Supplementary Material

G protein coupled receptor interactions with cholesterol deep in the membrane

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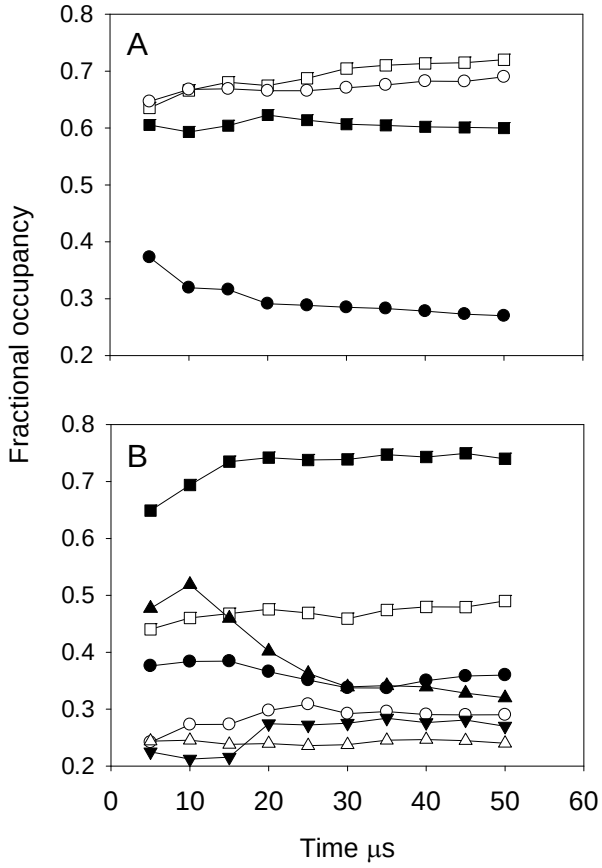

Fig. S1. Convergence of estimates of fractional occupancy

(A) β_2-adrenergic receptor and (B) A_{2A} receptor. The plot shows fractional occupancies calculated at 5 μs intervals throughout a 50 μs simulation for the clusters identified in Table 1: (A) cluster A1 (□) and A2 (○) for agonist-free β_2-adrenergic receptor, and cluster B1 (■) and B2 (●) for agonist-bound β_2-adrenergic receptor; (B) cluster C1 (□), C2 (○) and C3 (Δ) for the agonist-free A_{2A} receptor and cluster D1 (■), D2 (●), D3 (▲) and D4 (▼) for the agonist-bound A_{2A} receptor.

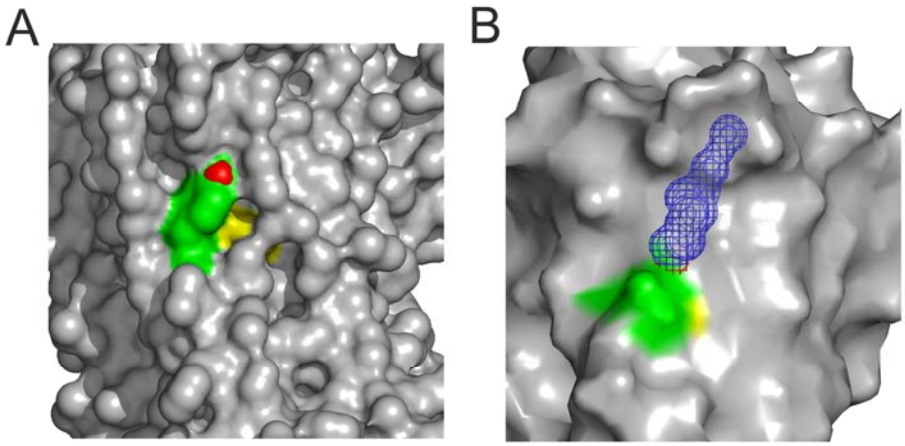

Fig. S2. Cluster B2 in the agonist-bound β_2-adrenergic receptor

(A) Crystal structure showing:Phe208 (yellow) and Tyr209 (green), with the-OH group of Tyr209 in red. (B) Of five snapshots from the simulations taken at 10 μs intervals between 10 and 50 μs only that at 20 μs shows a cholesterol molecule with its –OH group within 5 Å of the protein surface, shown here as a blue mesh with a red –OH group. Phe208 and Tyr209 are coloured as in (A).

