SUPPLEMENTARY INFORMATION

Crystal structure of the human β_2 adrenergic G protein coupled receptor

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SUPPLEMENTAL METHODS

Generation of β_2AR365 encoded baculovirus

The wild-type coding sequence of the human β_2AR (starting at Gly2) was cloned into the pFastbac1 Sf-9 expression vector (Invitrogen) with the HA signal sequence followed by the Flag epitope tag at the amino terminus. The mutation N187E was made to remove the third glycosylation site. A sequence encoding ENLYFQGP was introduced between the FLAG tag and the start of the receptor, in order to install a TEV protease site and a Phe residue to inhibit aminopeptidase activity. Finally, a TAA stop codon was placed between G365 and Y366, terminating translation without the 48 C-terminal residues of the wild-type β_2AR. The resulting construct is referred to as β_2AR365. The mutations and insertions described above were made using the Quickchange Multi protocol (Stratagene), and the resulting construct was confirmed by sequencing. Recombinant baculovirus was made using the Bac-to-Bac system (Invitrogen).

Preparation of Fab fragments

Monoclonal mouse immunoglobulins against the β2AR reconstituted in liposomes were raised as described [1]. Mab5 IgGs from mouse hybridoma cell culture supernatant were purified using a Protein G column (Pierce). Fab5 fragments were generated by papain proteolysis (Worthington) and purified by Mono Q chromatography. The fragments were concentrated to ~100 mg ml^{-1} with a Millipore concentrator (5 kDa molecular weight cut off) in a solution of 10 mM HEPES pH 7.5 and 100 mM NaCl.

Preparation of β_2AR365-Fab5 complexes

The β_2AR365 was expressed in Sf-9 insect cells infected with β_2AR365 baculovirus, and solubilized according to previously described methods[2]. Functional protein was obtained by M1 FLAG affinity chromatography (Sigma) prior to and following alprenolol-Sepharose

chromatography[2]. Receptor bound alprenolol was exchanged for carazolol on the second M1 resin. N-linked glycolsylations were removed by treatment with PNGaseF (NEB), and the FLAG epitope was removed by treatment with AcTEV protease (Invitrogen). Protein was concentrated to ~50 mg/ml with a 100 kDa molecular weight cut off Vivaspin concentrator (Vivascience) and mixed in stoichiometric excess of Fab5. The complex was purified on a Superdex-200 (10/300GL) column in a solution of 10 mM HEPES pH 7.5, 100 mM NaCl, 0.1 % dodecylmaltoside, and 10 μM carazolol. The purified β_2AR365-Fab5 complexes were concentrated to ~60 mg ml^{-1} using a Vivaspin concentrator.

LITERATURE CITED

1. Day, P. W., Rasmussen, S. G. F., Parnot, C., Fung, J. J., Masood, A., Kobilka, T. S., Yao, X. J., Choi, H. J., Weis, W. I., Rohrer, D. K. & Kobilka, B. K. A monoclonal antibody for G protein coupled receptor crystallography. Nat Methods (in Press) (2007).
2. Kobilka, B. K. Amino and carboxyl terminal modifications to facilitate the production and purification of a G protein-coupled receptor. Anal Biochem 231, 269-71 (1995).

Table S1 X-ray data collection and refinement statistics

	β_2AR365-Fab5	β_2AR24/365-Fab5
Data collection		
Space group	C2	C2
Cell dimensions		
a, *b*, *c* (Å)	338.4, 48.5, 89.4	338.4, 48.5, 89.4
α, β, γ (°)	90., 104.6, 90.	90., 104.6, 90.
Resolution (Å)	86.4.-3.4 (3.49-3.40)*	50-3.4 (3.52-3.40)*
R_{merge}	0.117 (0.407)	0.120 (0.456)
$I/\sigma I$	9.9 (2.3)	7.8 (2.6)
Completeness (%)	98.9 (94.9)	99.4 (98.4)
Multiplicity	3.3 (2.9)	4.1 (3.4)
Refinement		
Resolution (Å)	20.-3.4	20. – 3.4
No. reflections work/free	17658 / 1902	17458 / 1886
$R_{work/}$ R_{free}	0.216 / 0.269	0.226 / 0.279
No. atoms	4905	4887
Average B values (Å^2)		
Receptor	156.	187.
Fab5	67.	91.
Overall anisotropic B (Å^2)		
$B_{11}/B_{22}/B_{33}/B_{13}$	-27.1 / 31.3 / -4.2/ 4.4	-16.8 / 20.4 / -3.5 / 12.4
R.m.s deviations		
Bond lengths (Å)	0.007	0.008
Bond angles (°)	1.4	1.5
Ramachandran plot **		
receptor / Fab5		
% most favored	76.3 / 71.5	75.8 / 71.8
allowed	22.1 / 27.2	22.1 / 26.6
generously allowed	1.6/ 1.3	2.1 / 1.6
disallowed	0.0 / 0.0	0.0 / 0.0

