Drug Design for the Focal Adhesion Kinase FERM Domain

Conner Maly, Reece Riddle, Timothy Marlowe

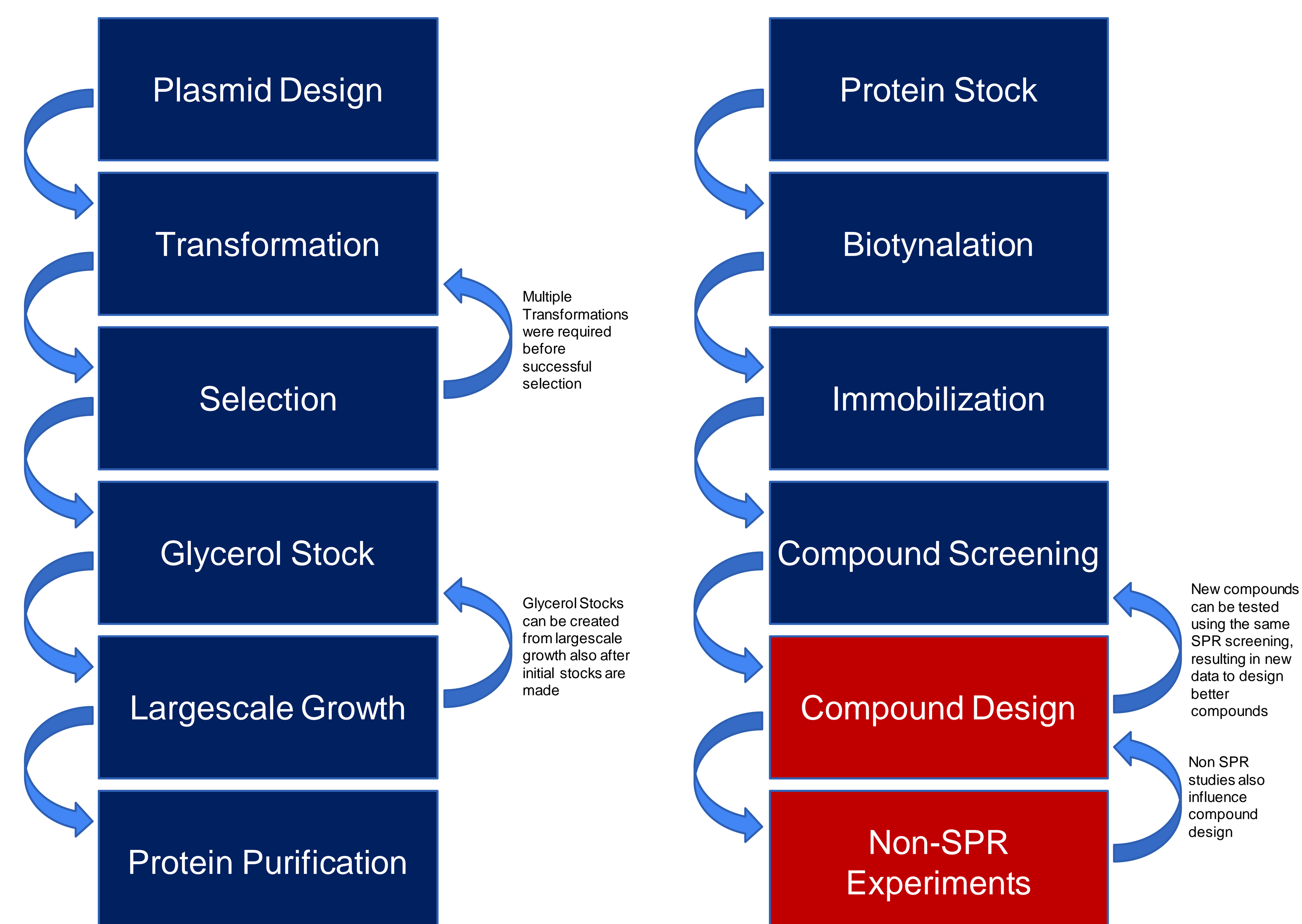
R. Ken Coit College of Pharmacy

Introduction

- Cancer cell activity is dictated by several intracellular processes, one of which involves the focal adhesion kinase (FAK) protein
- FAK is an intracellular scaffold and tyrosine kinase protein that binds to oncogenes, influencing downstream signal transduction and adhesion dynamics for the cell
- FAK is overexpressed in over 80% of solid malignant tumors, well validated in its role in human cancer progression
- FAK protein is composed of three primary domains: FERM, FAT, and a central kinase domain
- The central kinase domain has historically been found to be the primary "druggable" target within FAK, studies in mice having shown decreases in tumor growth, invasion, and metastasis resulting in loss of adhesion and subsequent induction of apoptosis in tumor cells.
- There is less ongoing research exploring the FAT domain and even less of the FERM domain
- Targeting FAK through the FERM domain provides an opportunity for a unique research focus with major clinical implications in the event of discovering druggable targets in cancer cell progression, ultimately improving and even saving patient lives

