Protein Engineering of Spectrin Isoforms

We investigate structural properties of proteins to understand disease mechanisms and to develop therapeutic agents. In this project, we focus on a family of proteins, spectrin isoforms, which are in most cell types. We study red blood cell and brain spectrin isoforms and their mutants. Spectrin maintains the unique shape of red blood cells. Mutations in spectrin cause hemolytic anemia diseases such as hereditary elliptocytosis. In neural cells, brain spectrin anchors membrane receptors to the cytoskeleton and regulates the function and/or morphology of excitatory synapses.[1-5] It is a major component of postsynaptic densities. It has been found that Alzheimer patients have more spectrin break down antibodies. The functional form of both red blood cell and brain spectrin isoforms is the spectrin tetramer. We study spectrin tetramer formation. We identified human brain proteins and other proteins that interact with spectrin. We study effects of these proteins on spectrin tetramer formation. We use biophysical methods to study structures, molecular interaction and protein-protein association affinities. The methods include EPR and fluorescence spectroscopies, with cysteine scanning for site directed labeling, small angle x-ray scattering, x-ray crystallography, solution NMR spectroscopy, homology modeling, and molecular dynamic simulation for structural and interaction information. We use isothermal titration calorimetry methods to measure equilibrium constant of association/dissociation of protein molecules. We use recombinant DNA methods to engineer mutations in model proteins of spectrin to study mutational effects on spectrin structure and function.[6-10]

Projects for REU students include protein engineering, protein purification, and biophysical studies of proteins. The students may be involved in one, more or all of these projects. For example, students may use homology modeling to design a mutation site for protein engineering, prepare engineered protein, and use isothermal titration calorimetry methods to study its association with its binding partner (Figure 6).

Figure 6. X-ray structure of the first 147 amino acid residues of brain α-spectrin

Students will learn about research techniques in biochemistry, biophysics and protein engineering. They will also learn how to design an experiment, to analyze data and present data. Students will also receive training in time management and scientific writing.

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