Bispecific Chemically Self-Assembling Nanorings (CSANs) with Engineered Fibronectins for Immunotherapy

Engineered non-antibody protein scaffolds have gained interest in recent years to overcome the challenges faced with antibody-based drugs. Several scaffolds have shown promise in both preclinical models and clinical trials. One of these promising scaffolds is the tenth type III domain of human fibronectin, which consists of seven β-strands connected with flexible loops. Analogous to the complimentarity determining regions of antibodies, the amino acid sequences of these loop regions can be engineered to provide target-specific binders with high affinity and specificity. One such target, epidermal growth factor receptor (EGFR) is a well-studied cancer biomarker and a promising target for cancer therapeutics. While some groups have already evolved EGFR-binding fibronectins, their use in cancer immunotherapy applications has not yet been explored.

Our lab has developed Chemically Self-Assembling Nanorings (CSANs) as a non-genetic approach to cell-directed immunotherapies. Multimeric CSANs are formed when a bis-methotrexate dimerizer induces the oligomerization of dihydrofolate reductase-dihydrofolate reductase ($DHFR^2$) fusion proteins. An advantage of the CSAN platform is that it can be easily expanded through fusion of additional protein domains or by chemical modifications to the dimerizer. By fusing an EGFR-targeting fibronectin to the $DHFR^2$ subunits, we formed fibronectin CSANs with both high affinity and high avidity for EGFR-overexpressing tumor cells. Furthermore, we have formed bispecific-CSANs by oligomerizing these fibronectin-$DHFR^2$ subunits alongside anti-CD3 scFv-$DHFR^2$ subunits. A unique advantage of this bispecific CSAN system is the ability to disassemble the CSANs *in vitro* and *in vivo* by introducing the FDA-approved antibiotic, trimethoprim, thus providing control over the T cell-directing activity of the CSAN.

Our previous work suggests that these EGFR/CD3 bispecific CSANs can direct reversible, therapeutic cell-cell interactions. Specifically, we hypothesize that T cells functionalized with EGFR/CD3 bispecific CSANs will be able to selectively recognize and eradicate EGFR-overexpressing tumor cells. Our extensive characterization of these fibronectin-$DHFR^2$ subunits and bispecific CSANs, including cell binding and functional activity experiments, will be presented

Justification:

Since I am a PhD student that will graduate in the next 1-2 years, I would like to use this conference as a way to connect with people in industry and hopefully get a chance to do an internship next summer and/or get a job afterwards. Even if not, this conference will be a good way to see the difference in research in academia vs industry.

As a student in medicinal chemistry department, we do not get seminars on the topics covered in this conference so I will like to get more of an exposure in the cutting-edge science related to my work. Also, our department has limited funding for conference attendance. This will be my first conference in graduate school so I hope to get this scholarship and be able to attend another conference before I graduate-which could likely be the next PEGS meeting.

This conference covers every aspect of my research so I am excited to get some new insights on my projects and potentially start collaborations-which happened to my labmates who attended this conference before. The relevance of my projects, that are not all covered in my poster, to the sections of this conference are as follows: engineering small protein scaffolds to bind to certain targets, looking at bispecific approach for both dual targeting of the tumor and immunotherapy (Engineering and immunotherapy stream), looking at soluble and insoluble proteins and different expression mechanisms, increasing protein yield for bioassays (expression stream), looking into the clinical relevance of the protein therapeutics and their immunogenecity (oncology stream and immunogenicity&bioassays stream), conjugating a drug molecule (doxorubicin) to the targeted nanoring construct to offer a novel antibody-drug conjugate type of approach (bioconjugates stream). Moreover, I could benefit other researchers by sharing my experiences and background reading in these areas.