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High-throughput Generation of Recombinant _{CCP}Fabs for Intransigent Protein Cocrystallization.

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Crystallization is often the rate-limiting factor in the production of protein atomic structures. The central hypothesis here is that many proteins will co-crystallize as binary complexes when they will not crystallize alone. Co-crystallization recombinant antibody fragments (_{CCP}Fabs) are antibody fragments derived from a parent antibody fragment that can be used to assemble a 3- dimensional matrix (crystal). We are developing such an antibody-based (Fab) system to generate co-crystallization proteins (CCPs), useful for co-crystallizing intransigent proteins. Several techniques and their optimizations such as use of auto induction media and “automated HPLC” with thiophilic adsorption and metal chelate chromatography have been used to isolate Fab4, our model antibody fragment at the level of 12mg/L. A previously built synthetic Fab library with phage display technology has been utilized to select for Fabs that recognize target proteins. Target proteins include prokaryotic transcriptional regulators such as MerR, FlgR, BenM, CatM as well as drug metabolic targets like cytochrome p450. The purified, isolated _{CCP}Fab is used to prepare target protein-_{CCP}Fab complexes. The application of high-throughput methods to the preparation, evaluation and crystallization of these target protein-_{CCP}Fabs will allow the production of at least 7 purified _{CCP}Fabs per day.