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Towards Structure Determination of Prostaglandin Receptors Targeted as Prostaglandin-mediated Chronic Inflammation.

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Abstract

Recent successes in the determination of G-protein coupled receptor (GPCR) structures have relied on the ability of receptor variants to overcome difficulties in expression and purification. Therefore, the quick screening of functionally expressed stable receptor variants is vital. We developed a platform using *Saccharomyces cerevisiae* for the rapid construction and evaluation of functional GPCR variants for structural studies. This platform enables us to perform a screening cycle from construction to evaluation of variants within 6-7 days. We firstly confirmed the functional expression of wild type prostanoid receptors, which are belonging to GPCR family, in this platform. Then, in order to improve the expression level and stability, we generated and evaluated the variants of them. These stabilized receptor variants improved the monodispersity, compared to that of wild type receptors in *S. cerevisiae* at first screening. The stabilized human thromboxane A_2 receptor (TP) and prostaglandin E_2 receptor EP4 subtype (EP4) were able to be purified for use in crystallization trials. We demonstrated that the *S. cerevisiae* system should serve as an easy-to-handle and rapid platform for the construction and evaluation of GPCR variants. This platform can be a powerful prescreening method to identify a suitable GPCR variant for crystallography.