

## Radioligand binding and functional autoradiography

### Competition and functional assays in human tissue membranes

Confirmation of the affinity and efficacy of a compound for the native human receptor is becoming increasingly important before progressing compounds to pre-clinical development. Receptor screening typically relies on recombinantly expressed human targets or native animal targets and sometimes, the apparent pharmacology at these can be different to that at the native human target Asterand has developed and validated a range of radioligand binding assays to demonstrate affinity and/or function of specific human targets:

- Radioligand competition binding assays
- [<sup>35</sup>S]-GTPγS functional binding assays
- [<sup>35</sup>S]-TBPS functional binding assays

[<sup>35</sup>S]-GTPγS binding can be used to measure agonist-stimulated activation of some Gprotein-coupled receptors in human tissue membranes. This is achieved by incubating tissue membranes with agonist in the presence of [<sup>35</sup>S]-GTPγS, a non-hydrolysable radiolabelled analogue of GTP. Following receptor activation by the agonist, [<sup>35</sup>S]-GTPγS binds irreversibly to the activated G-protein and this can be quantified by liquid scintillation counting.

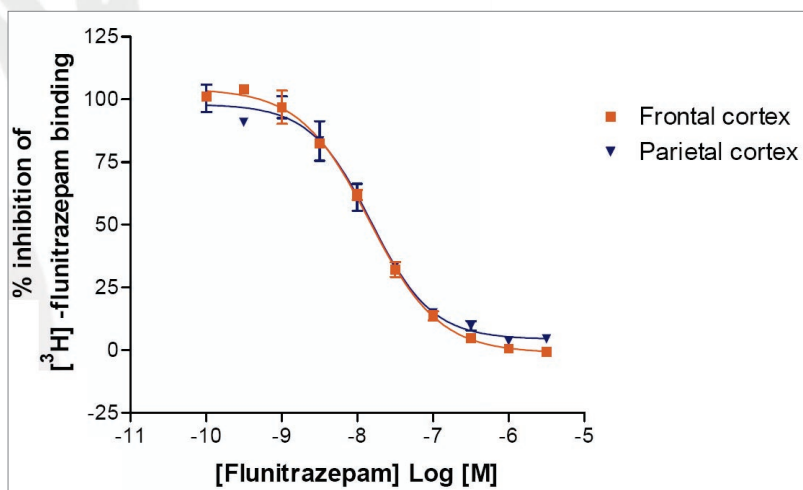
The assays that Asterand has developed and validated to date all use subcellular fractions prepared from human CNS and examples of data are shown below. We have previously performed assays to

- Confirm pharmacology (particularly agonist efficacy) at the native human receptor
- Identify any differences in pharmacology at a native human receptor in the disease state

Optimisation of assay conditions and assay validation for targets or tissues not shown can be provided as required.

Please contact your Business Development representative or e-mail [advantage@asterand.com](mailto:advantage@asterand.com) for further information

Competition binding to benzodiazepine receptors:



Competition binding of [<sup>3</sup>H]-flunitrazepam to benzodiazepine receptors in membranes prepared from non-diseased human brain (cortex).

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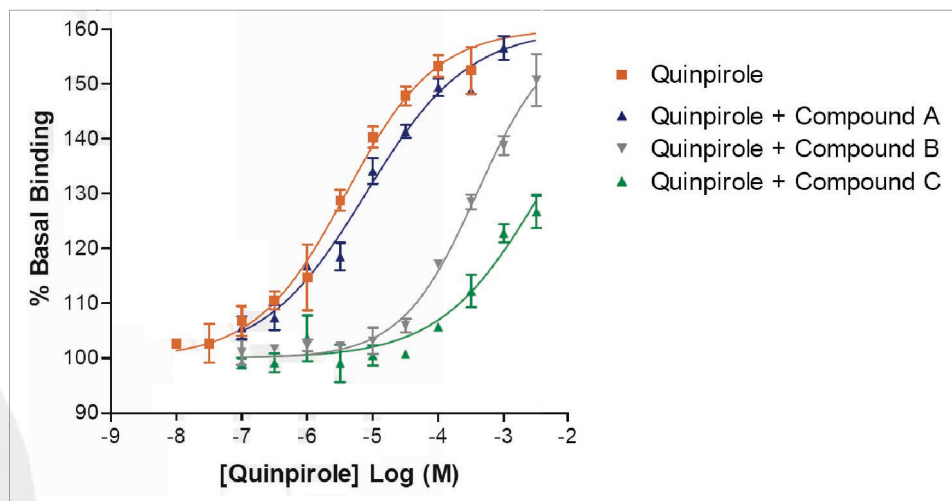
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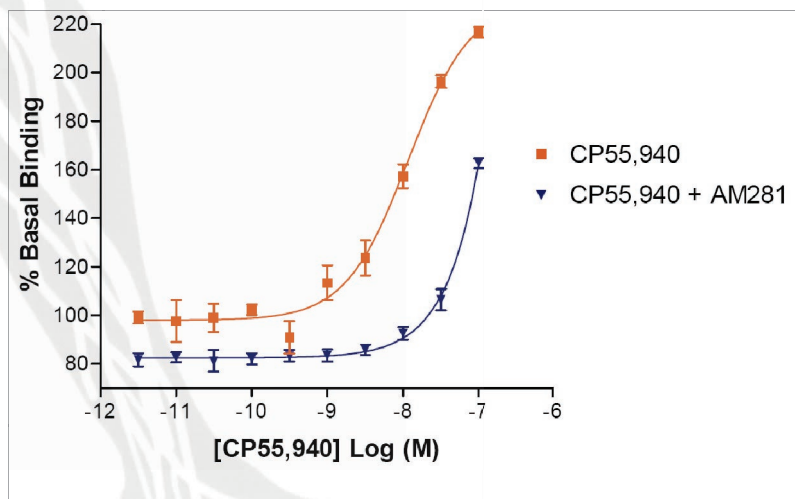
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Dopamine receptor-mediated [<sup>35</sup>S]-GTPγS binding:



