

**W0271**

**High-throughput Protein Production for Macromolecule Co-crystallization.** Ashwini Nadkarni, Cory Momany, Laura-Lee Clancy Kelley, Dept. of Pharmaceutical & Biomedical Sciences, College of Pharmacy, Univ. of Georgia, Athens, GA 30602 USA.

Crystallization as an intermediate to produce protein structures is often the rate-limiting factor. We are developing an antibody-based (Fab) system to make co-crystallization proteins (CCPs), useful for co-crystallizing intransigent proteins. Our antibody construct pET28-Fab4 has an engineered binding site which binds immobilized metal-chelate media. A combination of ammonium sulfate precipitation, thiophilic absorption and metal-chelate chromatography techniques help to isolate Fab4 directly from the media at about 5 mg/L. High level production of functional Fab fragments has been shown in an oxidizing bacterial cytoplasm. (Venturi *et al*; 2002 *J.Mol.Bio* 315, 1-8) This overcomes the need for secretion, leading to optimized Fab4 production. Other optimizations include the use of rare codon tRNA-expressing strains of *E. coli* and media modifications for IPTG-free induction. All these steps can be performed in a high-throughput production mode. A previously built phage display <sub>CCP</sub>Fab library was used to generate positive clones against 7 test proteins. (Kelley and Momany, 2003 *BioTechniques* 35:750-758). Implementation of the high-throughput methods will allow high yield production of at least 7 purified <sub>CCP</sub>Fabs per day.