*Highest resolution shell is shown in parenthesis.
** As defined in PROCHECK

SUPPLEMENTAL FIGURES

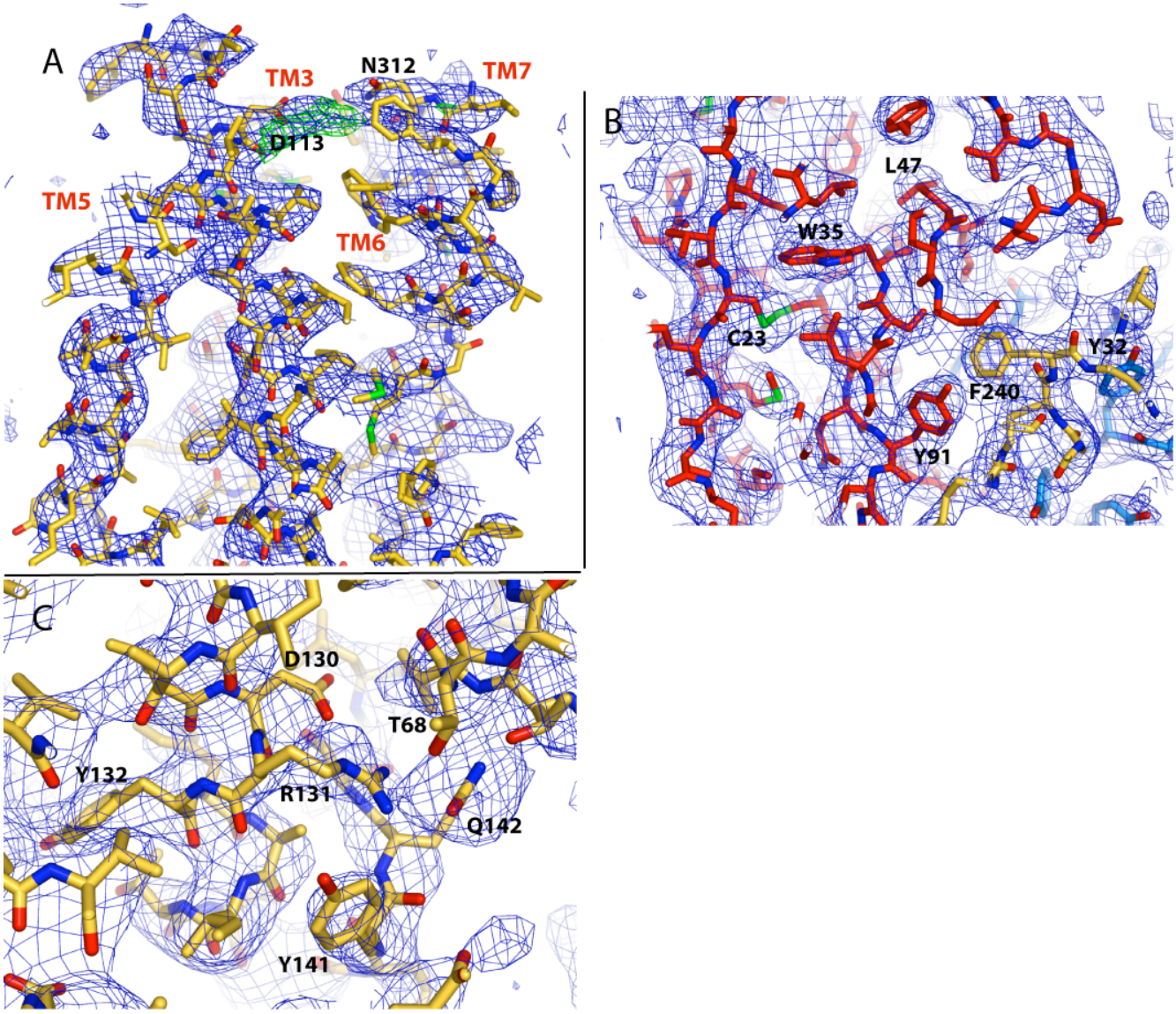

Figure S1. Views of the electron density. The final $2F_o$-F_c map contoured at 0.7σ (blue mesh) is shown in three regions of the structure. Note that this contour level is required to visualize the receptor molecule, as it is much more poorly ordered than the Fab. The receptor carbon atoms are gold, with red, blue and green atoms representing oxygen, nitrogen, and sulfur. A) TM segments 3,5,6,7 near the extracellular side of the receptor. B) The Fab-Intercellular loop 3 interface. The Fab5 light chain carbon atoms are shown in red, heavy chain carbon atoms in light blue. Selected side chains are labeled. C) The DRY region of TM6 and surroundings.

