MICROMAPPING WITH PEPTIDE LIGANDS

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Project Background and Aims

MicroMapping (µMap) is a new technology developed by Merck and Princeton University (1). It involves the utilization of a photocatalyst which, when excited by blue light, can catalyse reactions in a small radius. By appending this catalyst to a ligand, interactions involving the ligand can be investigated.

The aims of this project are to investigate the applicability of MicroMapping technology to peptide ligands. By attaching an iridium photocatalyst to a peptide with a binding affinity for a chosen protein, molecules containing diazirines can be selectively activated by the catalyst, labelling proteins in proximity to the peptide. By demonstrating that these molecules selectively label proteins with binding affinity for the peptide, it can be shown that this technology can be used to investigate these types of interactions.

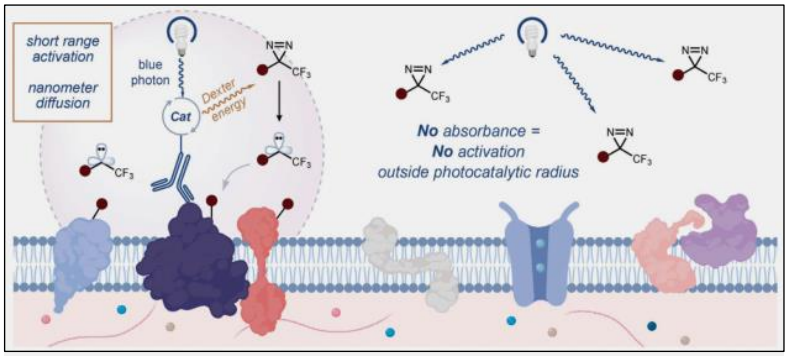
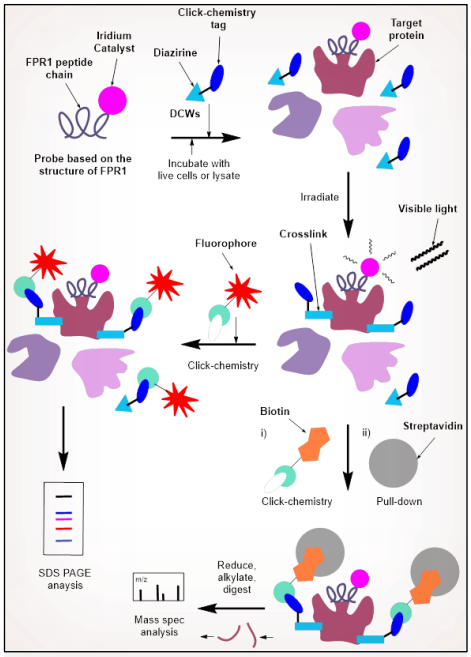


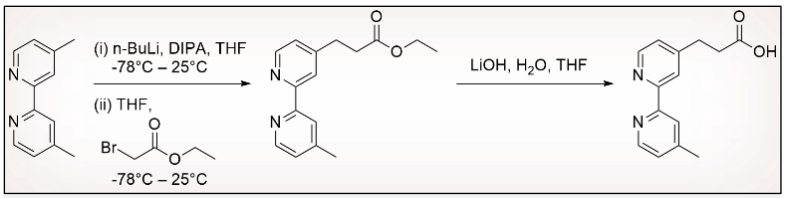
Figure 1: Protein labelling with diazirines can be achieved in proximity to the photocatalyst, while off-target labelling is mitigated, copied from reference (1).



Scheme 1: Project workflow from creation of peptide to SDS-PAGE and pull-down assays.

The workflow begins with the synthesis of the iridium catalyst to be attached to the peptide. This is done by first synthesising a bipyridine ligand with a carboxylic acid group that coordinates to the iridium. This allows the metal complex to be attached to a peptide via a peptide bond at the N-terminus.

The amended peptide is introduced to a cellular system or lysate, followed by diazirines with click-chemistry tags, at which point it is expected that the target proteins will be labelled. Corresponding click-chemistry tags can then be introduced to label proteins with either a fluorophore for SDS-PAGE assay or biotin for pull-down assay (Scheme 1).



