A new powerful research tool for GPCR structure determination

G-protein-coupled receptors (GPCRs) are the largest transmembrane receptor family in humans and consist of approximately 800 genes. They are involved in several physiological functions, such as immune response, blood pressure regulation, nerve stimulation, vision, and smell (Fig. 1) [1]. Hence, drugs that target GPCRs have been developed to treat multiple human diseases, such as central nervous system disorders, inflammatory diseases, metabolic imbalances, cardiac diseases, and cancer [2]. All GPCRs have conserved seven-pass transmembrane helices (TM1-7) that are connected by three extracellular loops (ECL1-3) and three intracellular loops (ICL1-3) (Fig. 1). The third intracellular loop (ICL3) is very flexible and interacts with G proteins, which are necessary for intracellular signal transduction [3].

During small molecule drug development, knowledge of the three-dimensional structure of the target protein with the candidate compound is very useful for refining the compound to improve its binding capacity. This method—structure-based drug design (SBDD)—has been widely used since the 1990s. By the SBDD approach, rational drug design is possible, which greatly improves the success rates of drug development. However, the structure determination of GPCRs is very difficult owing to their poor protein expression in native tissues or heterologous expression systems, low protein stability, and the presence of several receptor conformational states. Over the last ten years, several technologies for protein expression, purification, crystallization, and X-ray diffraction data collection have been developed [4]. To improve the stability of GPCRs for crystallization, ICLs are often replaced with soluble proteins, such as T4 lysozyme, thermostabilized apocytochrome b562 from *Escherichia coli* M7W/H102I/R106L (BRIL), or rubredoxin [4]. Such soluble proteins could also be attached to the N-terminus of GPCRs. In cases where the target GPCR cannot be successfully crystallized even after its fusion to soluble proteins, additional approaches are needed to enhance crystallizability, and antibodies that specifically bind to target GPCRs are often used for this purpose (Fig. 2(a)). Although this approach is useful to expand the soluble regions of the target for crystal packing [4], antibodies must be screened for each target GPCR to identify those with high binding affinity; this is very difficult and laborious. Another common approach to increase the thermal stabilities of GPCRs is to mutate the transmembrane helices [4]. In this method, the mutation sites are originally specified using the extensive knowledge of the active and inactive forms of the adenosine A_{2a} receptor. However, the screening of the mutation sites within the target GPCR is still necessary to determine those

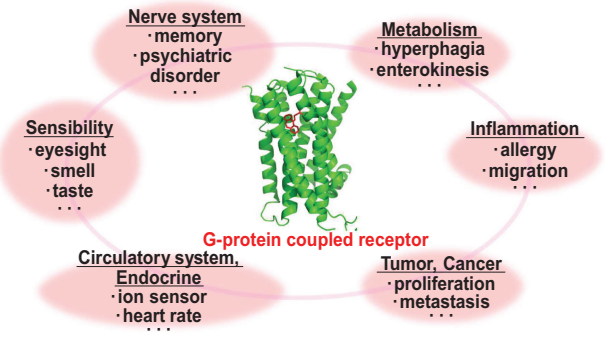

Fig. 1. GPCRs are involved in several physiological functions, such as immune response, blood pressure regulation, nerve stimulation, vision, and smell. The molecule at the center is a model of GPCR with seven-pass transmembrane helices.

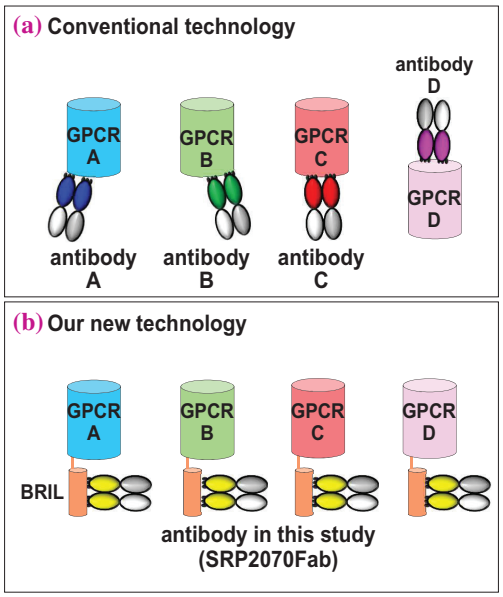

Fig. 2. Advantage of our new technology. **(a)** The conventional technology requires the preparation of antibodies for every GPCR. Producing antibodies that are useful for crystallization is very laborious work. **(b)** Our new antibody (SRP2070Fab) recognizes BRIL. Hence, once BRIL is attached to GPCRs, SRP2070Fab can be used for all GPCRs.