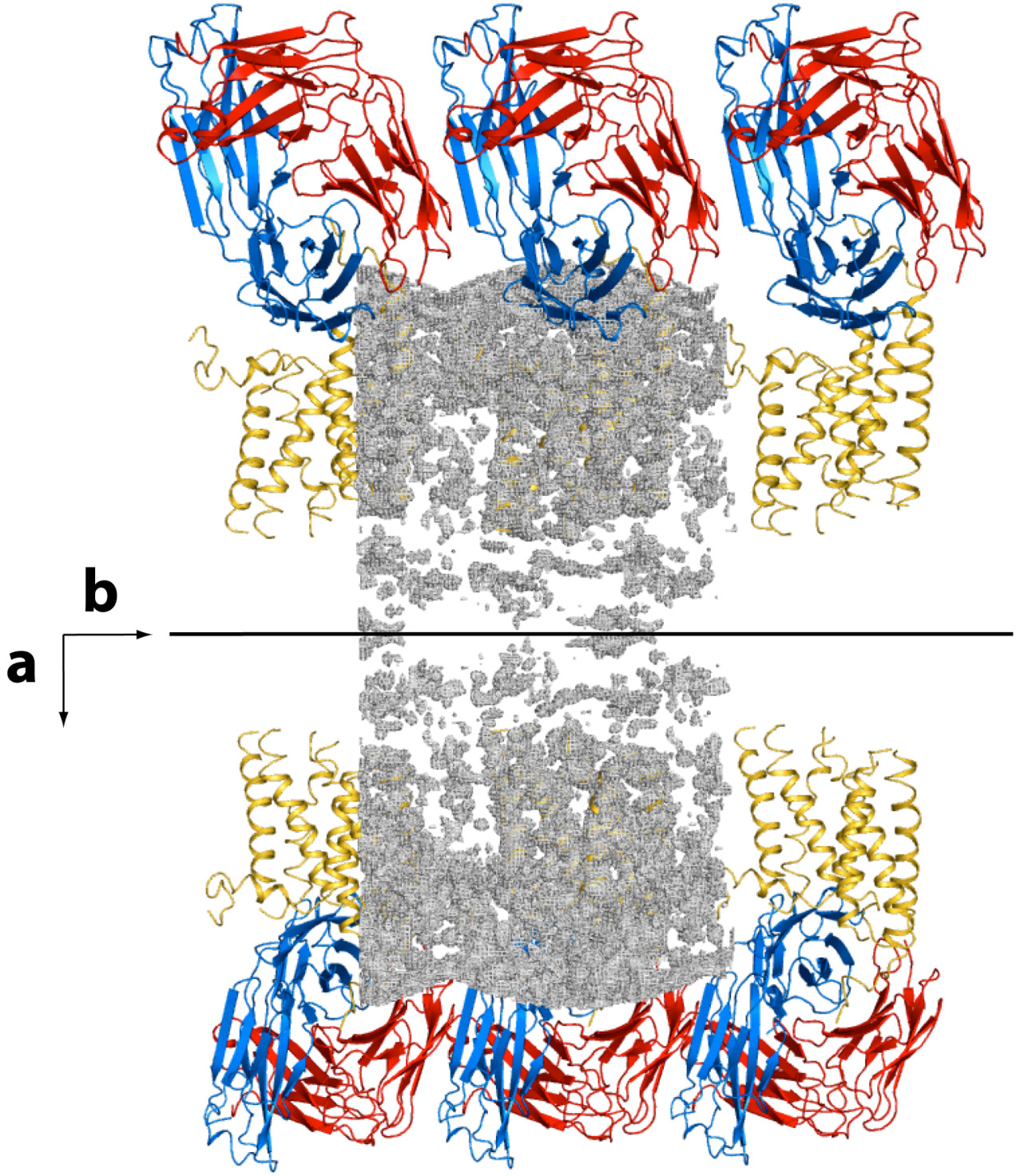

Figure S2. Weak electron density in the extracellular region of the β_2AR. The final $2F_o$-F_c map (grey mesh, contoured at 0.7σ) around two receptor molecules packed across the crystallographic twofold (*b*) axis (horizontal line). The view is the same as the left panel of Fig. 2a.

