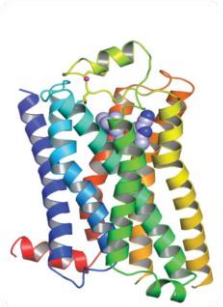
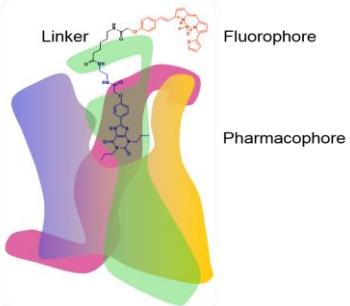
Fluorescent Ligands - Getting Started Guide

Our CellAura fluorescent ligands target G protein coupled receptors (GPCRs) and are comprised of 3 units:

- A pharmacophore - e.g. a synthetic agonist or antagonist
- A fluorescent dye (the fluorophore)
- A linker - which connects the pharmacophore with the dye



GPCR and Ligand Crystal Structure



GPCR and Fluorescent Ligand Schematic Structure

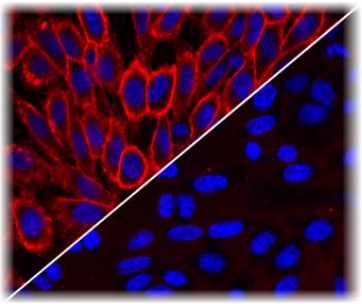
Visualization of the fluorescent tracer bound to a GPCR offers you several advantages compared to using conventional radioisotope-labeled ligands:

- Immediate 'real time' readout
- Visual confirmation of receptor localisation
- Multi-colour and multi-parameter data generation
- Miniaturisation capability (one cell per data point)
- No scintillation detection costs
- Enhanced safety with reduced costs - no radioisotope use and disposal

Applications

Each CellAura fluorescent ligand is characterised using live cell imaging and fcutional analysis to confirm its affinity and pharmacoglocial activity. They have been used successfully in a wide range of applications including:

- **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**



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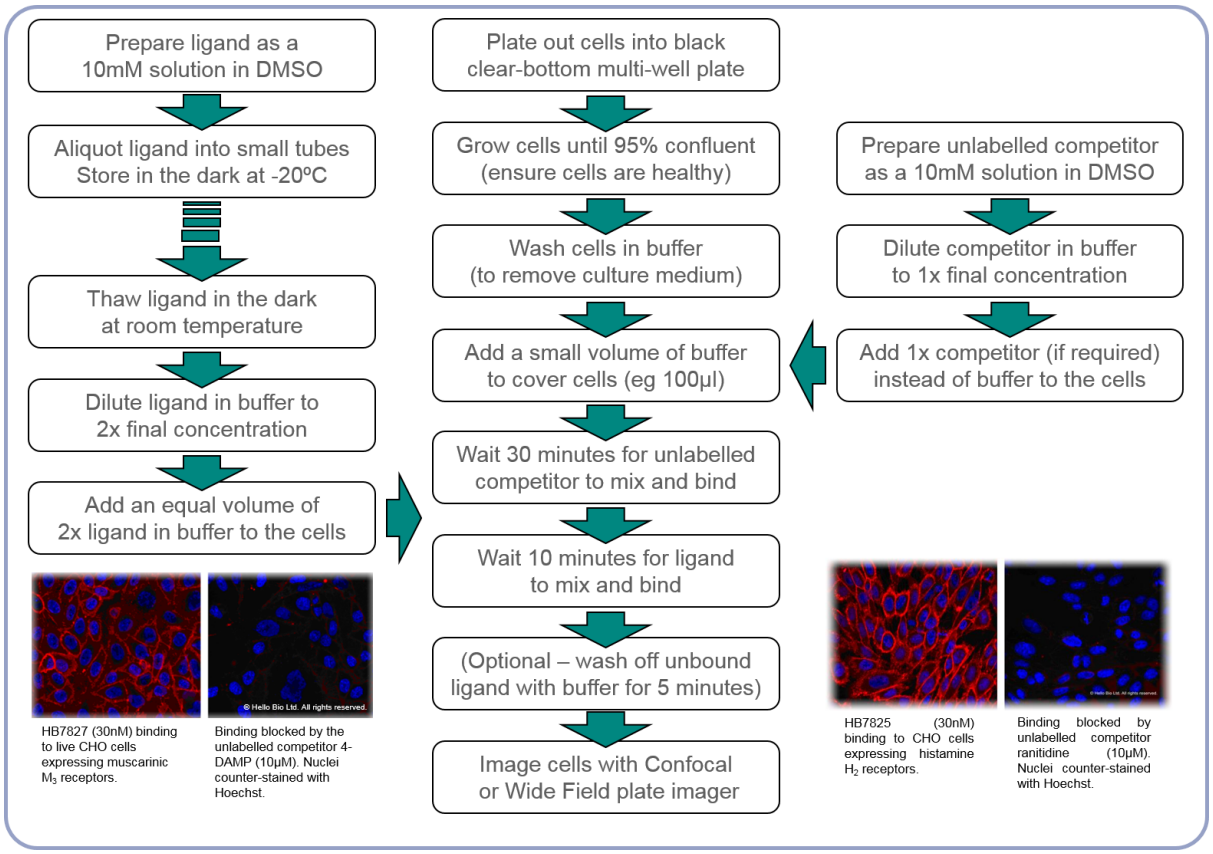
A Guide to Fluorescent Ligand Binding and Cell Imaging

