

Generation of antibody-Fab reagents to capture and stabilize functionally important conformational states of proteins to facilitate their structure determination by crystallography and single particle Cryo-EM

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A high throughput phage display pipeline has been established to generate Fab-based synthetic antibodies that have been successfully used as high performance crystallization chaperones. The phage selection strategies have been developed to target Fab binding to specific surface epitopes or to capture and stabilize functional conformational states to facilitate probing both the protein's static and dynamic features, as well as the transitions between states. In most cases, the high throughput capability of the pipeline provides researchers with at least 10 unique Fabs that perform a desired function. A further enhancement for the generation of Fabs for membrane proteins is the use of lipid-filled nanodiscs to provide membrane-like environments for membrane proteins during phage display selections. Fabs generated from nanodisc formats provide guidelines for which detergents best (and worst) mimic the protein's membrane-like lipid environment, which can guide choices for setting up crystallization and Cryo-EM experiments.