High Throughput Fluorescence-Based Live Cell Binding Assays For G Protein-Coupled Receptors

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During the last decade, it has been shown that fluorescent ligand binding assays are a reliable and cost-saving alternative to traditional radiolabelled ligand assays for the study of GPCR signalling (Briddon et al., 2011). We have previously described a competitive equilibrium fluorescent binding assay which can be performed in live cells using a high content screening platform (Stoddart et al., 2012). This fluorescence-based competition assay, originally designed for the adenosine-A_3 receptor, can be adapted to a high throughput screening platform yielding identical affinities values to those obtained in the high-content based method (Arruda et al., 2012). In this study, we have extended this high throughput screening approach to other human GPCRs, namely the dopamine-D_1 receptor and beta$_1$- and beta$_2$-adrenergic receptors.

Binding assays were carried out in CHO-K1 cells expressing the human dopamine-D_1, beta$_1$- or beta$_2$-adrenergic receptors. Cells were seeded into 96-well plates, and incubated with increasing concentrations of competing ligand (60 min, 37°C) followed by a further incubation (30min, 37°C) with the selective BODIPY-FL-labeled dopamine-D_1 receptor antagonist, CA200773 (CellAura Technologies Ltd; 50 nM) or the fluorescent beta-adrenoreceptor ligand BODIPY-TMR-CGP12177 (Baker et al, , 2003; 10 nM). Fluorescence intensity in each well was read on a BMG Pherastar FS plate reader. Optimal focal height was determined automatically and total fluorescence intensity was assessed taking 81 reads per well (read time <5 min per plate). Non-linear curve fitting was performed in GraphPad Prism 5.

Competition binding experiments using CA200773 yielded binding affinity constant (pK_i) values for known dopamine receptor ligands consistent with binding to the D_1-receptor (Clozapine, 7.31 ± 0.35; Haloperidol, 8.19 ± 0.19; mean± s.e. mean, n=3). Binding experiments using BODIPY-TMR-CGP12177 also generated pKi values consistent with the known pharmacology of either the beta$_1$-adrenoreceptor (Propranolol, 9.19 ± 0.34 ; CGP 20712A, 9.96 ± 0.24; ICI 118,551, 7.59 ± 0.22, n=3) or the beta$_2$-adrenoreceptor (Propranolol, 8.98 ± 0.07; CGP 20712A, 5.68 ± 0.06; ICI 118,551, 8.72 ± 0.06; n=3)

Taken together, our data strongly support the potential of the Pherastar FS plate reader as a high-throughput screening platform for competitive fluorescent binding assays for GPCRs, being highly suitable for the purposes of hit discovery and lead optimization. Binding studies using the same approach for other GPCRs such as the dopamine-D_2, serotonin $5HT_{1A}$, as well as miniaturization of this assay (384-well plate) and its suitability for screening compound libraries are under investigation.

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