13e Engineering of High Affinity Binding Peptides Using N-Terminal Bacterial Display

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Display library technologies play a central role in protein engineering applications, from drug discovery to biomineralization. Bacterial display holds the potential to expedite the process of isolating desired binding proteins, but has been slow to mature owing to technical limitations. One such limitation is the inability to display passengers efficiently as N-terminal fusions. To address this issue, a large constrained peptide library ($X_2C(X)_7CX_2$) (10^{10} variants) was displayed via the N-terminus of a circularly permuted variant of outer membrane protein X (OmpX) in *E. coli*. For comparison, using native OmpX protein an otherwise identical peptide library was constructed within an extracellular loop as a sandwich fusion. To evaluate the two display platforms, each library was screened against streptavidin as a model target using sequential magnetic and fluorescence activated cell sorting. Individual binding clones were obtained in just two to three days. A common three-residue streptavidin binding motif, HPQ, and a similar motif, HPM, were isolated using both the sandwich fusion and N-terminal display scaffolds. The N-terminal display format yielded a unique, high affinity motif, CGWMYYXEC, having six consensus residues. One of the isolated peptides, expressed as a terminal fusion to a monomeric fluorescent protein, possessed a dissociation rate constant of $2x10^{-3}$ s^{-1}, a value twenty fold improved relative to the best clone with an HPQ motif. In addition to displaying peptides that allow for single disulfide constrained loops, peptide scaffolds that incorporate multiple cysteines have been used, such as an α-conotoxin and cyclotide scaffold. Within these scaffolds libraries were created with select residues randomized. Both libraries were successfully screened for peptides binding to streptavidin, and strong consensus sequences emerged. The peptide display libraries were also used to isolate sequences that bind specifically to pharmaceutically interesting proteins, such as VEGF. To our knowledge, this is the first successful demonstration of an N-terminal bacterial display peptide library using a circularly permuted transmembrane protein, and the first successful use of libraries incorporating a cyclotide scaffold. This novel bacterial display platform offers new opportunities for investigating protein-peptide interactions as well as a means to rapidly isolate and engineer high-affinity, specific protein binding peptides.