Methods

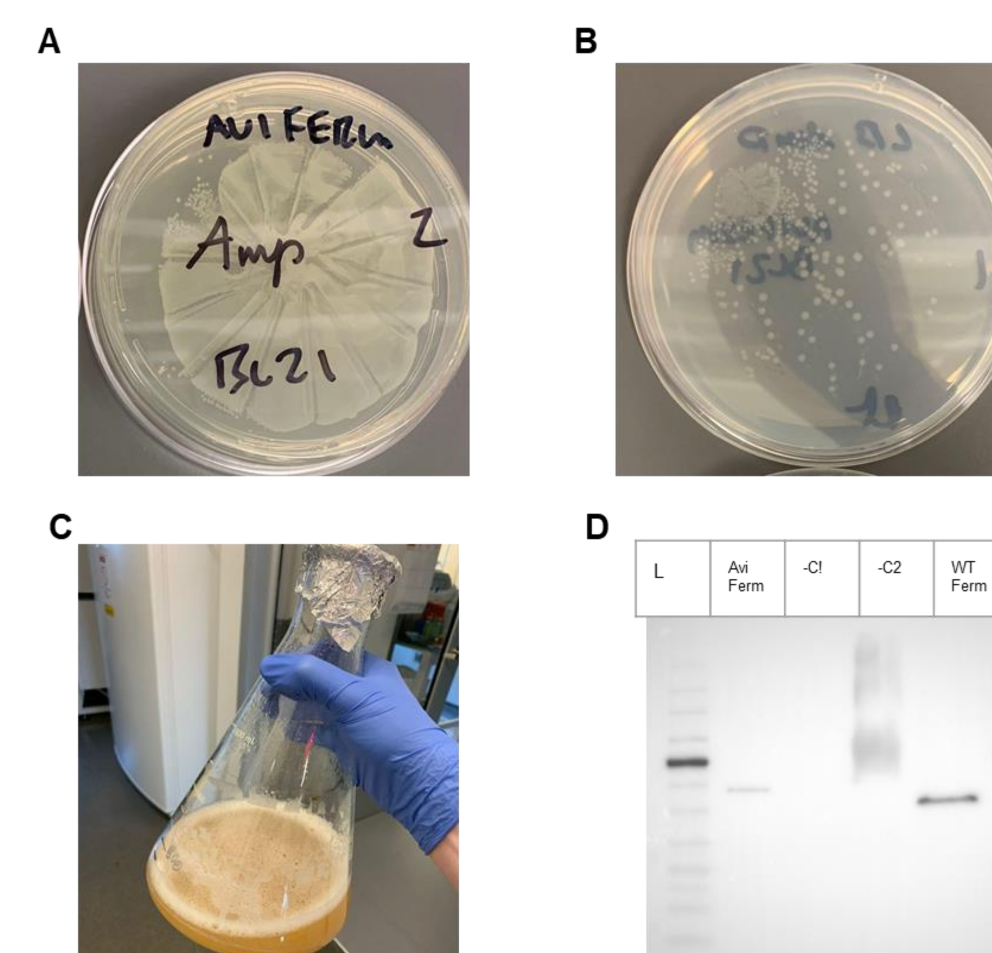
This study was a wet lab pharmacological study where the affinity of molecules was measured directly in a controlled environment.



