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Supplementary Discussion

There are two $A_{2A}R$–mini-G_s complexes per crystallographic asymmetric unit composed of either chains A and C (complex AC) or chains B and D (complex BD). The best electron density was observed for complex AC (chain A, $A_{2A}R$; chain C, mini-G_s), and included density for the agonist NECA bound to $A_{2A}R$ and density for a molecule of GDP bound to mini-G_s (Extended Data Fig. 3). Chain B ($A_{2A}R$) in complex BD also contained density for NECA, but chain D (mini-G_s) did not contain density corresponding to GDP. It is unclear from the structure why the two molecules of mini-G_s differ in GDP occupancy, because the structures are virtually identical (rmsd 0.12 Å over 1127 atoms) and although the GDP site in chain D is in the vicinity of extracellular loop 2 of a symmetry related receptor (chain A), this loop is disordered and it is unlikely that it would prevent nucleotide binding. The presence of GDP in the mini-G_s structure is a reflection of the properties of the engineered G protein, which, after complex formation, is insensitive to GTPγS-mediated dissociation[3]. Mini-Gs is in a conformation virtually identical to that observed in the β_2AR–Gs structure (see below) and therefore represents the active state of the G protein, consistent with its ability to couple to $A_{2A}R$ and induce high-affinity agonist binding (Fig. 1). The two $A_{2A}R$ molecules in the asymmetric unit are also virtually identical (rmsd 0.05 Å over 1665 atoms), but differ significantly to previously determined $A_{2A}R$ structures due to the outward movement of the cytoplasmic end of H6. Structural alignment of complex AC with complex BD showed that mini-G_s was oriented slightly differently in the two receptors with a rotation of the GTPase domain by 3˚ relative to the receptors. This results in slightly different packing between mini-G_s and $A_{2A}R$. While it is possible that this is due to the presence of GDP in one complex but not the other, it seems more likely that it arises from differences in lattice contacts. Even so, this may represent a natural variation in the interface between mini-G_s and $A_{2A}R$ due to the flexible nature of the activated G protein and the receptor.