

High Throughput Production of Fabs for Macromolecular Cocrystallization

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Background

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 ($_{\text{CCP}}$ Fabs) are antibody fragments derived from a parent antibody fragment that can be used to assemble a 3- dimensional matrix (crystal). We are developing an antibody-based (Fab) system to make co-crystallization proteins (CCPs) useful for co-crystallizing intransigent proteins.

Methods

We have generated a non-immune phagemid recombinant antibody fragment (Fab) library derived from a mouse monoclonal antibody. Several target proteins such as prokaryotic transcriptional regulators like MerR, FlgR, BenM, and CatM have been panned against this library to produce rFab fragments specific to these proteins. Paramagnetic bead (Dynabeads[®]) immobilization of targets and URSA (Ultra Rapid Selection of Antibodies) were used to make the selection (panning) process scalable to high throughput and to ensure selection of antibodies to proteins that are in as “native” a state as possible. High throughput production of these Fabs has been optimized using our model antibody construct pET28-Fab4. This construct has an engineered binding site that binds to metal-chelate media. 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 highly purified Fab4.

Results

1. Using the phage display library, we have generated positive clones against 7 test proteins. (Kelley and Momany, 2003 *BioTechniques* 35:750-758).
2. We have optimized our production protocol to isolate Fab4, our model antibody fragment at the level of 12 mg/L.
3. All these steps are designed to be performed in a high-throughput production mode.

Conclusion

Implementation of the high-throughput methods will allow high yield production of at least 7 purified $_{\text{CCP}}$ Fabs per day in quantities useful for cocrystallization. The Fab reagents generated could also be downstream in proteomic projects as biochemical probes and biosensors. Fabs to specific proteins can also be useful in areas like micelle production for therapeutics.