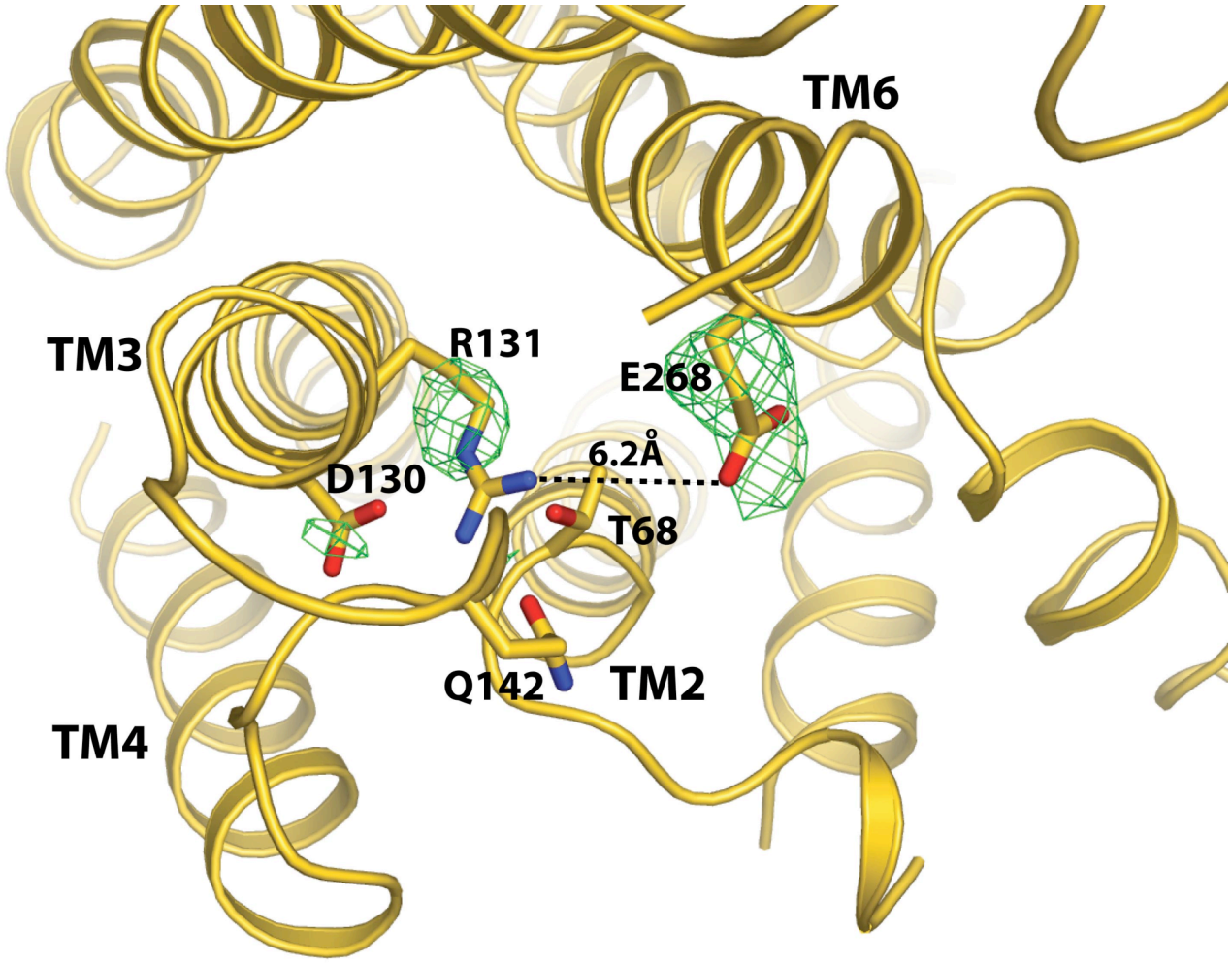

Figure S3. F_o-F_c omit map, contoured at 2.7σ, made by deleting all side chain atoms from D130, R131 and E268 and refining the structure.

