CellAura Technologies

Getting Started Guide

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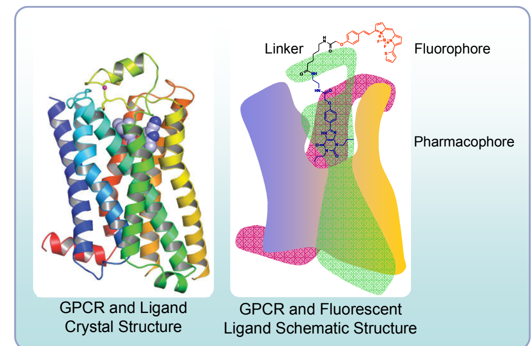
Do you need alternatives to radio-labelled ligands for GPCRs?

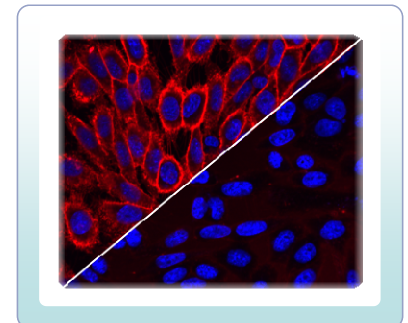
Fluorescent ligands for G-protein coupled receptors (GPCR) [also known as 7 transmembrane domain (7-TM) receptors] comprise synthetic receptor agonists or antagonists (the pharmacophore) linked to a fluorophore dye. Visualisation of the fluorescent tracer bound to the GPCR has several advantages over conventional radioisotope-labelled ligands:



- **Immediate 'real-time' readout**
- **Visual confirmation of receptor localisation**
- **Multi-colour, multi-parameter data generation**
- **Miniaturisation capability – down to one cell per data point**
- **No scintillation detection costs**
- **No radioisotope use and disposal – enhanced safety and reduced costs**

Fluorescent GPCR ligands as alternatives in Life Science and Drug Discovery

CellAura's fluorescent GPCR ligands comprise pharmacologically-characterised small molecule compounds linked to a range of fluorescent dyes. Each fluorescent ligand is characterised using live cell imaging and functional assays to confirm its affinity and antagonist or agonist activity.
CellAura's ligands have been used successfully for:

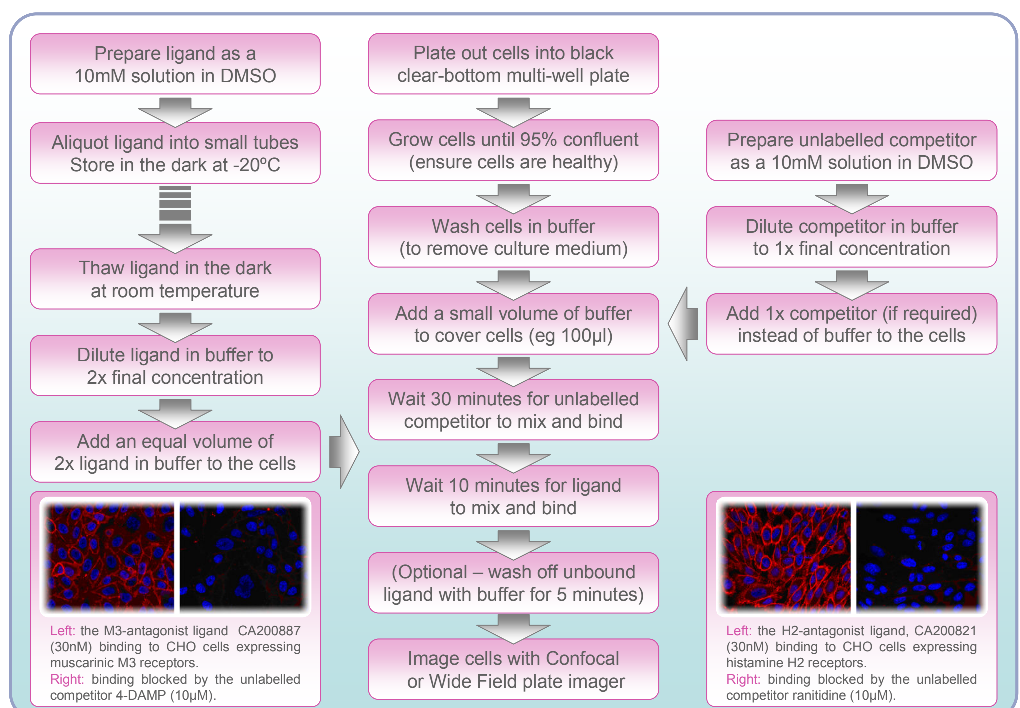


- **Fluorescence Microscopy (Confocal and Wide-field)**
- **High Content Screening / Analysis (HCS / HCA)**
- **Equilibrium and Kinetic Ligand Binding**
- **Fluorescence Activated Cell Sorting (FACS)**
- **Fluorescence Correlation Spectroscopy (FCS)**
- **Dual readout Binding and Function**