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.

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FAQs and Solutions

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 you use?

Each of our fluorescent ligands come with a Technical Datasheet which recommends the appropriate excitation wavelength and emission filter sets. You can also find these on our website. These filter sets have been tested to ensure that you are able to achieve optimal fluorescence detection of our fluorescent ligands.

If the excitation wavelength and/or emission filter set is not set as recommended then the fluorescence intensity and detection sensitivity of our fluorescent ligands are reduced. An incorrect set up may lead to signal cross-talk with other fluorescent dyes in multi-colour imaging or fluorescence resonance energy transfer (FRET) assay formats.

My cells don't seem to be binding my fluorescent ligand

This suggests that your cells are no longer expressing the receptor you're wishing to target. You should check that your cells are healthy and that the target you are investigating is being expressed, you can do this by trying an alternative assay. Don't forget our fluorescent ligands can also be used to select cells expressing your target receptor from a mixed cell population using FACS.

Other explanations for this could be that your receptor isn't reaching the cell membrane. This can be confirmed by an antibody approach with fixed, permeabilised cells.

The receptor may also be unable to bind our fluorescent ligand because either too much solvent (DMSO) in buffer is being used causing damage to the cells

The CellAura fluorescent ligands are not designed to work on fixed cells as this can prevent binding of the fluorescent ligands.

My cells appear to label everywhere (cytoplasm and nucleus) and not just the membrane

The most likely reason for this is because your cells aren't healthy so have they may have taken up the fluorescent ligand non-specifically. A way of overcoming this is to use cell cultures that are just approaching confluence instead of using cells which have been confluent for a day or two. This reduces the number of dead cells (that show non–specific staining) within your cell culture populations. We also suggest checking your assay conditions (e.g. correct pH for isotonic buffer), that minimal solvent is used and that the cells haven't been fixed or permeabilised.

My control cells (non-target expressing) appear to be labelled with your fluorescent ligand

This suggests that the fluorescent ligand is binding non-specifically to the host cells you are using as well as your target cells. This could either be because both target and control cells aren't healthy (see above question) or because our fluorescent ligand is being used at a concentration higher than recommended (see below). It could also be that both the control and target cells are expressing the endogenous receptor that you're wishing to investigate so our fluorescent ligand is binding to both. Another explanation could be that our fluorescent ligands are binding to other receptors. We provide some cross-selectivity data but you should also use the pharmacology of the unlabelled drug from which our fluorescent ligand is derived as a guide to potential additional targets that our ligand may bind to.

I'm experiencing a very high background fluorescence

The most likely cause of this is due to the fluorescence of the ligand in solution around your cells. To overcome this, try using our fluorescent ligand at a lower final concentration. To achieve the best background to signal ratio we recommend using a concentration somewhere in the range of 30 to 100 nM. If you are still experiencing a high background fluorescence try a brief (10 minute) gentle wash step to reduce to fluorescence of our ligand in solution.

My cells label well, but the maximal saturation binding is lower than I expected

This could be because the fluorescence detector you are using is reaching saturation rather than your cells reaching a binding saturation. We suggest reducing the gain on the detector or using an alternative instrument to take measurements.

In my cell cultures there are bright fluorescent particles, why?

The most likely reason for this is because the florescent ligand wasn't dissolved completely in DMSO before you used. We recommend sonicating the DMSO stock aliquot after thawing before dilution to two times the final concentration.

We strongly advise that you do not repeatedly freeze or thaw the stock ligand aliquots or that you do not freeze or thaw the final ligand-buffer solutions as these actions can cause crystal formation.

If you have any questions or enquiries that re not covered in the above FAQs then please contact us.