Results

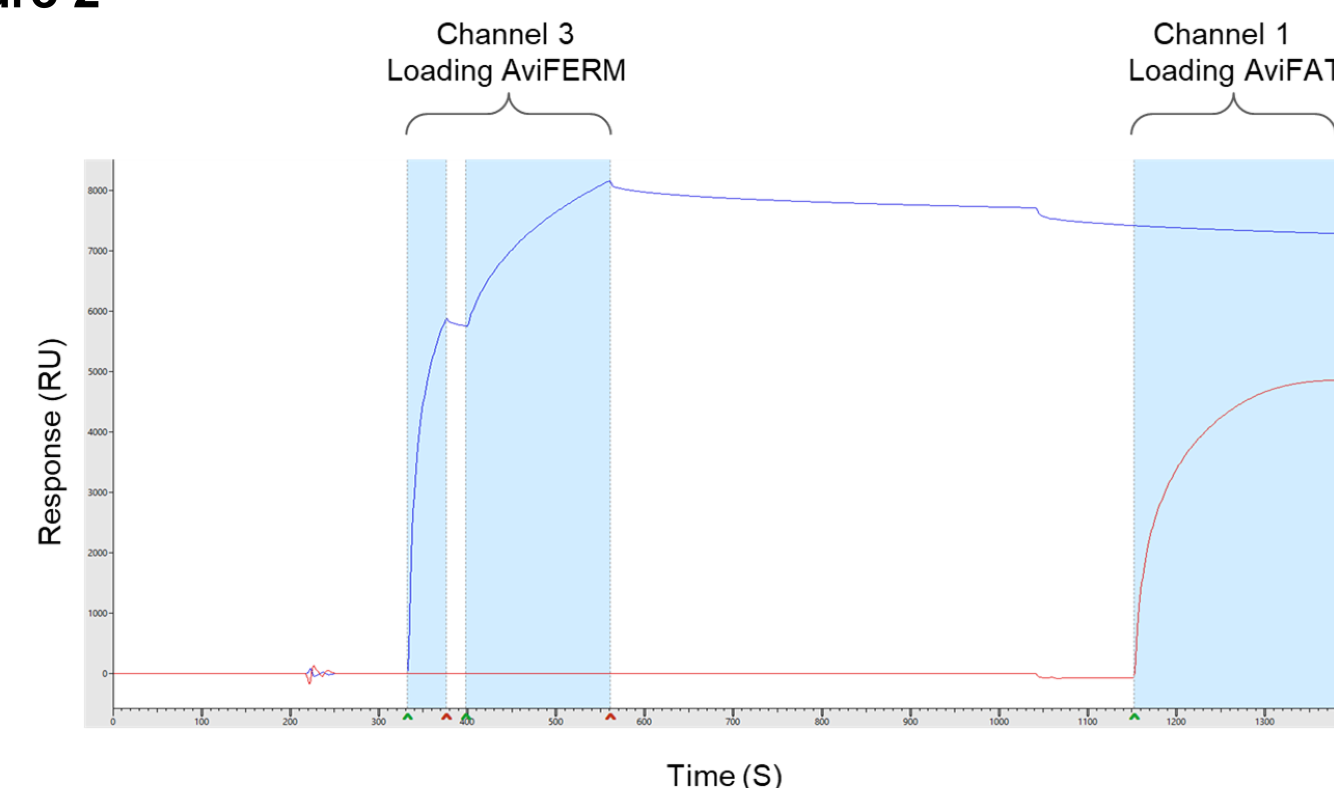
- The AVIFERM plasmid with antibiotic resistance was successfully used to transform E Coli, expression resulting in sufficient amounts of protein for use in the biotinylation procedure (Figure 1).
- Successful biotinylation was confirmed by binding the FERM protein to the SPR chip, measuring ~8000 RU of AVIFERM bound (Figure 2), sufficient for small molecule experiments.
- ~200 molecules from a commercial fragment library were screened for binding (Appendix A). Several fragments showed favorable binding though very few were able to stay bound for long. The most promising fragments were found to have a strong binding and slower dissociation (Figure 3).

