

530f Engineering and Selection of Ligand-Binding Gfp Variants and Antibodies Via Tat Display

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“Fluorobodies” – hybrid molecules that combine the affinity of an antibody with the intrinsic fluorescence of GFP – hold great promise for diagnostics applications. However, so far it has not been possible to engineer fluorescent protein mutants with ligand binding affinity. This is in part due to biosynthetic limitations that preclude the display of fluorescent GFP libraries and the subsequent selection of binding mutants. We developed a Twin Arginine Translocation (Tat)-dependent filamentous phage display platform that allows the GFP to properly fold in its fluorescent form in the cytoplasm prior to its export via the Tat export pathway. In this manner libraries of random peptides could be inserted into loops of GFP for the selection of mutant proteins exhibiting both ligand binding and high fluorescence. Similarly, we have developed display systems that capitalize on the *Escherichia coli* Tat secretion pathway for display of scFv antibodies, thus affording the isolation of clones with unique features.