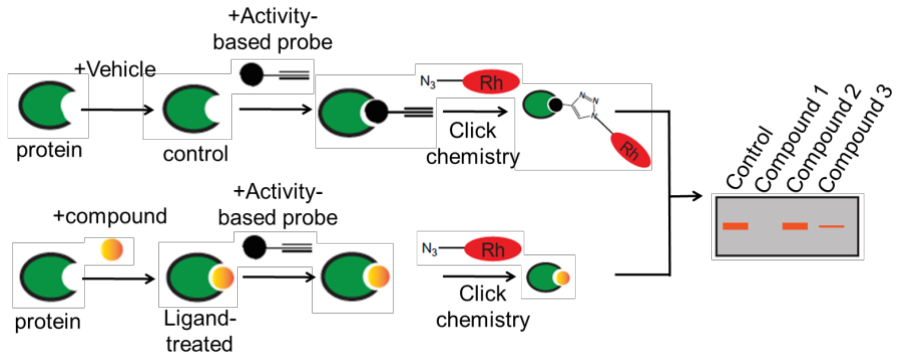
The identification of cancer-related protein is a breakthrough for targeted cancer treatments as it gives rise to the possibility for drug development. However, many of these proteins are traditionally considered to be "undruggable" due to its structure-function considerations. Despite this, through the use of cysteine-reactive compounds, provides the possibility to turn the proteins into "druggable" with its sensitivity to different chemical pathways.

In the human body, there is a low abundance of cysteine, but at functionally important sites, it is highly conserved; thus, allowing it to facilitate essential protein functions. As a result of cysteines' unique structure and chemistry, it can show specific chemical reactions with potential compounds thus increasing binding selectivity within the biological system. These properties make this amino acid a favourable target for modification in proteins.[1]

To better understand the interaction mechanism between the cysteine-reactive compounds and protein, activity-based protein profiling (ABPP) is used. This technology utilises a probe which consists of a reactive group to label proteins containing reactive amino acids, such as cysteine in this study. The probe also contains a linker site and a reporter group which allows further functionalization for detection and analysis. In the gel-based ABPP experiment, the reporter group is an alkyne which can undergo click chemistry with fluorophore-azide. Therefore, proteins labelled with the probe will show strong fluorescence. On the other hand, if our cysteine-reactive compounds can bind onto the protein, this will compete with the probe binding, resulting in a significant diminishment of fluorescence from the protein[2] as illustrated in in figure 1. When ABPP is coupled with mass spectrometry, the exact binding site of the compounds on the protein can also

be identified, and this can provide important information for unravelling mechanism of actions of the compounds.

Figure 1: Gel-based activity-based protein profiling



I will investigate anticancer properties of novel cysteine-reactive compounds by first synthesizing new cysteine-reactive compounds during the first two weeks using chemistry techniques. Following weeks 3 to 5, I will then apply gel-based ABPP experiment to investigate in vitro binding of the new compounds with cancer-associated proteins such as NRF2, which has been found to show higher expression in aggressive cancers. Then, I will conduct live-cell experiments to study anticancer properties and the compounds' effectiveness through cell viability assay and western blotting in week 6 to 8. Throughout the project, data will be collected and analysed at different stages to ensure the reliability of the results.

If this project goes smoothly and the abovementioned experiments can be finished earlier, MS-based ABPP experiment will be performed in the final stage of this project to investigate selectivity profile of the compounds toward the cancer-associated proteins. This is critical because humans are made of a complex system consisting of a myriad of proteins and my lead compound may show binding to other proteins that can lead to undesirable side effects. By the MS-based ABPP experiment, these off-target proteins, if any, can be identified and this can help to provide insights onto future medicinal chemistry and lead optimization to improve efficacy and reduce toxic side effects.

This project aims to synthesize cysteine-reactive compounds to target cancer-associated proteins and investigate their anticancer properties as well as the mechanism of actions. This expands the library of the potential compounds to treat cancer in humans and further understand the biological functions of the targeted protein. By doing so, other forms of research and drug discovery can be expedited, when done in a collaborative manner.

Reference:

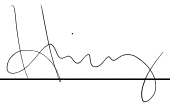
1. Weerapana, E., Wang, C., Simon, G., Richter, F., Khare, S., & Dillon, M. et al. (2010). Quantitative reactivity profiling predicts functional cysteines in proteomes. *Nature*, *468*(7325), 790-795. doi: 10.1038/nature09472
2. Wang, S., Tian, Y., Wang, M., Wang, M., Sun, G., & Sun, X. (2018). Advanced Activity-Based Protein Profiling Application Strategies for Drug Development. *Frontiers In Pharmacology*, *9*. doi: 10.3389/fphar.2018.00353

4. Details of supervision arrangements (e.g., how are you going to conduct your research? Will focused group/survey be conducted? How often will you meet your supervisor? Is laboratory work required? Etc.)

Notes:
If you wish to pursue a research opportunity at University of Leeds or University of St. Andrews, please name three professors with whom you would like to conduct your research. Once you are shortlisted for the award, the Horizons Office will match your proposal to a potential host supervisor according to your preferences.

This project will be supervised by Dr Chung Yik Shum Clive from the School of Biomedical Sciences at HKU. I will be conducting the investigation in his laboratory; thus, wet-lab experiments related to gel-based ABPP screening experiments, cell culture, cell viability assay, and some chemistry to synthesize cysteine-reactive compounds will be conducted. As these are new experiments to me, I will conduct prior research before attempting them and working with a PhD student who has research experience in applying ABPP and MS-based platforms throughout my internship. Furthermore, I will be meeting with Dr Chung once per week for individual meetings along with monthly group meetings to present research findings within the team. Moreover, a weekly journal club will be required to discuss exciting papers in the field.

Name of applicant: Yip Yui Yan Hillary

Signature of applicant:  Date: 6/2/2021