Probing neuroreceptors and ion channels with non-canonical amino acids

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We describe one strategy for incorporating non-canonical amino acids site-specifically into proteins expressed in living cells, involving organic synthesis to chemically aminoacylate a suppressor tRNA, protein expression in *Xenopus* oocytes, and, primarily, monitoring protein function by electrophysiology. With this protocol, a very wide range of non-canonical amino acids can be employed, allowing both systematic structure-function studies and the incorporation of reactive functionality. Here we present an overview of the methodology and examples meant to illustrate the versatility and power of the method as a tool for investigating protein structure and function. Our focus has been on neuroreceptors and ion channels, complex integral membrane proteins for which structural data are limited. A special emphasis has been the cation-π interaction, and we have been able to use non-canonical amino acids to establish the cation-π interaction as a general binding motif for neuroreceptors. In addition, we have used non-canonical amino acids to probe hydrogen bonding to the protein backbone and to incorporate fluorescent amino acids.