

Supplementary Information

Article title: The discovery of a new antibody for BRIL-fused GPCR structure determination

Authors: Hikaru Miyagi^{1, †}, Hidetsugu Asada^{2, †}, Michihiko Suzuki³, Yuichi Takahashi³, Mai Yasunaga³, Chiyo Suno², So Iwata^{2,4} and Jun-ichi Saito^{3*}

Affiliations: ¹R&D Division, Kyowa Kirin Co., Ltd., Tokyo, Japan.
²Department of Cell Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan.
³R&D Division, Kyowa Kirin Co., Ltd., Shizuoka, Japan.
⁴RIKEN, SPring-8 Center, Hyogo, Japan.

[†]These authors contributed equally to this work.

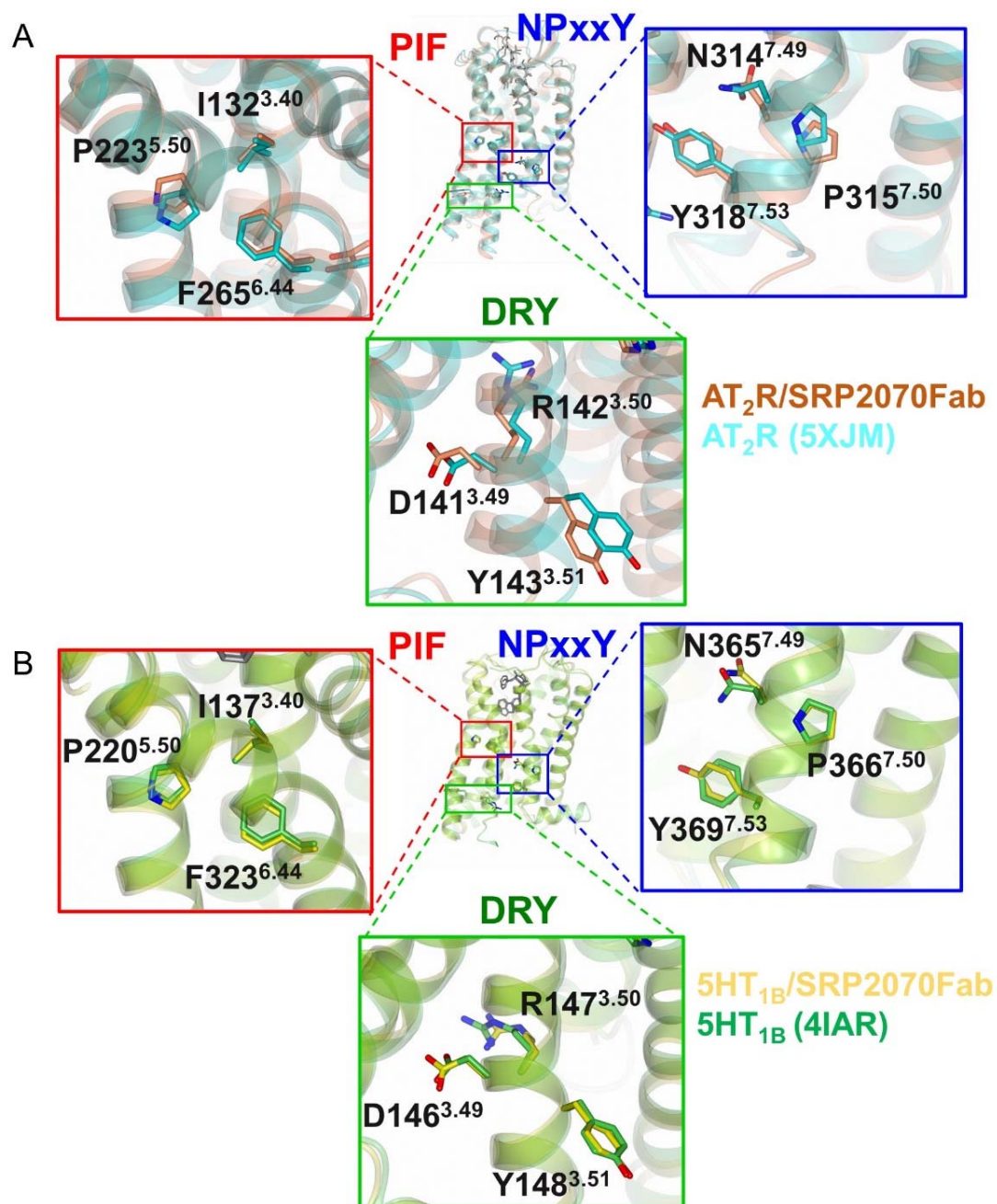
*Corresponding author: Jun-ichi Saito

Email: jun.saito.su@kyowakirin.com

Phone: 81-55-989-2004; Fax:81-55-986-7430;

