GPCRs
new strategies for an old target class

GPCRs are the most studied drug target class and have a proven record as valuable drug targets, with 40-50% of marketed drugs being modulators of GPCR function. Despite the intense effort focused on these targets by the pharmaceutical industry over many years, numerous challenges remain. Evolving technologies for GPCRs present new strategies for exploiting this rich target class. Here we present preliminary results from HighTech Business Decisions' recently conducted interviews with HTS directors as part of its global study 'High Throughput Screening 2007: New Strategies, Success Rates and Use of Enabling Technologies' and discuss trends in GPCR research including deorphanisation, identification on partial agonists, antagonists and inverse agonists, receptor oligomerisation and heterodimerisation and the development of screening panels of GPCRs including whole families.

By William Downey and Dr Shauna Farr-Jones

HTS directors were asked to list the types of targets they screen and to provide the percentage each represents of the total wells screened per year for 2007 and then forecast for 2009. The averaged percentages are shown in **Table 1** along with the number of respondents using each target type.

Based on the 39 respondents, the most prevalently screened target types are protein kinases and GPCRs. GPCRs now represent the largest target class screened with an average of 22.4%, slightly higher than protein kinases at 22.2% average use. The use of protein kinases is expected to decrease further in 2009. GPCRs, at an average percentage use of 20.7%, are expected to increase slightly in use over the next two years. Other target types with expected increases in use are voltage and ligand-gated ion channels, and protein-protein interactions. Novel, specialty targets are expected to grow substantially in use. Altogether, 39 respondents provided data for both 2007 and 2009. Those respondents not providing data for 2006 indicate that the target types provided by therapeutic groups fluctuate dramatically year to year and they cannot predict these shifts in target types. Several respondents mentioned the increasing use of phenotypic assays and high content screening (HCS) in the future.

Functional GPCR assay technologies

GPCRs are so important pharmacologically because they are cell surface receptors that respond to a variety of extra-cellular stimuli (drugs, neurotransmitters, hormones, odours and others) and transmit responses initiating intracellular signalling events that lead to a host of physiological effects. Functional assays for this crucial target class require cells and, in recent years, mammalian cellular expression systems have become the standard for GPCR screening.

Because GPCRs are such a rich source of drug targets, researchers have developed many approaches to GPCR screening. Some optical-readout assay systems that do not require displacement

of a known ligand to measure activity include PathHunter™ β-arrestin translocation chemiluminescence technology (DiscoveRx) and the imaging-based Transfluor technology (Molecular Devices) have unique advantages in orphan GPCR assays because displacement of a known ligand is not required. However, assay development is challenging for both of these technologies. Each of these assays have advantages and disadvantages. For example, while imaging-based detection gives information of intracellular translocation, imaging is much slower than Ca++ assays, making imaging-based assays less suitable for primary screening than a single readout.

The IP3 detection technology (HTRF® from Cisbio) can also be used for orphan receptors if they have constitutive activity. HTRF can be run in high throughput and used to identify agonists, antagonists and inverse agonists. Recently, Cisbio reported that its IP-One HTRF assay could be used for detecting slow-binding agonists. IP1 is a metabolite in the inositol phosphate pathway and is a better assay for detecting slow binding agonists than Ca++, which has a very short read out and cannot be used for long compound incubation. However, Ca++ is more sensitive than IP3.

In our survey we asked HTS lab directors about the types of cell-based assays they used. Based on our interim findings of 39 respondents, the types of cell-based assays are reported in **Table 2**. The average percentage of wells screened for GPCR activation or inactivation is expected to go down slightly from 17.9% in 2007 to 16.3% in 2008. Likewise translocation/redistribution assays are expected to decrease for 13.1% in 2007 to 7.7% in 2008

GPCR dimerisation, heterodimerisation and oligomerisation

While compounds binding to GPCRs are typically classified as agonists, partial agonists or antagonists, GPCR signalling may involve more varied mechanisms than a simple two-state model of active and inactive forms would suggest. Other forms of regulation of receptor signalling have been recognised over the past decade that involved receptor dimerisation, heterodimerisation, interaction with non-GPCR effector proteins and other mechanisms. Each of these different active states can display different ligand binding profiles, and *in vivo*, different pharmacology. Thus to the extent that cell-based functional assay systems can recapitulate this complex biology, they will provide assays that are more predictive of clinical results.

