Probing GPCR Signaling with Genetically-Encoded Non-Natural Amino Acids

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We are interested in uncovering the principles that underlie ligand recognition in heptahelical G protein-coupled receptors (GPCRs) and to understand with chemical precision how receptors change conformation in the membrane bilayer when ligands bind. We have developed an interdisciplinary approach that employs a number of new converging technologies: i) all atom and coarse grain molecular dynamics (MD) computer simulations of GPCRs in membrane bilayers in concert with experimental validation, ii) unnatural amino acid mutagenesis of GPCRs using amber codon suppression technology, iii) targeted photocrosslinking and bioorthogonal chemical labeling of engineered GPCRs, iv) interrogation of receptor dynamics using advanced FTIR (Fourier-transform infrared spectroscopy) and solid state NMR methods, v) use of nanoscale apolipoprotein bound bilayers (NABBs) as membrane mimic support structures for GPCRs. Our near-term aim is to employ single-molecule detection (SMD) of GPCRs by TIRF (total-internal reflectance fluorescence) microscopy in self-assembling oriented tethered bilayers or in NABBs using microfluidics. This talk will focus on unnatural amino acid mutagenesis as a tool for targeted photocrosslinking methods and bioorthogonal labeling of heptahelical receptors in live cells.