Dopamine D<sub>2</sub> receptor mediated [<sup>35</sup>S]-GTPγS binding in membranes prepared from non-diseased human brain (caudate). Data show concentration-effect curves for quinpirole in the presence and absence of three test compounds.

Dopamine receptor-mediated [<sup>35</sup>S]-GTPγS binding:



Functional binding of [<sup>35</sup>S]-TBPS to benzodiazepine activated chloride ion channel binding sites in membranes prepared from non-diseased human brain (frontal cortex).

### Competition binding autoradiography

In addition to being able to use radioligands to visualise binding sites in frozen human tissue sections using autoradiography, this approach can also be used to investigate the localization of effects of test compounds on radioligand binding.

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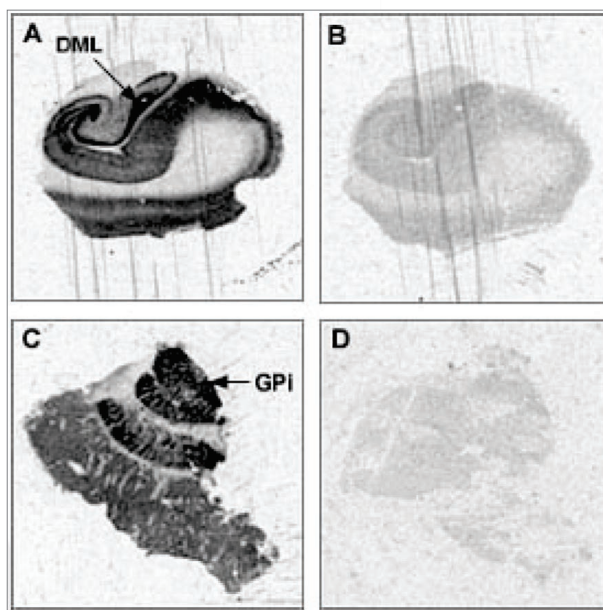
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Binding of the tritiated cannabinoid receptor agonist, [<sup>3</sup>H]-CP55,940, to sections of human hippocampus (panel A) and basal ganglia (panel C). Dark grey areas represent high levels of binding. Non-specific binding of [<sup>3</sup>H]-CP55,940 is defined in adjacent sections of hippocampus (panel B) and basal ganglia (panel D) incubated in the presence of excess unlabelled CP55,940. DML = molecular layer of the Dentate Gyrus, GPI = internal compartment of the Globus Pallidus.