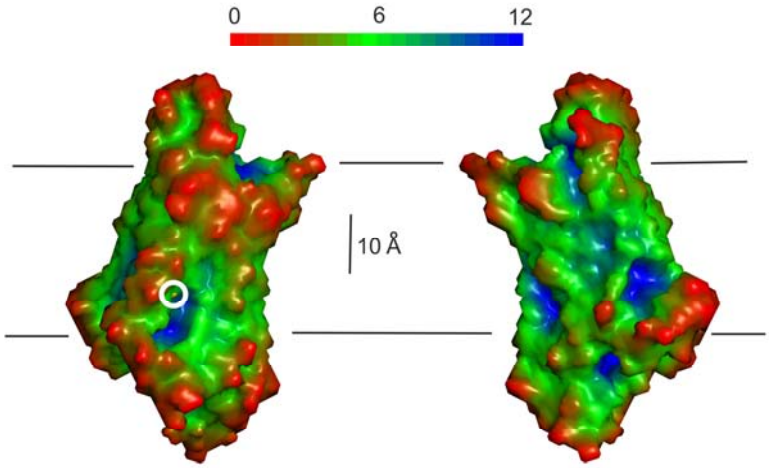

Fig. S3. Travel Depth views of the surface of the agonist-free A_{2A} receptor.
The two views are related by a 180^{o} rotation around the bilayer normal. The horizontal lines indicate the approximate positions of the glycerol backbone regions of the lipid bilayer, taken from the OPM database [http://opm.phar.umich.edu/]. The protein has been tilted by 18^{o} relative to the crystallographic z axis to account for the tilted orientation of the protein in the bilayer reported in the OPM data base. The view on the left shows the cleft close to the exposed –SH group of Cys128, shown in yellow, with a VDW radius scaled by a factor of 1.1; the location of the –SH group is marked by the white ring.

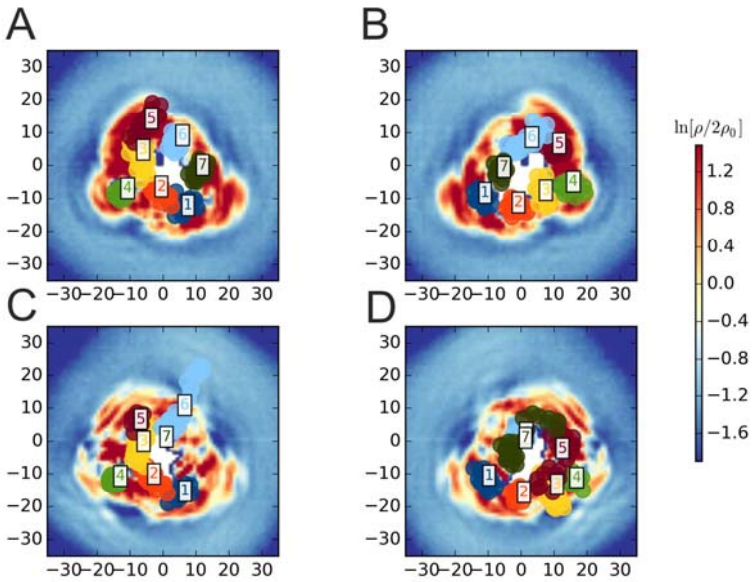

Fig. S4. Spatial distributions of –OH groups of deep cholesterol molecules around the A_{2A} receptor.
Distributions are shown for agonist-free (A, B) and agonist-bound (C,D) receptor, projected on to the plane of the membrane. The views in (A) and (C) are from the IC side, and in (B) and (D) are from the EC side. Note that the density scale is different from that in Fig. 2.

