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Engineering a Bispecific Protein from a Single-Domain, Bivalent Protein Scaffold

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In medical applications, bispecific proteins offer both increased selectivity to disease sites and increased efficacy through engaging or inhibiting more than one biological pathway. Traditionally, researchers have created bispecific molecules by genetically or chemically linking together protein domains such as antibody fragments, each with a single binding epitope. As an alternative to this method, we explored the possibility of using a single-domain scaffold with dual binding epitopes. The advantages of this approach include maintaining a constant molecular size in converting from a monospecific into a bispecific protein and bypassing the need to optimize the linkage of distinct scaffold parts. This study represents a unique example in which the binding epitopes of a naturally bivalent scaffold are utilized as engineering starting points for a bispecific protein.

Our model scaffold is a monospecific, bivalent protein with an epitope on each end that recognizes an essential angiogenic cell surface receptor. The scaffold is thermally and chemically stable and is readily produced by microbial expression, rendering it amenable for therapeutic development. First, we demonstrated the ability of this bivalent protein to tolerate extreme mutagenesis in one of its binding epitopes while maintaining structural integrity and binding on the non-mutated end towards its native receptor. Then, we affinity engineered the mutated binding epitope towards the model proteins maltose-binding protein and streptavidin, as well as a clinically-relevant target. These experiments showed that the scaffold behaved well when used to create a stable naïve protein library, and can be used to discover protein variants that bind various medically-relevant targets on one end, while retaining high affinity binding to the native receptor on the other. More generally, this study expands the concept of a protein scaffold to include protein domains with multiple functional interfaces and suggests a novel strategy of engineering bispecific proteins.