

E0061

Structural and Kinetic Studies of the "Kindling Fluorescent Protein" (KFP). Nathan Henderson, Mike Quillin, Xiaokun Shu, Jim Remington, Univ. of Oregon.

asFP595 is a GFP-like protein that displays unusual fluorescence properties. In the dark-adapted state the protein is essentially nonfluorescent but when exposed to light in the range of 540-570 nm, fluorescence intensity gradually increases. Due to this unique behavior, asFP595 is referred to as the "kindling fluorescent protein" (KFP). Kindled asFP595 thermally relaxes back to the ground state with a time constant of ~100 seconds at room temperature, or it can be instantly quenched by illumination at 450 nm. It has been proposed that transition of KFP from the dark-adapted to light-activated state involves a chromophore *cis-trans* isomerization. Consistent with this hypothesis, microspectrophotometer experiments reveal an energy barrier to thermal relaxation of kindled KFP of ~71 kJ/mol, compared to ~55 kJ/mol observed for *cis-trans* isomerization of a model GFP chromophore in solution.

We are studying the kindling phenomenon with a combination of x-ray crystallography, site-directed mutagenesis, spectroscopy, and kinetic analysis. The dark state structure of KFP has been solved to 1.38 Å and structural studies of the light-activated structure are underway.