Although it has been known for many years that

Table 1: Selected target types by averaged percentage use: 2007 and 2009 from 39 respondents

SELECTED TARGET TYPES	% CURRENT USE (2007)	% PREDICTED USE (2009)
Kinases (protein and lipid)	23.2	20
GPCR	22.4	22.6
Phenotypic assays	10.1	11.3
Ion channels	10	12.2
Protein-Protein/peptide Interactions	6.3	7
Nuclear receptors	4.6	3.0
Protease	4.5	4.5
Cytokine receptors (non-GPCR)	2.4	2.3
Phosphatase	1.7	1.3
Protein-nucleic acid Interactions	1.6	1.7
Transporters	0.9	0.5
Polymerase	0.4	0.4

Source: HighTech Business Decisions

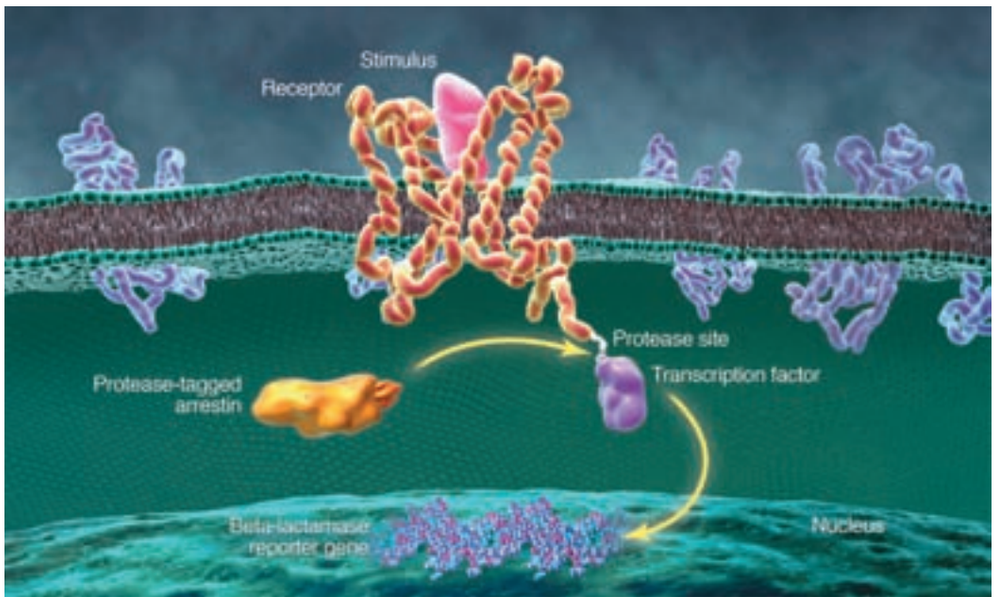


Figure 1

GPCRs can multimerise, the pharmaceutical industry has only recently begun to explore this property. Multimerisation is part of the normal trafficking and function of GPCR may provide a new approach to these targets. Furthermore, understanding multimerisation of GPCRs may explain pharmacologic properties of ligands and drugs.

Invitrogen technology provides competitive binding assay pharmacology in a cell-based assay platform. Tango™ GPCR assays allow isolation of target GPCR signal to be separated from the background of endogenous GPCRs, thus reducing the number of false positives during HTS.

In early 2007 Invitrogen acquired Sentigen/CMT, obtaining capability in cell scale-up and production for cell based assays and Tango™ Assay, a protein-protein interaction assay applied to GPCR screening. The Sentigen/CMT acquisition provided Invitrogen with an entry into the GPCR assay and screening market, which the company reports will be a major focus in the next two to three years.

The engineered Tango assay, illustrated in **Figure 1**, uses a reporter gene readout. When the GPCR is activated, it binds an intracellular protein (arrestin) with a protease activity. When the arrestin binds to the GPCR a tethered transcription factor is released via protease cleavage, allowing the transcription factor to enter the cell nucleus, bind DNA and initiate transcription. One of the advantages of the Tango assay is that it is highly specific to the receptor under investigation, reducing background signal and identification of false positive hits through endogenous receptors.

GPCR assay panels

GPCR assay panels allow determination of specificity and selectivity of leads, allowing researchers to determine potential off-target effects, which may suggest adverse effects clinically. Assay panels are very useful for primary screening, secondary screening and compound profiling. Furthermore, inclusion of orphan receptors in panels facilitates deorphanisation.

Multiple companies offer screening of GPCR panels, including Millipore, Multispan, DiscoveRx, Novascreen (a division of Caliper), Invitrogen, MDS Pharma Services and others.

