Crystal structure of the small-molecule-bound apelin receptor

Apelin receptor (APJR) belongs to the class A G-protein coupled receptor (GPCR) group, which is closely related to cardiac development, cardiovascular homeostasis and cardiac contraction [1,2]. The activation of the APJR signaling pathway can alleviate or treat many cardiovascular-related diseases such as heart failure and hypertension (Fig. 1). Many pharmaceutical companies, such as AMGEN, BMS and Sanofi, are trying to develop effective APJR agonist ligands; however, no drug has been successfully marketed until now. To develop new agonists of APJR, especially small-molecule drugs, understanding the molecular mechanism of the small-molecule binding mode is of critical importance.

To investigate the molecular mechanism of a small-molecule ligand, we chose a potent small-molecule agonist, (1S,2R)-N-(4-(2,6-dimethoxyphenyl)-5-(6-methylpyridin-2-yl)-4H-1,2,4-triazol-3-yl)-1-hydroxy-1-(5-methylpyrimidin-2-yl) propane-2-sulfonamide (termed cmpd644), which is similar to a clinical-stage drug candidate developed for treating heart failure (US patent WO2016187308A1, Amgen Inc.). After several rounds of optimization, we obtained cmpd644-bound APJR structures in a complex with its downstream heterotrimeric $G\alpha_iG\beta\gamma$ by cryo-EM methods [3]. Interestingly, we observed two types of APJR-Gi coupling stoichiometry from one cryo-EM dataset. Dimeric APJR and monomeric APJR adopt 2:1 and 1:1 (receptor: G protein) stoichiometric ratios, respectively (Fig. 2). This provides the first direct structural evidence of the coexistence of homodimers and monomers in the ligand-bound and G protein-coupled APJR signaling complexes.

To understand the small-molecule ligand binding mode for APJR in greater detail, we also solved the co-crystal structure of APJR in a complex with cmpd644 at a high resolution (2.7 Å) in the absence of Gi protein by X-ray crystallography. To obtain a stable APJR protein for structural determination, we first back-mutated W261K to the wild type employing the previous construct used for obtaining the APJR-peptide co-crystal structure [4]. Then, we generated and screened multiple constructs and finally introduced three new mutations (i.e., E174C, M217C, and I250C) based on the wild-type APJR sequence to further stabilize the receptor, and conducted crystallization trials by the LCP method. Finally, with the help of beamline scientists at SPring-8 **BL41XU**, we obtained high-quality X-ray diffraction data sufficient for solving the structure.

This is the first reported small-molecule-bound APJR co-crystal structure and we named it $_{xtal}APJ^{cmpd644}$ (Fig. 3(a)). Alignment with our previously solved peptide-bound AMG3054-APJR co-crystal structure revealed an almost identical receptor conformation (r.m.s.d. 0.529Å) [4]. We are curious about the distinction between peptide and small-molecule binding modes. Upon close examination of the binding pocket, we found that cmpd644 only occupied “site 1” of the pocket and inserted itself deeper when aligning with the C-terminal portion of the peptide ligand, which is different from the two-site binding mode for the peptide ligand (Fig. 3(b)). Moreover, the dimethoxyphenyl group of cmpd644 simulates the phenyl ring of F17 in AMG3054, interacting with $W85^{2.60}$, $I109^{3.32}$, $F110^{3.33}$, and $F291^{7.35}$ (Fig. 3(c)). There is an extended subpocket formed by the interaction between the methylpyridine ring in

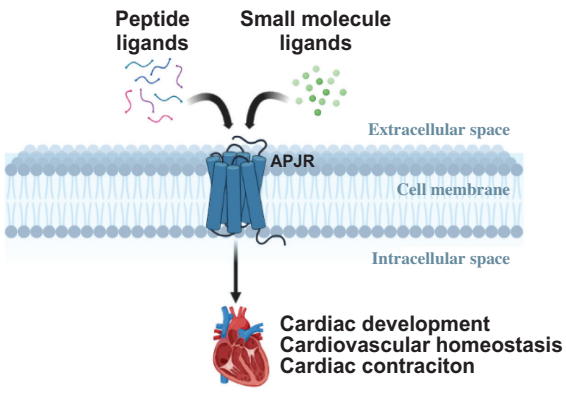

Fig. 1. Simplified illustration of APJR functional pathway. Peptide and small-molecule ligands can activate APJR signaling, which leads to cardiac development, cardiovascular homeostasis and cardiac contraction [1,2]. This figure was created at BioRender.com.

