STRATEGIES TO ENGINEER G PROTEIN-COUPLED RECEPTOR LIGAND BINDING PROPERTIES

Justin Yoo, Department of Chemical Engineering, University of California Santa Barbara
Justin_yoo@umail.ucsb.edu
Patrick Daugherty, Department of Chemical Engineering, University of California Santa Barbara
Michelle O'Malley, Department of Chemical Engineering, University of California Santa Barbara

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G protein-coupled receptors (GPCRs) comprise a family of integral membrane proteins that mediate eukaryotic cells' responses to a wide array of extracellular signals. As a result of their ligand specificity, sensitivity, and capacity for signal transduction, GPCRs have great potential as biosensors for a wide range of molecules (e.g. toxins, value-added products, biomarkers for disease). Despite their inherent advantages, many GPCRs are difficult to express functionally and in high numbers within heterologous hosts such as yeast. Thus, there is a need to engineer functionally expressed GPCRs to bind ligands of interest with high affinities and specificities. Towards this end, we have optimized a high-throughput screening methodology to engineer variants of the human adenosine A_{2A} receptor ($hA_{2A}R$) with improved binding affinity towards a target ligand. Specifically, a fluorescent ligand binding assay was used in concert with fluorescence-activated cell sorting (FACS) to isolate yeast cells expressing desirable $hA_{2A}R$ mutants. After four rounds of sorting, we observed convergence of mutated residues towards a consensus sequence and a 3.5-fold increase in cellular mean fluorescence intensity upon incubation with fluorescent ligand. Additionally, we demonstrate the importance of vector choice and concomitant mitotic stability in influencing $hA_{2A}R$ yield and cellular homogeneity in yeast. The use of a mitotically stable integrating vector results in increased GPCR yield compared to non-integrating (i.e. centromeric and episomal) vectors. Yields of $hA_{2A}R$ are improved further by increasing vector integration frequency, where gene copy number is shown to have a greater effect on protein yield at lower relative copy numbers. Further, the growth of cells in raffinose-containing media prior to gene induction is shown to improve cellular homogeneity of yeast expressing $hA_{2A}R$ under the control of an inducible galactose promoter. In all, these results are envisioned to benefit both GPCR expression and engineering in yeast. The use of this platform to further evolve and isolate improved $hA_{2A}R$ variants is expected to generate mutants with even greater binding affinity and specificity towards ligands of interest.

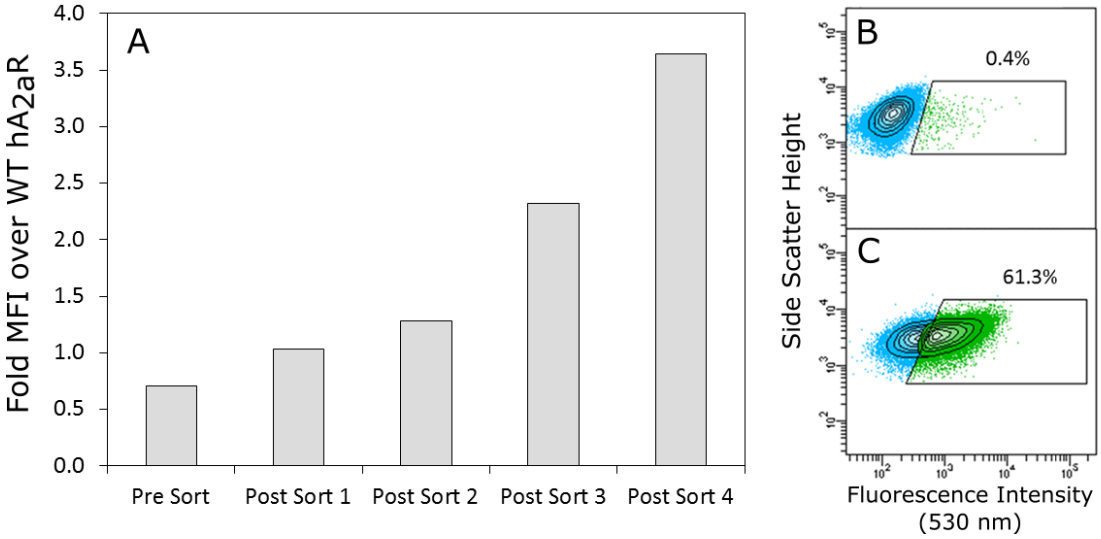

Figure 1 – Fluorescence-activated cell sorting of yeast harboring an $hA_{2A}R$ library. (A) Fold mean fluorescence intensity of cell populations over that of wild type $hA_{2A}R$ upon incubation with fluorescent ligand. FACS dot plots of cell populations (B) prior to sorting and (C) after 4 rounds of sorting show rapid enrichment of improved $hA_{2A}R$ variants.