Table 2: Cell-based assay types (based on results from 39 HTS lab director interviews)

ASSAY TYPE	AVERAGE OF 2006 % WELLS SCREENED	AVERAGE OF 2008 % WELLS SCREENED
GPCR activation/ inactivation	17.9	16.3
Other receptor binding	16.7	13.6
Second messenger – cAMP or inositol phosphate	21.2	20.7
Translocation/redistribution	13.1	7.7
Protein or Enzyme complementation	5.0	5.5

Source: HighTech Business Decisions

DiscoveRx, whose products are distributed by GE Healthcare, has launched a GPCR profiling service using its PathHunter™ beta-arrestin technology platform for 32 ligand families. PathHunter™ Arrestin technology measures GPCR activation without imaging or second messenger detection, furthermore, the target does not need to be overexpressed. The PathHunter™ platform can be used in studies of dose response, agonist/antagonist activity and allosteric modulation.

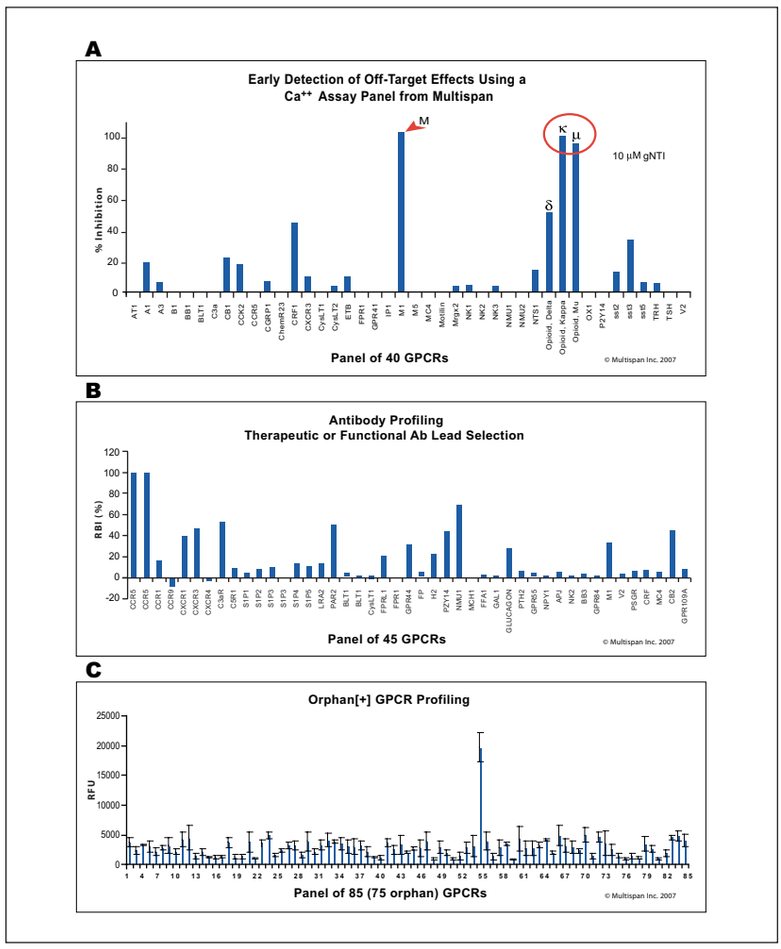


Figure 2: A Antagonist lead compound against Opioid Receptor Kappa (κ) was profiled on a panel of 40 GPCRs that includes closely-related Opioid Receptor Mu (μ) and Delta (δ). Our result demonstrates that the lead antagonises both the Opioid Receptor κ and μ with similar potency and Receptor δ with a lesser and yet significant effect. Unexpectedly, this compound also demonstrates great off-target cross-reactivity to Muscarinic receptor M1 with even higher potency than to any of the 3 Opioid Receptors.
B Binding of a test mAb to the GPCR receptor panel is measured by FACS analysis using Geometric Mean of fluorescence intensity. RBI (Relative Binding Index) is determined by normalising Geometric Mean of the test mAb binding to that of anti-tag mAb (N-terminal FLAG tag) binding. RBI for the test mAb to the target GPCR is set at 100%.
C One lead compound tested for agonistic activity in a panel of 85 GPCRs including 75 orphan GPCRs using Ca++ assay. All GPCRs are cloned into Multispan's proprietary vector and confirmed for expression by FACS (data not shown). Activities of non-orphan GPCRs have also been confirmed by known ligands (data not shown).

GPCR assays historically involved competition assays using radioactive labels. This approach, besides having the inconvenience and hazard of radioactivity, requires a known ligand. This is not always the case and many orphan receptors remain. Now fluorescent assays predominate, as illustrated in **Table 3**, based on interviews with 39 respondents, 13 of whom reported their GPCR assay detection mode.