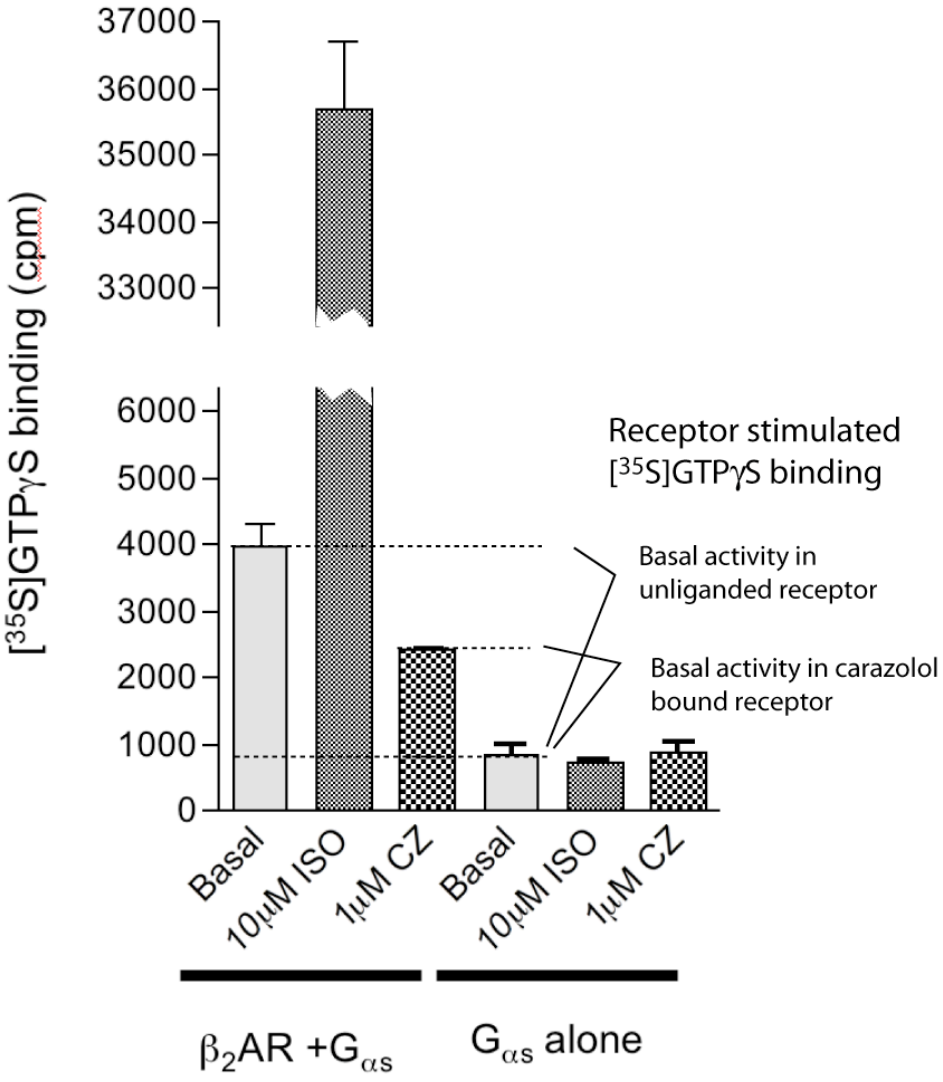

Figure S4. Basal activity of carazolol-bound β_2AR. Purified Gs was reconstituted into phospholipid vesicles in the presence and absence of purified β_2AR. [^{35}S]GTPγS binding was measured in the absence of ligand, the presence of the