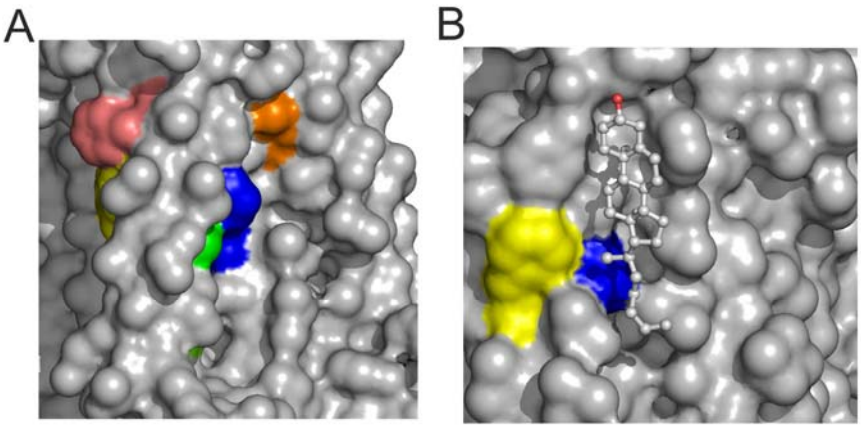

Fig. S5. Crystal structures showing clusters C2 and C3 on the agonist-free A_{2A} adenosine receptor.
(A) Cluster C2 relative to the binding site shown in Figure 6C: Phe133 (salmon) and Trp129 (yellow), with other residues coloured as in Fig. 6C.
(B) Cluster C3 showing the cholesterol molecule (ball-and-stick representation) observed in the crystal structure: Phe182 (blue) and Phe183 (yellow).

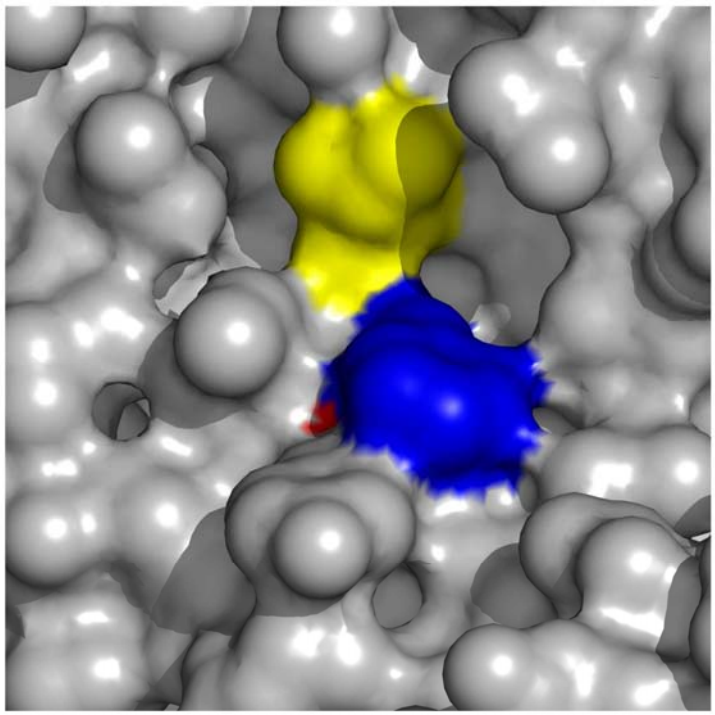

Fig. S6. Crystal structure showing cluster D4 on the agonist-bound A_{2A} receptor. Residues are coloured: Val283 (yellow) and Phe286 (blue). The backbone oxygen of Phe286, shown in red, is predicted to be located in the glycerol backbone region of the surrounding lipid bilayer, on the IC side.

Table S1. Average numbers of deep cholesterol molecules[a]

System	Number not in contact[b]	Number in contact[b]
Agonist-free β_2 receptor	1.4	2.6
Agonist-bound β_2 receptor	1.4	2.2
Agonist-free A_{2A} receptor	1.7	2.5
Agonist-bound A_{2A} receptor	1.7	2.8

[a] A cholesterol is defined as deep if its –OH group is in the central 8 Å core of the membrane; the total number of cholesterol molecules in all simulations was 60.

[b] A deep cholesterol is defined as in contact with the receptor if its –OH group is within 5 Å of any protein residue. The uncertainty is < 0.1.