Figure 1



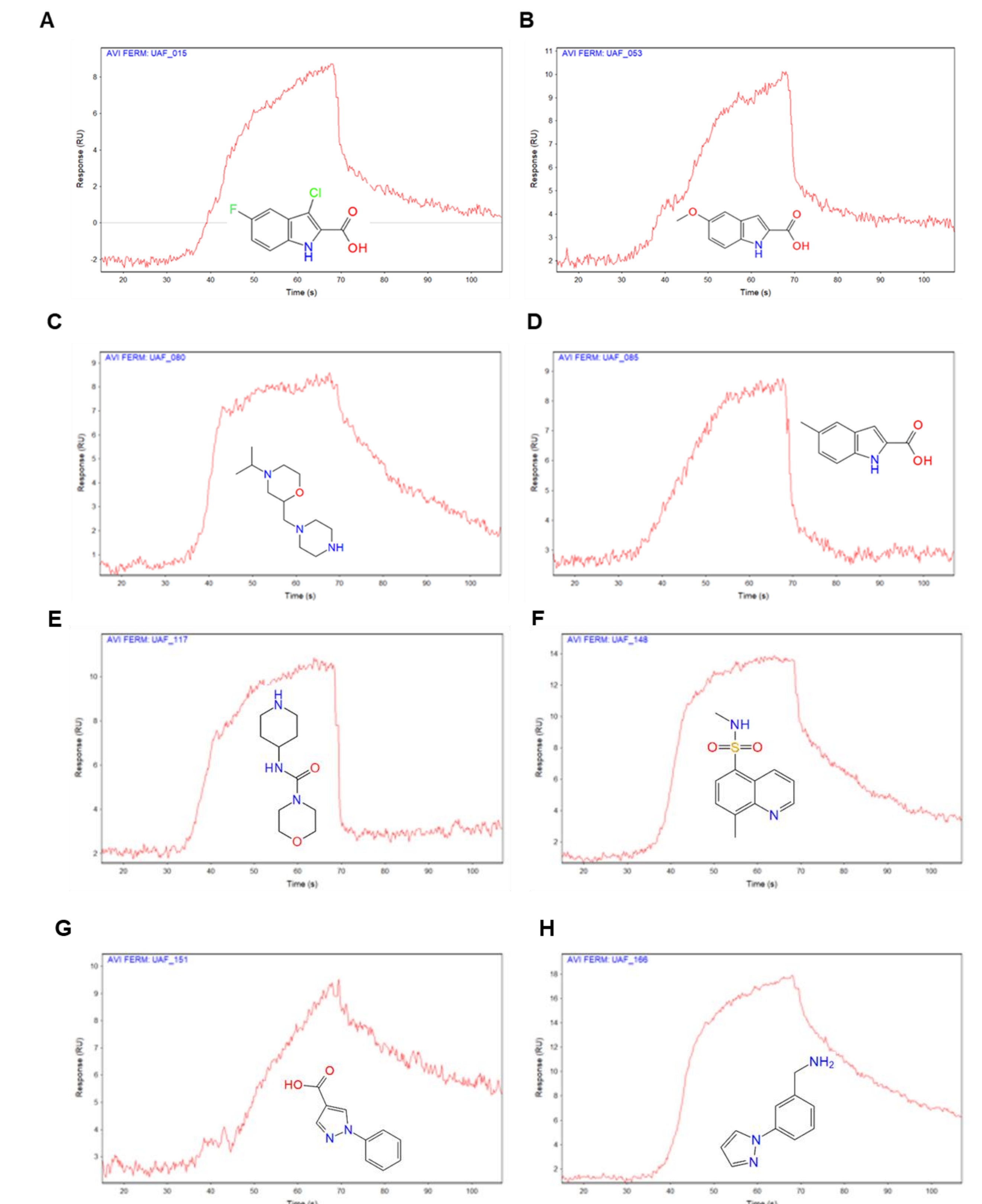
The ampicillin plates (B) showed few selected colonies compared to control plates (A). Colonies from (B) were picked and used in large scale expression in 1-liter Erlenmeyer flasks (C). After purification, the identity of the isolated protein was confirmed to be avi-tagged FERM with western blot (D). The faint band in the AVIFERM column is slightly heavier than the WTFERM band due to the addition of the avitag.

Figure 2



The graph shows the density of the chip over time during the protein loading procedure. White areas indicate washing with buffer, blue areas show when the chip is flooded with protein. AVIFERM was loaded first causing an increase in the RU on the third channel, remaining even after being washed with buffer, indicating that the protein was successfully loaded to the chip.

Figure 3



Curves shows the density of the chip over time. The density on the chip is proportional to the amount of fragment bound to the AVIFERM protein. The fragment molecules are loaded onto the chip until a plateau (Rmax) is reached. The protein is then washed with a buffer, slowly removing fragments, resulting in decreased RU over time. Promising fragments were selected based off of an RUmax of 8 or greater and a slow dissociation.

Discussion and Conclusion

- Several in-vitro binding hits were confirmed in a first screening experiment using a novel avi-tagged FERM domain protein construct, paving the way for future drug discovery in the FERM domain.
- The successful design, expression, characterization, and proof-of-concept experimentation with the novel construct will allow it to be used confidently in future experiments.
- The eight drug-like fragments identified are promising candidates for FERM drug discovery and warrant further research.