Reversible Cell Membrane Engineering with EpCAM-Targeted Fibronectin Domains

Clifford M. Csizmar[1], Jacob R. Petersburg[1], Lawrence A. Stern[2], Benjamin J. Hackel[2], & Carston R. Wagner[1]

Departments of Medicinal Chemistry[1] and Chemical Engineering and Material Science[2]
University of Minnesota, Minneapolis, MN 55455

Targeted molecular recognition continues to be a cornerstone of contemporary therapeutics and diagnostics. Though antibodies are commonly employed as targeting scaffolds, their large size, long plasma half life, and lack of facile bioconjugation make antibody-based ligands less than ideal for many basic and clinical applications. As the repertoire of clinically relevant biomarkers continues to grow, there is an increasing need to develop new agents capable of selectively identifying and targeting these markers.

To meet this need, we have engineered a targeted protein scaffold based upon the human tenth type III fibronectin domain that binds the highly overexpressed carcinoma antigen epithelial cell adhesion molecule (EpCAM). Using yeast surface display, mammalian cell panning, and a novel titratable avidity-reduction selection technique, we successfully evolved fibronectin clones exhibiting high affinity (11 nM) and robust selectivity for cellular EpCAM. These new selection methodologies can be applied to other cellular biomarkers and should increase the success rate of isolating scaffolds whose binding efficacy translates to *in vivo* applications.

Furthermore, we incorporated these EpCAM-targeting ligands into a multivalent chemically self-assembled nanoring (CSAN) for use as a cell-directing scaffold. When the CSAN is functionalized with our anti-EpCAM fibronectins and monovalent streptavidin, heterobifunctional CSANs are formed. These bispecific nanorings are capable of interfacing between EpCAM-expressing tumor cells and biotinylated surfaces – including other cells whose membranes have been viably modified with biotinylated phospholipids.

Importantly, the CSAN scaffold can be disassembled via exposure to the FDA-approved antibiotic trimethoprim, providing a pharmacologic mechanism for reversing the interactions. Thus, we hypothesize that these bispecific CSANs can serve as a modular scaffold for directing reversible, therapeutic cell-cell interactions. Our work to develop this platform will be presented.