G protein coupled receptors (GPCRs) constitute the largest group of cell-surface receptors found in nature. GPCRs are involved in all kinds of signaling processes, giving this class of proteins enormous pharmacological relevance. However, our understanding of GPCR architecture and mechanism has remained limited, and the design features of agonists and antagonists for the diverse set of receptors have remained mostly enigmatic.

Previous data suggested that the main limitations in GPCR research, namely low expression levels and instability, might be attributable to poor biophysical properties. Directed evolution approaches conducted in our laboratory were successful, but not exhaustive enough to understand why certain sequences were selected. Hence, we performed saturation mutagenesis of every position of the neurotensin receptor 1 individually. 380 libraries were expressed in *E. coli* and selected for high expression using a FACS-based approach. Selected variants were analyzed using ultra-deep 454-sequencing. From this study we could deduce, for each position, which amino acids are not acceptable, acceptable and preferred. The results gave insight into the influence of every single position on expression level and biophysical stability of the receptor. Advantageous mutations were combined and shuffled by *in vitro*-DNA recombination and selected for high functional expression. The isolated mutants display expression levels of up to 25,000 functional receptors in *E. coli*, and are by far more detergent-stable than any previously reported mutated receptor, while retaining G-protein coupling.

The knowledge gained from this study can be used to make the GPCR family more accessible to functional and structural studies.