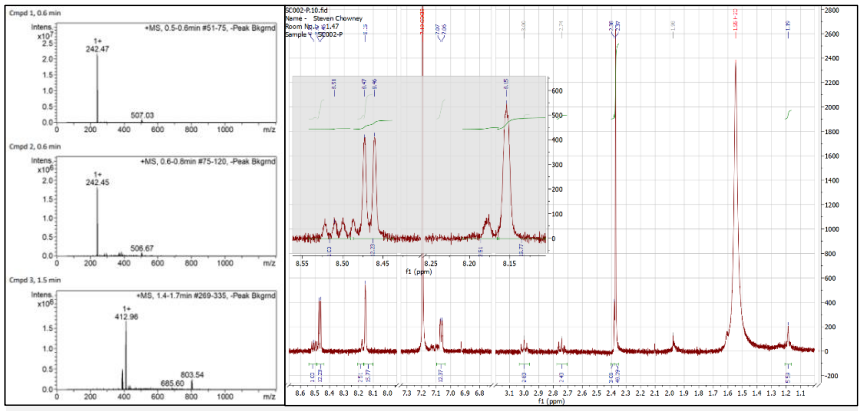
Scheme 2: Synthesis of 3-(4'-Methyl-[2,2'-bipyridin]-4-yl)propanoic acid from 4,4'-dimethyl2,2'-bipyridyl in two steps as described by Trowbridge et al. (2)

Description of work

4'-Methyl-2,2'-bipyridine-4-propionic acid was prepared in a two-step synthesis by the condensation of 4,4'-dimethyl-2,2'-bipyridyl and ethyl 2-bromoacetate followed by ester hydrolysis (Scheme 2), as described by Trowbridge et al. (2). The molecule is highly polar; the pKa of the conjugate acid of bipyridine is 4.3 while carboxylic acid has a pKa of 4.8. This causes the ligand to be difficult to extract from water unless at a specific pH range of

around 4-5. The recommended use of ammonium chloride salts to acidify the solution proved problematic as the quantity of salt required greatly exceeded the mass of the product.

Mass spectroscopy and ^{1}H NMR of the product showed the synthesis had been completed successfully, but isolating it from the salts proved to be impossible. Further attempts to synthesise the compound were made with varied success; the reaction is sensitive to conditions leading either to poor conversion or polymerisation.



Figures 2 and 3: Mass spectrum and ^{1}H NMR spectrum of 4'-methyl-2,2'-bipyridine-4-propionic acid showing successful synthesis.

Discussion of Results

Difficulties with the synthesis of the bipyridine ligand hindered progress with the project substantially. Steps in the synthetic process outlined in the literature, such as acidification of the intermediate compound, did not work as described. For this reason, the project was stuck in it's initial stages for the duration of the studentship as efforts were made to refine the process and yield a suitable compound for further study. The key insights obtained regarding the synthesis of 4'-methyl-2,2'-bipyridine-4-propionic acid is that it is both very sensitive to the ideal reaction conditions, and that acidification and extraction of the product may be best achieved by the careful addition of a strong acid.

The question of whether MicroMap technology can be applied to protein-peptide interactions is yet to be determined, but the opportunities to investigate this are not limited to the scope of this project. The workflow can be easily modified to be compatible with a wide variety of similar projects.

Value of studentship to the student and to the lab

I began my undergraduate studies with the intention of becoming an academic research scientist, and this studentship was my first opportunity to get hands-on experience with my chosen career. Even though little progress had been made in the project by the end of the six-week period, I enjoyed my time in the lab immensely and left with only enthusiasm to continue. From a practical perspective, the studentship has helped me to acquire and hone several skills important to chemical synthesis which will no doubt improve my work throughout my studies and early career.

Steven Chowney (left) and Jacob Webb (right)

The synthetic process I developed during my studentship was frustrated by a lack of accuracy or clarification in the literature, and I hope that the work I have done will ensure that future work undertaken in this area will benefit from my elucidation of these pitfalls.

References

1. Huth, S. W., Oakley, J. V., Seath, C. P., Geri, J. B., Trowbridge, A. D., Parker, D. L., Rodriguez-Rivera, F. P., Schwaid, A. G., Ramil, C., Ryu, K. A., White, C. H., Fadeyi, O. O., Oslund, R. C., & MacMillan, D. W. C. (2023). MMAP Photoproximity labeling enables small molecule binding site mapping. Journal of the American Chemical Society, 145(30), 16289–16296. https://doi.org/10.1021/jacs.3c03325
2. Trowbridge, A. D., Seath, C. P., Rodriguez-Rivera, F. P., Li, B. X., Dul, B. E., Schwaid, A. G., Buksh, B. F., Geri, J. B., Oakley, J. V., Fadeyi, O. O., Oslund, R. C., Ryu, K. A., White, C., Reyes-Robles, T., Tawa, P., Parker, D. L., & MacMillan, D. W. C. (2022). Small molecule photocatalysis enables drug target identification via energy transfer. Proceedings of the National Academy of Sciences, 119(34). https://doi.org/10.1073/pnas.2208077119