Abstract:

EXPRESION AND PURIFICATION OF ARRESTIN MUTANTS

Kirsten A. Carlson[1], Derek J. Francis[2], Candice S. Klug*[2] [1]Department of Chemistry, Ripon College, Ripon, WI 54971
[2]Department of Biophysics, Medical College of Wisconsin, Milwaukee, WI 53222,
candice@mcw.edu

Arrestin is a protein that is involved in signaling pathways in the cell. Visual arrestin is important in the eye because of its interactions with rhodopsin and involvement in the visual signal transduction pathway. A crystal structure for arrestin has been solved but there is some discrepancy about the secondary structure of several key regions of the protein. The main focus of this study was to determine the secondary structure of one of these regions in solution using site-directed spin labeling electron paramagnetic resonance (EPR) spectroscopy. The focus of this presentation is the preparation of protein samples to be used in the EPR studies, including the expression, purification, and spin labeling of several different mutant proteins. Total amounts of the three mutants purified are given, as well as an explanation of expected results had EPR been performed.