Multispan has launched a few unique GPCR panels for compound profiling – the FFA (free fatty acid) receptor family which is typically difficult to develop for HTS. Multispan has the entire FFA family with a signal to noise ratio of 10. Multispan has also launched a prostanoid family panel of nine receptors. This family is involved in inflammation, cancer, cardiovascular disease and a wide range of disease indications. The Multispan assays use FLIPR readouts of calcium signalling. **Figure 2** illustrates the range, diversity and sensitivity of Multispan's GPCR assays.

Another challenging family for scientists is the glutamate receptor family, also called the metabotropic receptor family which has eight members. Multispan plans to launch a panel of the complete family by the end of 2007.

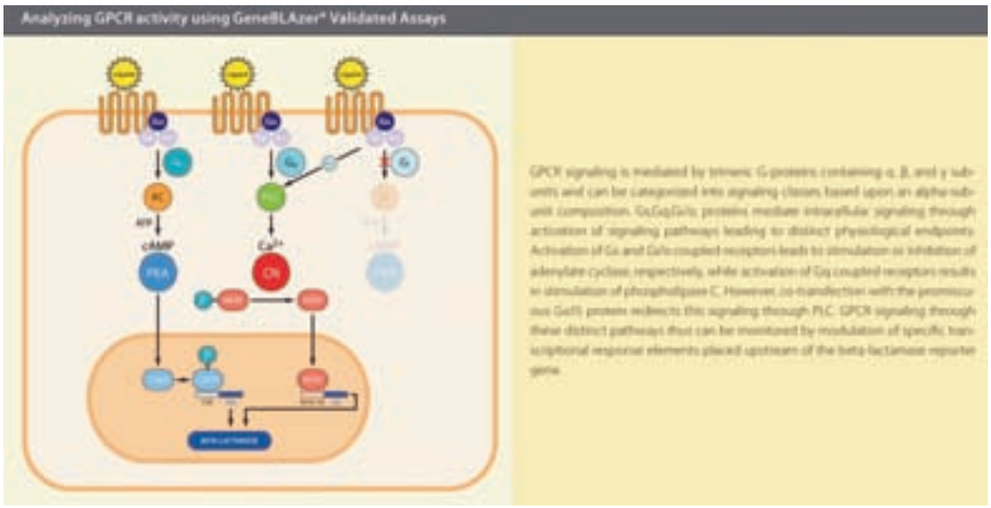
One particularly challenging target family is the somatostatin receptors (SST). The hormone somatostatin inhibits secretion of growth hormone, insulin, gastrin, secretin, cholecystokinin, vasoactive intestinal peptide, glucagons and others, making the SST receptor family an especially interesting target for drug discovery. SST receptor binders may have applications for treating cancer. Multispan recently launched a panel of five SST receptors (SST1-5).

Deorphanisation

GPCRs with no known ligands (orphan receptors) present a significant opportunity for drug discovery because it opens up new, unexploited targets. Furthermore, identifying agonists or antagonists may lead to discovery of the endogenous ligands and reveal insight into the function of orphan receptors. GPCR panels for orphan screening include known GPCRs as positive controls. However, ligand displacement assays cannot be used for deorphanisaion as known ligands are not available.

Repurposing

Drug repurposing is finding new uses for existing drugs with known safety. Access to panels of GPCRs including those with unknown function offers the opportunity for drug repurposing, an important trend in an industry trying to circumvent the need for expensive discovery efforts and safety testing. Drugs with known clinical safety



profiles may be found to have application for other, unexpected indications. Access to large panels of GPCRs including complete GPCR families provides the ability to explore in great detail the selectivity and specificity of GPCR ligands, and potentially uncover new uses for existing drugs.

Summary

While a very old and intensively studied target, technology for assaying GPCRs continues to evolve and pharmaceutical and biotechnology companies continue to search for drugs to this target class. We anticipate that more comprehensive assay panels will be assembled, and assays with the ability to explore the more subtle regulatory mechanisms of oligomerisation and allosteric modulation will be developed. DDW

Table 3: Detection methods used for GPCR assays (13 respondents)

DETECTION MODES USED FOR GPCR ASSAYS	COMPANIES REPORTING
FLIPR (Molecular Devices)	5
Confocal microscopy, HCS	2
Scintillation proximity assay (GE Healthcare)	2
TR-FRET (cAMP, IP3)	2
Unspecified fluorescence	2
Unspecified luminescence	2
LumiLux™ (PerkinElmer)	1
HTRF™ (Cisbio)	1
EFC (DiscoveRx)	1
Unspecified detection method	4

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