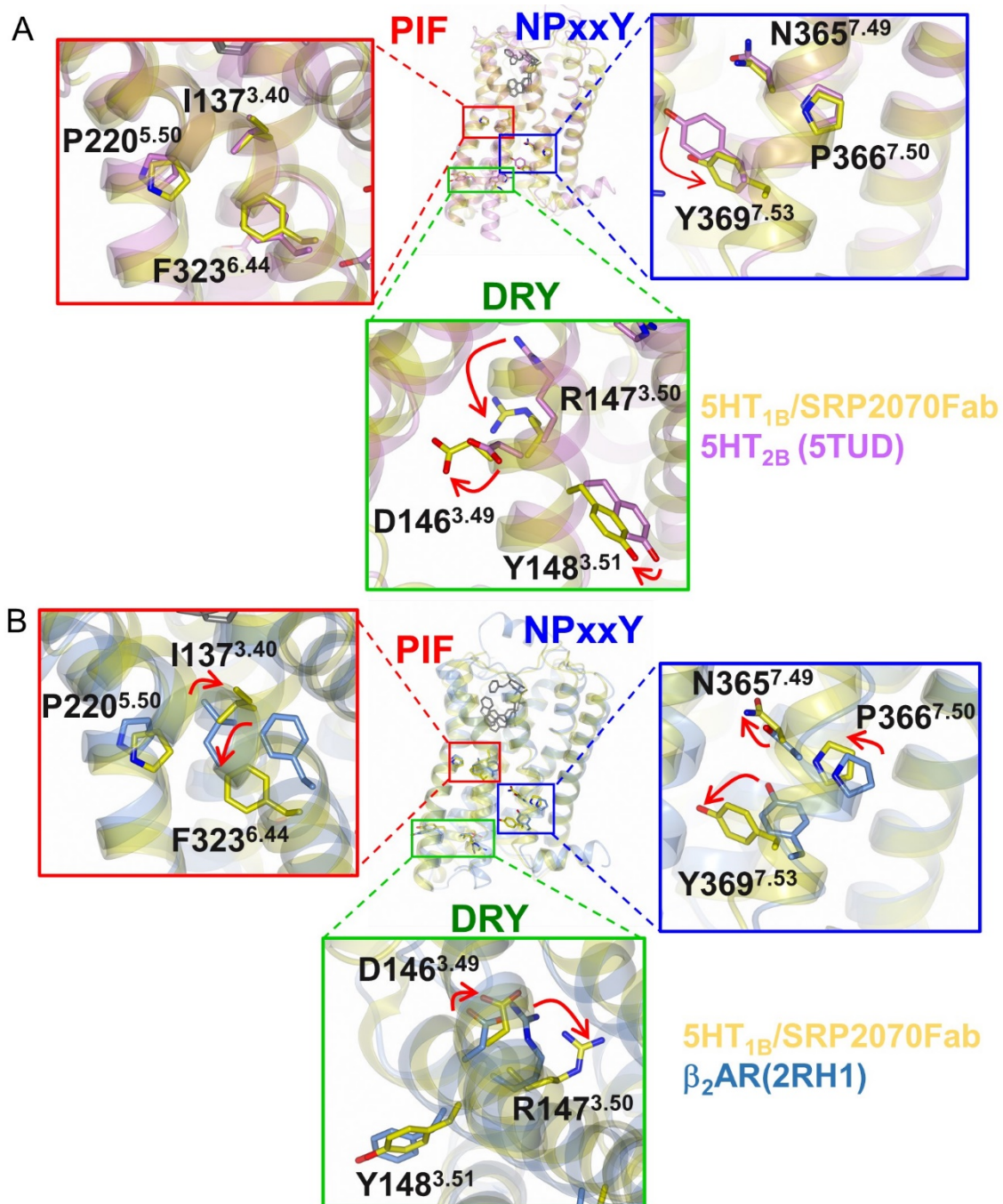
Supplementary Table 1S: The crystal packing calculations

