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Generation of designer receptors exclusively activated by designer drugs (DREADDs) using directed molecular evolution

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Abstract

G protein-coupled receptors (GPCRs) and their signal transductions are important for both physiological and pathological processes in neuron systems. Neuronal GPCRs activated by synthetic ligands have been created by designed mutagenesis for studying their functions and signal pathways. However, these engineered GPCRs have problems, such as their high constitutive activity. To overcome this drawback, a new generation of receptors termed designer receptors exclusively activated by designer drugs (DREADDs), have been designed. DREADDs are exclusively activated by synthetic ligands, but are insensitive to their endogenous ligand and have no constitutive activity, which provides the ability to selectively modulate signal transduction of certain GPCRs in vitro and in vivo. This protocol provides detailed instructions for creating DREADDs using directed molecular evolution. The procedures to generate DREADDS include GPCR functional expression in yeast, mutant GPCR library generation, and high-throughput yeast screening. These methods are general and suitable for any GPCRs that can be functionally expressed in yeast.