agonist isoproterenol or the inverse agonist carazolol. Basal activity is determined relative to [^{35}S]GTPγS binding to Gs in the absence of β_2AR.

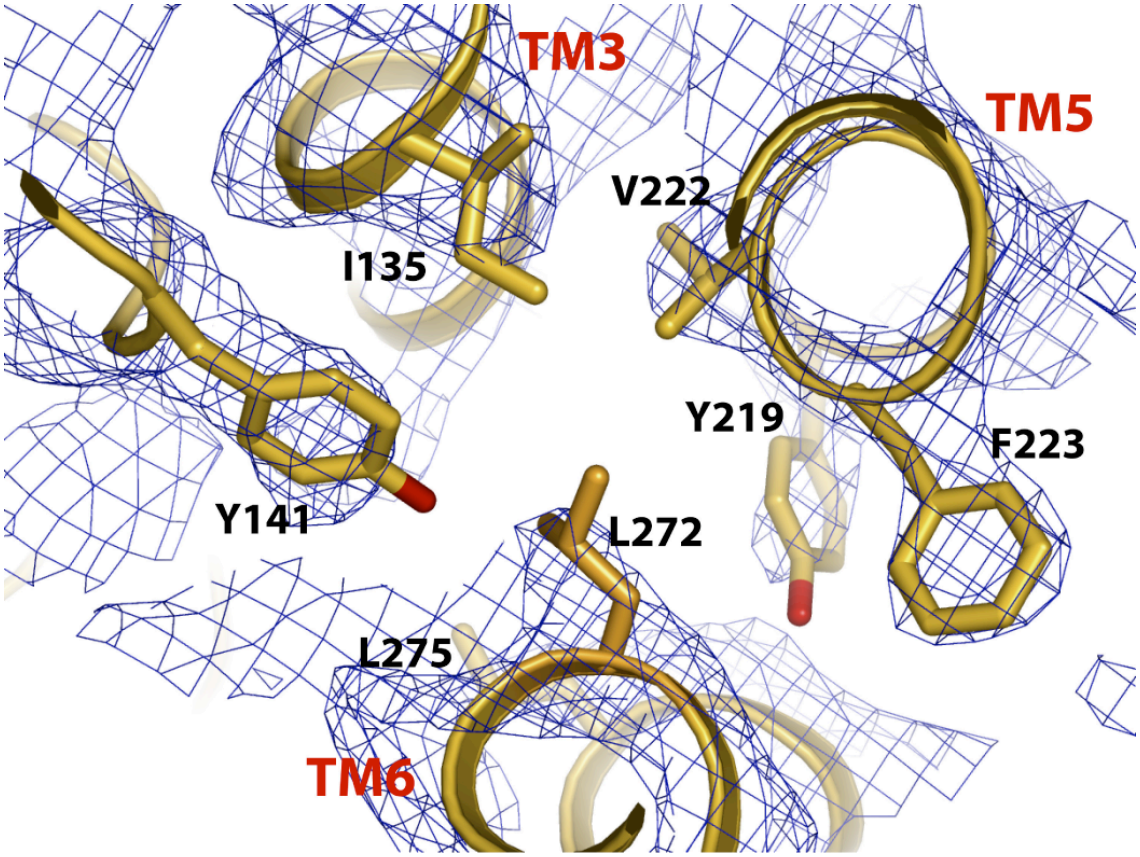

Figure S5. $2F_o$-F_c electron density map, contoured at 0.7σ, in the vicinity of L272.