	AT ₂ R-BRIL/s-ArgII/SRP2070Fab			5HT _{1B} -BRIL/s-ArgII/SRP2070Fab		
	AT ₂ R	BRIL	SRP2070 Fab	5HT _{1B}	BRIL	SRP2070 Fab
total SA (Å ²)	14983.4	5618.1	19757.9	14822.5	5745.0	19609.3
buried SA by crystal contact (Å ²)	1329.7	510.1	2293.8	2206.9	713.4	2225.2



Supplementary Figure 1S: Structural comparison of the activation motifs

(A) Superpositions of AT₂R /s-Ang II /SRP2070Fab (light brown; this study), and AT₂R/s-Ang II with antibody (light blue; PDB ID: 5XJM). The activation motifs PIF, NPxxY, and DRY are shown as red, blue, and green boxes, respectively. (B) Superpositions of 5HT_{1B}/ERG/SRP2070Fab (yellow; this study), and 5HT_{1B}/ERG (green; PDB ID: 4IAR). The activation motifs PIF, NPxxY, and DRY are shown as red, blue, and green boxes, respectively.



Supplementary Figure 2S: Structural comparison of the activation motifs

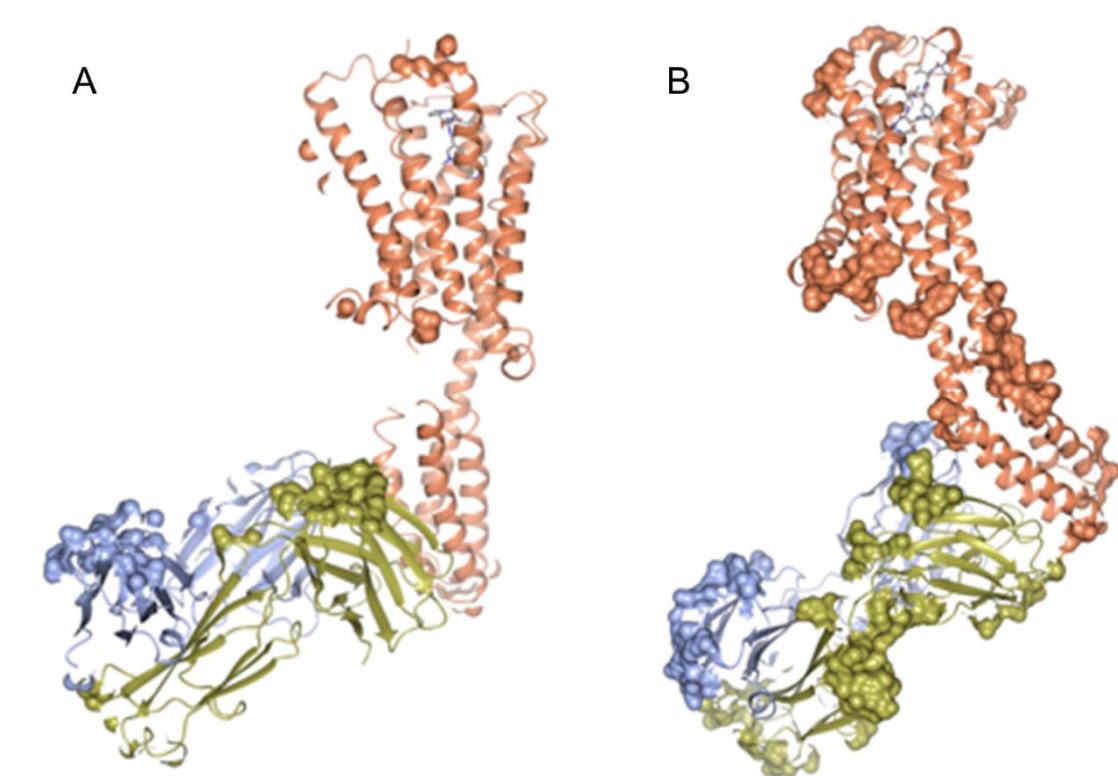
(A) Superpositions of 5HT_{1B}/ERG/SRP2070Fab (yellow; this study), and 5HT_{2B}/ERG (magenta; PDB ID: 5TUD). The activation motifs PIF, NPxxY, and DRY are shown as red, blue, and green boxes, respectively. (B) Superpositions of 5HT_{1B}/ERG/SRP2070Fab (yellow; this study), and β_2 AR (light blue; PDB ID: 2RH1). The activation motifs PIF, NPxxY, and DRY are shown as red, blue, and green boxes, respectively.

