Electronic Supplementary Material (ESI) for Nanoscale.

Electronic Supplementary Information

Single molecule binding of a ligand to a G-protein-coupled receptor in real time using fluorescence correlation spectroscopy, rendered possible by nano-encapsulation in styrene maleic acid lipid particles

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SUPPORTING EXPERIMENTAL

SDS-PAGE analysis of purified A_{2A}R-SMALP

Purified A_{2A}R-SMALP was analysed by SDS-PAGE using 12% (w/v) acrylamide gels run at 160 V for 60 min at room temperature. Resolved protein bands were visualised using Coomassie Brilliant Blue.

Cell culture

Chinese hamster ovary (CHO) cells stably expressing a cAMP response element-secreted placental alkaline phosphatase (CRE-SPAP) reporter gene expressing the human A_{2A}R were produced as previously described[1] and were maintained in Dulbecco's Modified Eagle Medium:Nutrient Mixture F-12 (DMEM/F12) medium containing 10% FCS and 2 mM L-glutamine at 37 ^{o}C in humidified atmosphere of air/CO_2 (19:1).

CRE-SPAP gene transcription assay

A_{2A}R CRE-SPAP cells were grown to confluence in clear 96-well plates. On the day prior to analysis, normal growth medium was removed and replaced with serum-free medium (SFM; DMEM/F12 supplemented with 2 mM L-glutamine). On the day of the experiment, fresh SFM was added to the cells with or without the required concentration of CA200645 and incubated for 30 min at 37 ^{o}C. After 30 min, increasing concentrations of NECA were added and cells were then incubated for 5 h at 37 ^{o}C. After the 5 h incubation, all medium was removed from the cells, replaced with 40 µL of SFM and incubated for a further 1 h. The plates were then incubated at 65 ^{o}C for 30 min to inactivate the endogenous alkaline phosphatases. After cooling the plates to room temperature, 5 mM 4-nitrophenyl phosphate in a diethanolamine-containing buffer (10% (v/v) diethanolamine, 280 mM NaCl, 500 µM $MgCl_2$, pH 9.85) was added to each well. Plates were incubated for 10 min at 37 ^{o}C and then the absorbance at 405 nm was measured using a Dynex MRX plate reader (Chelmsford, MA).

Data analysis

All data were fitted using Prism 7 (GraphPad Software). NECA concentration-response curves in the absence and presence of a range of concentrations CA200645 were globally fitted to the following interaction model.

$$\text{Response} = \frac{E_{max} \times [A]}{([A] + EC_{50} \times \left(1 + \frac{[B]}{K_D}\right)^S)}$$

E_{max} is the maximal response to NECA, EC_{50} is the molar concentration of NECA [A] that gives half maximal response in the absence of test compound, [B] is the concentration of test compound, K_D is the antagonist equilibrium dissociation constant and S is the Schild slope.

SUPPORTING DATA

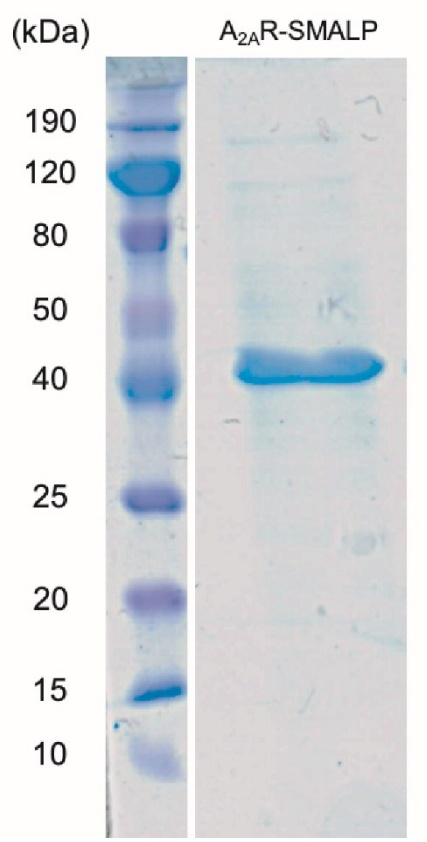

Fig. S1 Purified A_{2A}R-SMALP. A sample of A_{2A}R-SMALP purified by Ni^{2+}-NTA resin was analysed by SDS-PAGE using 12% (w/v) acrylamide gel, then visualised by staining with Coomassie Brilliant Blue. Right-hand lane, purified A_{2A}R-SMALP; left-hand lane, molecular weight markers.

