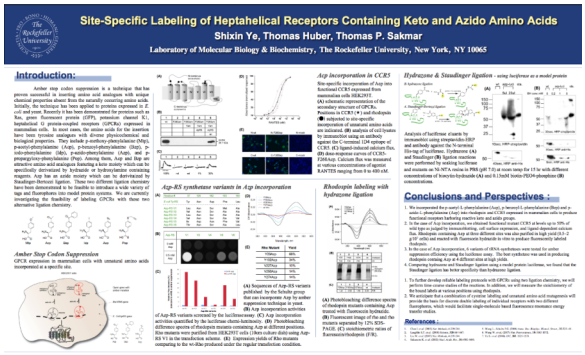
Application of unnatural amino acid mutagenesis in studies of G protein-coupled receptors.



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G protein-coupled receptors (GPCRs) comprise a superfamily that mediates important and diverse actions of extracellular signals. Activation of these receptors requires protein conformational changes. The lack of general biophysical and biochemical techniques make the study of the conformational changes of GPCR activation difficult. We are currently developing chemical tools that allow us to introduce spectroscopic probes and fluorescent labels by inserting an unnatural amino acid (UAA) at unique sites in a receptor. The incorporation of a UAA is achieved by the amber codon suppression technology, which involves read-through of the amber stop codon in an mRNA by a UAA aminoacylated suppressor tRNA. In this presentation, I will focus on our progress in the incorporation of fluorescent probes for the structure and functional studies of rhodopsin, a prototypical GPCR responsible for the dim light vision. We believe that the generality of the approach will make it feasible to the studies of other GPCRs. This will substantially advance both the analysis of the mechanism of receptor activation and the use of fluorescent methods in ligand screening.