5HT_{1B}/SRP2070Fab_TM6
5HT_{2B} (5TUD)_TM6 (active)
β₂AR (2RH1)_TM6 (inactive)



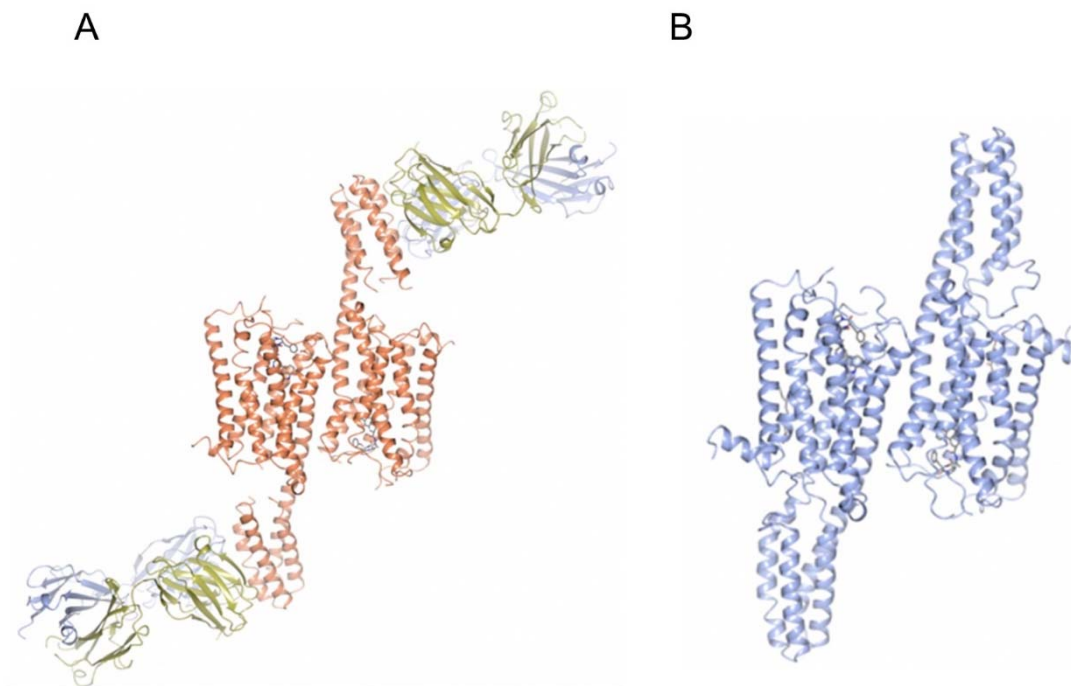
Supplementary Figure 3S: Structural comparison of TM6 helices

Superpositions of 5HT_{1B}/ERG/SRP2070Fab, 5HT_{2B}/ERG (PDB ID: 5TUD), and β₂AR (PDB ID: 2RH1). The TM6 helices are colored, 5HT_{1B}/ERG/SRP2070Fab (yellow), 5HT_{2B}/ERG (magenta), and β₂AR (blue), respectively.



Supplementary Figure 4S: Analysis of crystal packing contact areas

(A) Structure of 5HT_{1B}-BRIL/ERG/SRP2070Fab, 5HT_{1B} and BRIL (red), the light chain of SRP2070Fab (light purple), and the heavy chain of SRP2070Fab (yellow). The contact areas in the crystal packing are shown by the surface map. (B) Structure of AT₂R -BRIL/s-Ang II /SRP2070Fab, AT₂R and BRIL (red), the light chain of SRP2070Fab (light purple), and the heavy chain of SRP2070Fab (yellow). The contact areas in the crystal packing are shown by the surface map.



Supplementary Figure 5S: Structural comparison of 5HT_{1B}-BRIL/ERG/SRP2070Fab and 5HT_{1B}-BRIL/ERG (4IAR) in the crystal packing

(A) Structure of 5HT_{1B}-BRIL/ERG/SRP2070Fab, 5HT_{1B} and BRIL (orange), L chain of SRP2070Fab (light purple), and H chain of SRP2070Fab (yellow). (B) Structure of 4IAR, 5HT_{1B} and BRIL (light purple).