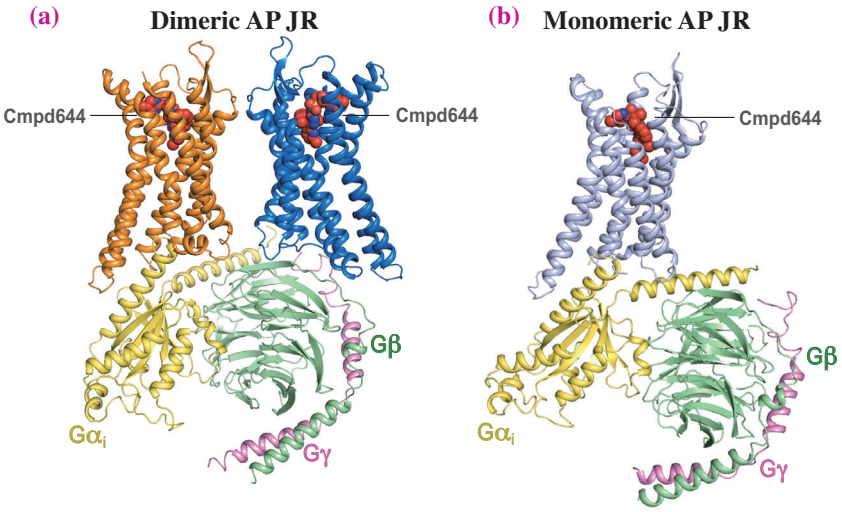

Fig. 2. Cryo-EM structures of the dimeric APJR-Gi **(a)** and monomeric APJR-Gi **(b)** complexes in the presence of cmpd644.

cmpd644 and the surrounding hydrophobic residues of F78$^{2.53}$, F110$^{3.33}$, Y264$^{6.51}$, and Y299$^{7.43}$ (Fig. 3(c)). The interacting residues W85$^{2.60}$, F110$^{3.33}$, K268$^{6.55}$, Y271$^{6.58}$, M288$^{7.32}$, and F291$^{7.35}$ are consistent with the reported functional assay for the small-molecule AM-8123, which shares a similar scaffold with cmpd644 [5].

We report the first co-crystal structure of APJR in a complex with the drug-candidate small-molecule compound, providing a precise molecular template for further pharmacology studies on treating cardiovascular disease. Additionally, together with the G-protein-bound cryo-EM structures in both 2:1 and 1:1 stoichiometric ratios, we provide a different, exciting view of the class A GPCR signaling mechanism.

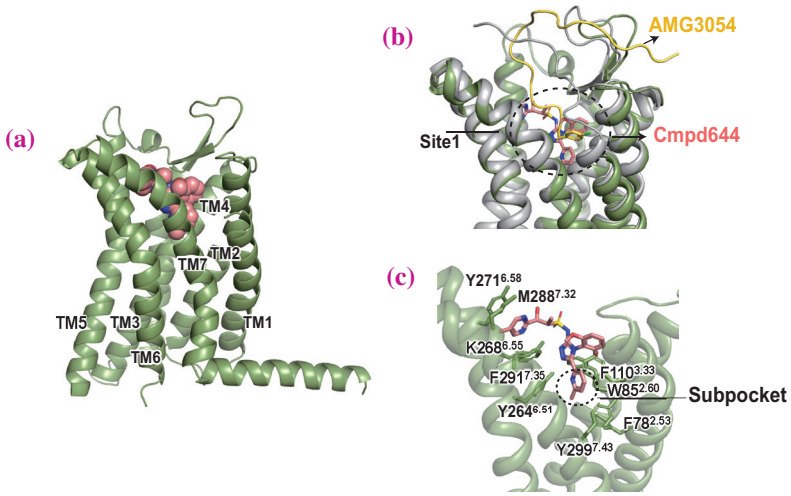

Fig. 3. **(a)** Overall structure of $_{xtal}$APJ^{cmpd644}. APJR is colored green and cmpd644 pink. **(b)** Comparison of $_{xtal}$APJ^{cmpd644} with AMG3054-APJR co-crystal structure (PDB ID: 5VBL, AMG3054 is colored yellow and APJR gray). Site 1 is circled with dashed lines. **(c)** Binding pocket of cmpd644 in $_{xtal}$APJ^{cmpd644}. Interacting residues of APJR are shown as sticks. The subpocket is circled with dashed lines.

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