

E0015

Chaperone-Assisted Crystallography. A. Kossiakoff¹, S. Koide¹, F. Fellouse², D. Sidhu². ¹Dept. of Biochemistry and Molecular Biology, Univ. of Chicago, Chicago, IL 60637, ²Dept. of Protein Engineering, Genentech, 1 DNA Way S. San Francisco, 94080 USA.

We have developed a powerful methodology called “chaperone-assisted crystallography” (CAC) to facilitate the determination of the most challenging classes of structural biology problems. CAC is based on using antibody fragments (Fabs) targeted to a specific protein entity to act as potential crystallization chaperones. The concept has been shown to work spectacularly well, especially with regard to membrane proteins; however, the previously used hybridoma methodology to produce Fabs has been fraught with technical difficulties. We have overcome those difficulties using a novel phage display approach that exploits the use of a “reduced genetic code” in an innovative way to greatly expand the number of sites that can be randomized in the phage display selections. This represents a complete paradigm shift in phage display strategies. We have designed and tested libraries using only 2-4 amino acid types that have produced nM Fab binders to soluble proteins, membrane proteins and functional RNAs. SeMet sites are incorporated in the Fab fragments eliminating the need to introduce them in the protein target itself. The phasing power of the SeMet + molecular replacement contributions from the Fab greatly simplify structure solution.