Protein Engineering of G Protein-Coupled Receptors Using Directed Evolution in Saccharomyces cerevisiae

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Background

G Protein-Coupled Receptors (GPCRs) as Drug Targets
- GPCRs are the largest superfamily of cell surface receptors
- Their central role in disease is underlined by the large numbers of human diseases linked to dysfunctional GPCR signalling, and that 35 % of all FDA approved drugs target GPCRs

GPCRs are inherently unstable
- Biophysical and structural characterisation of protein-drug interactions requires the expression and often purification of stable and folded protein
- GPCRs are a notoriously difficult to express and unstable class of membrane proteins, particularly when solubilised in short-chain detergents amenable to crystallisation

Stabilised Receptors (StaR®) Enable Structure-Based Drug Design
- Sosei Heptares focus on the engineering of stabilised GPCRs (StaR® proteins) in both agonist or antagonist bound conformations
- StaR® proteins enable determination of 3D structures of GPCRs by X-ray crystallography, Cryo-EM and Biophysical Mapping™ unlocking the potential of otherwise undruggable targets in health and disease

Saccharomyces cerevisiae-based receptor evolution - SaBRE

1 Preparation of Yeast Cells for Directed Evolution
- Wild-type GPCR sequence is amplified using error-prone PCR to generate a diverse library of mutants
- Naïve library is transformed into yeast and the protein is expressed
- Cells are permeabilised to allow the fluorescent ligand to penetrate through the cell wall

2 Fluorescent Ligand Selection

Figure 2: Tag-Lite assay to assess fluorescent ligand binding to a receptor in HEK293T cells.
- Directed evolution using the SaBRE method requires a fluorescent ligand to select for functional receptors
- Initially, wild-type receptors are expressed in mammalian cells as expression of wild-type receptors in yeast can be low
- The specificity and affinity of fluorescent ligand binding is assessed by Tag-Lite assay
- An excess of unlabelled ligand is used as a non-specific control
- The representative binding profile (Figure 2, right panel) shows specific, high affinity binding

Sosei Heptares StaR® and SaBRE platforms

Stabilised Receptor – StaR®
- Sosei Heptares StaR® technology allows the stabilisation of GPCRs by engineering a small number of single point mutations outside of the ligand-binding site
- These mutations allow for the structural determination of otherwise inaccessible targets, revealing atomic detail of receptor drug interactions, enabling structure-based drug design

Saccharomyces Based Receptor Evolution - SaBRE
- SaBRE technology combines the utility of S. cerevisiae as a microbial eukaryotic expression system with directed evolution, enabling the investigation of a vast sequence space for stabilising mutations
- The fittest receptor variants are selected by Fluorescent Activated Cell Sorting (FACS) using fluorescent ligand binding to a receptor library expressed in S. cerevisiae

Figure 3: Receptor expression and sorting by FACS
- The yeast cells expressing the receptor are incubated with the fluorescent ligand under a selection pressure
- The most fluorescent yeast cells, and therefore fittest receptors, are enriched using FACS and taken forward to the next round of enrichment or DNA isolation for library analysis

Figure 4: Tag-lite assay fluorescent ligand expression and thermostability analysis of library mutants expressed in HEK293T cells and solubilised in octylglucoside
- The sorted library is transformed into E. coli and several colonies picked for sequencing
- The mutated receptors (1-12) are assessed for expression, affinity and thermostability in a range of harsh detergents using the Tag-Lite assay
- Here, the cells were solubilised in octylglucoside, a harsh short-chain detergent that would unfold the wild-type receptor
- Several of the evolved mutants show high stability and expression (e.g. 1, 2, 4 and 6)
- The fittest receptors either undergo another round of error-prone PCR to re-diversify the library to allow further adaptation, or they can be worked-up in a suitable large scale expression system for biophysical or structural analysis

5 Biophysics and Structural Biology at Sosei Heptares
- Sosei Heptares platform utilises mammalian and insect cell culture for large-scale purification
- The SaBRE technology allows access to otherwise poorly expressed and unstable receptors for biophysical characterisation (e.g. Surface Plasmon Resonance) and structure determination by Cryo-EM or X-ray crystallography enabling GPCR structure-based drug design

Figure 1: SaBRE technology overview (adapted from Schütz et al. 2016)