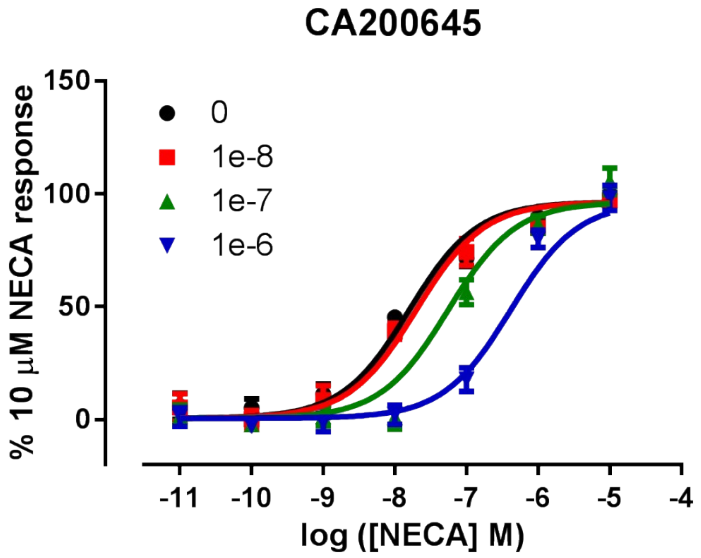

Fig. S2 CA200645 binds to the $A_{2A}R$. The concentration-response curve for the agonist NECA was determined in the presence of different concentrations of CA200645 as indicated, producing a parallel rightward shift in the curve. These data indicate that the competitive antagonist CA200645 binds to the $A_{2A}R$.[2] The NECA pEC_{50} = 7.91 ± 0.16 and the pA_2 value for CA200645 = 7.37 ± 0.17 (n = 6). The pA2 is the negative logarithm of the K_D of the antagonist. Data shown are the combined data from six independent experiments.

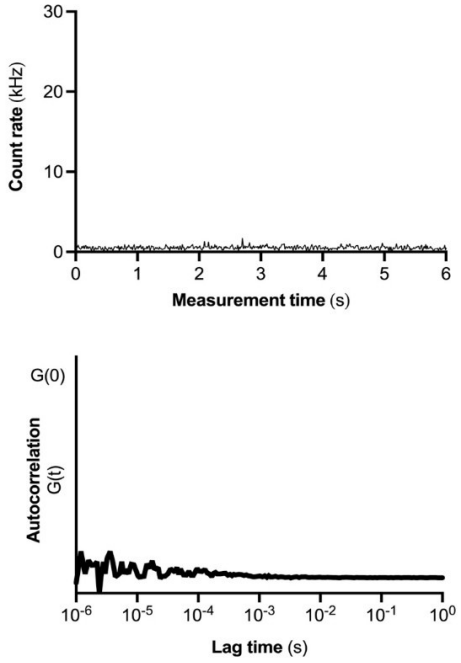

Fig. S3 FCS of A_{2A}R-SMALP alone. A_{2A}R-SMALPs were analysed by FCS in the absence of CA200645. Upper panel, background count rate showing an absence of autofluorescence; Lower panel, data did not autocorrelate to any observable diffusion model.

SUPPORTING REFERENCES

1 L. A. Stoddart, A. J. Vernall, S. J. Briddon, B. Kellam and S. J. Hill, *Neuropharmacology,* 2015, **98**, 68-77.

2 H. O. Schild, *Br J Pharmacol Chemother,* 1947, **2**, 189-206.