Engineering hyper-crystallizable single chain antibodies for crystallization scaffold

Jennifer C. Pai[1] and Jennifer Maynard[1], (1)Department of Chemical Engineering, University of Texas at Austin, Austin, TX, 78752

Crystallization of protein has been limited by expression and purification of the protein as well as the identification of conditions that promote crystal growth. Using protein engineering, the protein can become the variable in crystallography thereby offering a promising new pathway to the production of high-quality crystals. Varying amino acids result in altering the biophysical parameters of the protein which include, but are not limited to, expression level, ligand binding affinity, stability and immunogenicity. Manipulation of amino acid sequence was done with both directed and random evolution with the purpose of creating a hyper-crystallizable single chain antibody.

Genes for a hyper-crystallizable single chain antibody (scFv/scAb), were cloned and expressed in E. coli bacteria. The antibody was engineered with the capability of crystallizing in multiple space groups and lattice structures and intended for the development of a crystallization scaffold. The utility of this protein is its specificity and binding for a peptide tag (EE tag; amino acid sequence EYMPME), which can be appended to any protein of interest. CDRs with this EE-tag specificity have been grafted onto frameworks of previously crystallized antibodies. Biophysical properties of these constructs were characterized and recorded. The antibodies were evolved using mutator cells with multiple rounds of growth, selecting for variants with improved biophysical characteristics correlating with crystallizability. Directed evolution was performed to preserve amino acids that were significant in maintaining crystal lattice contacts. *CCP4 program suite for protein crystallography* was used to derive locations for directed evolution. These locations were selected based on surface area energetic and crystallographic work on the original antibodies from which the grafts derived their framework. Variants selected by phage display had significant EE-tag binding affinity, soluble expression and high stability. The engineered single chain antibodies produced were tested for their ability to crystallize independently using the Phoenix robot at the University of Texas.

The generation of a hypercrystallizable antibody will allow for a readily formed complex with any second protein of interest by simply attaching the EE-tag. Its use can then be extended to create scaffolds aiding the crystallization of proteins that are otherwise difficult to crystallize, producing a general engineering approach to creating crystalline proteins. This work allowed better understanding of the correlates for crystallization from biophysical parameters and energetics of proteins. The ability to crystallize biomolecular structures will expand our ultimate goal of understanding and manipulating proteins for improved properties and therapeutic treatments. Long term goals are the development of crystal based separation processes; potentially allowing us to separate out distinct proteins of interest regardless of its size.