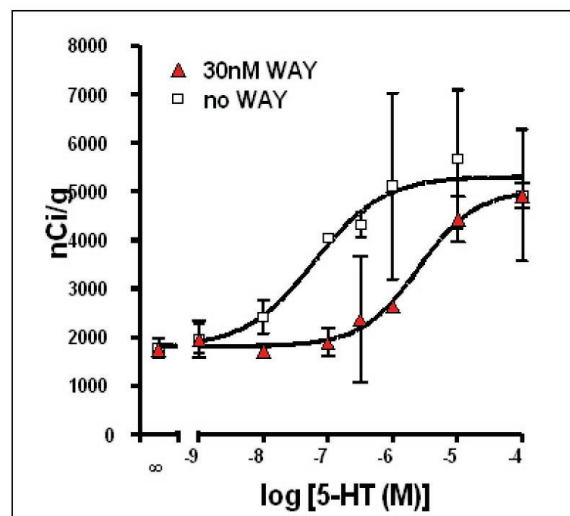
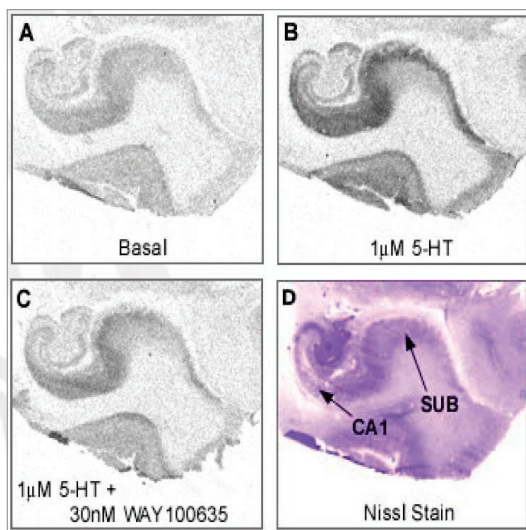
Typically such studies will involve 3 stages to:

- Preselect donor tissues and determine K<sub>d</sub> and B<sub>max</sub>
- Quantify extent of ligand binding through generation of grey-scale calibration curves and generate histochemical reference data in adjacent tissue sections
- Evaluate effects of test compounds on radioligand binding

Densitometric analysis is used to assess changes in radioligand binding and hence estimate IC<sub>50</sub> and pK<sub>i</sub> values.

### Functional [<sup>35</sup>S]-GTPγS binding autoradiography

[<sup>35</sup>S]-GTPγS autoradiography can be used to visualize agonist-stimulated activation of some G-protein-coupled receptors in sections of tissue. This is achieved by incubating tissue sections with agonist in the presence of [<sup>35</sup>S]-GTPγS, a non-hydrolysable radiolabelled analogue of GTP. Following receptor activation by the agonist, [<sup>35</sup>S]-GTPγS binds irreversibly to the activated G-protein and this can be detected by autoradiography.



*5-HT-stimulated [<sup>35</sup>S]-GTP $\gamma$ S accumulation in human hippocampus. 5-HT (1  $\mu$ M, panel B) caused an increase in [<sup>35</sup>S]-GTP $\gamma$ S accumulation in discrete areas of the hippocampus compared to basal levels of binding (panel A). The greatest increases in binding were observed in the CA1 region and the subiculum (SUB) regions identified in Nissl-stained section (panel D). 5-HT-stimulated [<sup>35</sup>S]-GTP $\gamma$ S binding was inhibited by the selective 5-HT<sub>1A</sub> receptor antagonist, WAY100635 (30nM, panel C). Using a calibrated grey-scale, the extent of antagonism by different concentrations of WAY100635 was quantified and antagonist potency and effect measured (right hand panel).*

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Donor selection and a full assay optimization are standard Stage 1 development work procedures for all autoradiographic studies. Assay optimization includes titration of GDP and ligand concentration against [<sup>35</sup>S]-GTP $\gamma$ S binding and titration of DTT to reduce non specific [<sup>35</sup>S]-GTP $\gamma$ S binding.

Optimal assay conditions are used in Stage 2 to determine the binding constant and receptor localization for test compound(s).

These scientific posters have been presented at scientific meetings by Asterand, some in conjunction with our customers.

**Intrinsic activity of S-8510, a benzodiazepine inverse agonist, in the cortex of Alzheimer's patients.**

Presented at Neurosciences, November 2005. This was a joint poster with Shionogi (Japan & US).

**Aripiprazole is a D2 receptor antagonist in human striatal membranes, as measured using [<sup>35</sup>S]-GTP $\gamma$ S binding.**

Presented at Neurosciences, November 2006. This was a joint poster with GSK.

**Visualisation and characterization of functional 5-HT receptors in the human dorsal raphe nucleus by [<sup>35</sup>S]-GTP $\gamma$ S autoradiography.**

Presented at the Federation of European Neuroscience Societies Forum, June 2000. This was a joint poster with SmithKline Beecham.

**Visualisation of functional CB1 cannabinoid receptors in the human CNS by [<sup>35</sup>S]-GTP $\gamma$ S autoradiography.**

Presented at the Society for Neurosciences meeting, 1999. This poster presented inhouse data comparing functional [<sup>35</sup>S]-GTP $\gamma$ S and classical radioligand binding autoradiography in human CNS.

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