P-1.10

Directed Evolution of the Human Kappa Opioid Receptor for Enhanced Solubility

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G-protein-coupled receptors (GPCRs) are membrane proteins that mediate cellular responses to various signaling molecules, including hormones, neurotransmitters, and environmental stimuli. Due to their transmembrane structure, working with GPCRs outside the membrane is challenging, as their structure often destabilizes, making it difficult to determine their biophysical properties. Our goal is to develop soluble human kappa opioid receptors (hKOR) using directed evolution methods to facilitate the analysis of their biophysical features. Using ribosome display enabled us to narrow down a library of variants. In the next step, selection was performed using yeast display, where variants underwent selective pressure through fluorescence-activated cell sorting (FACS). After successfully introducing the hKOR gene into the Saccharomyces cerevisiae strain EBY100 via homologous recombination, full-length and correctly folded hKOR variants were selected by FACS. In the next phase, we will focus on selecting variants that bind the natural hKOR ligand, dynorphin, through iterative rounds of FACS. This approach aims to isolate functional receptor variants with improved solubility and enhanced ligand-binding properties. Funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under project No. 09I01-03-V04-00041.