that stabilize the active or inactive state before the construct can be used for crystallization experiments. As this approach is also very strenuous and time-consuming, there is an urgent need for optimization methods that are more efficient and can be widely applied to various GPCR targets for crystallography.

To solve this problem, we produced a new antibody (SRP2070) targeting BRIL [5]. We found that a fragment of this antibody (SRP2070Fab) facilitated the crystallization of the BRIL-tagged, ligand-bound GPCRs (Fig. 2(b)) and determined the structures of 5-hydroxytryptamine receptor 1B ($5HT_{1B}$) with ergotamine (PDB:6JXB) and a Type-2 angiotensin II receptor (AT_2R) with [Sar1, Ile8]-angiotensin II (s-Ang II) (PDB:6JXO) (Fig. 3) [5]. These diffraction data were collected at SPring-8 **BL32XU**. To use SRP2070Fab for SBDD, it is very important to confirm that SRP2070Fab does not adversely affect the conformation of the ligand. We compared the ligand electron density maps with and without SRP2070Fab for both $5HT_{1B}$/ergotamine and AT_2R/s-Ang II [5]. There was no difference for either $5HT_{1B}$/ergotamine or AT_2R/s-Ang II. In short, although SRP2070Fab does not affect GPCR structures, it can improve the crystal packing. Considering these results, SRP2070Fab might be a universal chaperone for the crystallization of BRIL-fused GPCRs.

In addition, we anticipate that SRP2070Fab may also be used for cryo-EM analyses. In recent years, the resolution of cryo-electron microscopy has improved, and several structures of GPCRs have been determined by this technique [6]. By attaching SRP2070Fab to BRIL-fused GPCRs, the molecular weight is increased and the SRP2070Fab shape may be useful as a fiducial marker. Hence, we are convinced that this new tool (SRP2070Fab) will significantly accelerate structure determination and the design of small molecular drugs targeting proteins.

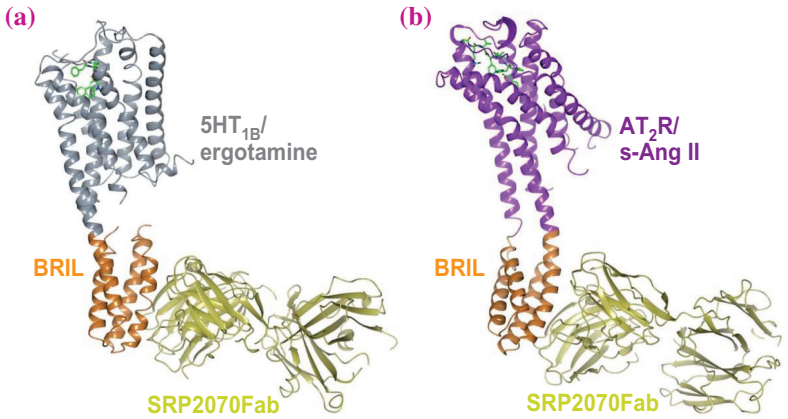

Fig. 3. Overall structures of 5HT1B-BRIL/ergotamine/SRP2070Fab and AT2R-BRIL/s-Ang II/SRP2070Fab. **(a)** Overall structure of 5HT1B -BRIL/ergotamine/SRP2070Fab. Ergotamine is shown as sticks. Each molecule is colored as follows: 5HT1B (light purple), BRIL (light brown), and SRP2070Fab (yellow). **(b)** Overall structure of AT2R-BRIL/s-Ang II/SRP2070Fab. s-Ang II is shown as sticks. Each molecule is colored as follows: AT2R (purple), BRIL (light brown), and SRP2070Fab (yellow).

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