A Guide to Fluorescent Ligand Binding and Cell Imaging

Shown here is a schematic representation of a typical fluorescent ligand binding and displacement assay, measuring membrane-bound fluorescence intensity with confocal or wide-field high content analysis (HCA) instrumentation.

This protocol can be readily adapted to other fluorescent assay formats.



Left: the M3-antagonist ligand CA200887 (30nM) binding to CHO cells expressing muscarinic M3 receptors.
Right: binding blocked by the unlabelled competitor 4-DAMP (10µM).

Left: the H2-antagonist ligand, CA200821 (30nM) binding to CHO cells expressing histamine H2 receptors.
Right: binding blocked by the unlabelled competitor ranitidine (10µM).

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FAQs and Solutions

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Fluorescent GPCR ligands are as easy to use as radioligands, without the inherent safety issues associated with handling radioisotopes. Techniques and assay formats used to overcome issues experienced with radioligands (eg. high non-specific binding, high background signals), can also be applied to fluorescent ligand binding – with the advantage that it is often possible to visualise immediately the cause of the problem.

Technical problems can arise using fluorescent GPCR ligands in live cells expressing either recombinant or endogenous GPCRs, These most commonly relate to the health of the cells and their expression of the target receptor. Solutions to FAQs and commonly encountered technical problems are listed below.

- **What excitation and emission wavelengths and filter sets should be used?**
  - Each CellAura product is provided with a Technical Datasheet that recommends the appropriate excitation wavelength and emission filter-set for optimal fluorescence detection of the product. Use of the incorrect excitation wavelength and/or emission filter-set will reduce fluorescence intensity and detection sensitivity. It may also lead to signal cross-talk with other fluorescent dyes in multi-colour imaging or fluorescence resonance energy transfer (FRET) assay formats.
- **Cells don't bind the fluorescent ligand**
  - This suggests the cells are no longer expressing the receptor. Use an alternative assay to verify that the receptor is present in the cells, and check that they are healthy. If necessary, the fluorescent ligand can be used to select cells expressing the receptor from a mixed cell population using FACS.
- **Cells label everywhere (cytoplasm, nucleus), not just the membrane**
  - This is most likely to be because the cells are not healthy and have taken up the ligand non-specifically. Even in a healthy culture, there will be a few dead cells that show this non-specific staining. To maintain the health of the cells, it is best to use cultures that are approaching confluence, rather than those that have been at confluence for a day or more.
- **Control (non target-expressing) cells label with the fluorescent ligand**
  - This suggests the ligand is binding non-specifically to the host cells as well as the target cells. This is either because both cell types are not healthy (see above), or because the ligand is being used at too high a concentration (see below), or because both cell types express an endogenous receptor to which the ligand is binding. Limited receptor selectivity information is provided by CellAura, but a comprehensive selectivity profile for each ligand has not been produced. Use the pharmacology of the pharmacophore from which the ligand is derivatised as a guide to potential additional targets to which the ligand may be binding.
- **There is very high background fluorescence**
  - This is likely to be due to fluorescence of the ligand in solution around the cells. Try using the ligand at a lower final concentration (30 – 100nM gives the best signal : background). It may also be worthwhile trying a brief wash step to reduce the background fluorescence of the ligand in solution.
- **Cells label well, but the maximal saturation binding level is lower than expected**
  - This may be due to the fluorescence detector reaching saturation, rather than the cells reaching binding saturation. Try taking measurements on an alternative instrument.
- **There are bright fluorescent particles in the cell cultures**
  - This is likely to be because the ligand was not completely dissolved in DMSO before use. It is advisable to sonicate the DMSO stock aliquot after thawing, before dilution to 2x final concentration.

If you have any questions or enquiries that are not covered in the FAQ section, please contact us.

Find Out More …

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