

Photo Crosslinking Rhodopsin to Transducin

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While in Gobind's lab I worked on developing a chemical approach to determining contact sites between rhodopsin to its G-protein transducin. We prepared rhodopsin mutants that contained a single reactive cysteine residue per rhodopsin molecule at position 65, 140, 240, or 316 on the cytoplasmic face. A carbene-generating photoactivatable group was linked by a disulfide bond to the cysteine sulfhydryl group of each of the rhodopsin mutants. The resulting derivative was then light-activated at $\lambda > 495$ nm to form the metarhodopsin II intermediate, which bound transducin. Subsequent photoactivation (355 nm) of the carbene-generating group resulted in crosslinking of the rhodopsin mutant carrying a cysteine residue at position 240 to transducin. This crosslinking was determined to be specifically with the alpha subunit of transducin. An alternative reaction observed during photolysis of the rhodopsin mutants was intramolecular insertion of the carbene into rhodopsin.