<u>Screen Design and Assay Technology Special Interest Group</u>
Monday, April 7, 2008
Open Discussion Forum
Kenda Evans, Chair

All preregistered attendees had been asked to submit questions for discussion during this Open discussion forum. The questions were asked as they had been submitted and the discussion is as follows:

1. Cellular Imaging as a Screening Platform – challenges, advantages and disadvantages. What will it take for broad adoption in primary screening and what is the current thought around the quality of the leads generated from cellular screening?

When creating the tools for these assays, one must ask oneself "what is the most physiological assay?" The challenge is target identification. It is hard to drive the research if you don't know the exact target.
Need to have assays that increase the physiological relevance.

An individual made the comment that when working with automated microscopy you are fully dependent on cells that are fully attached to have staining.

One researcher mentioned that live cell staining is Ok for a total fluorescent readout. HTS FACs can do this well.

2. "Promiscuous Inhibitors"
Promiscuous inhibitors are sometimes promiscuous only in relation to target – when they show enzyme concentration dependent inhibition.

Usually this is seen as being a compound dependent issue but it is not always just the compound but can also been seen with the enzyme.

3. There is a huge impact of artificial interferences on any fluorescent readout such as a large hit rate and a lot of false positives.
What are the TRUE experiences on most common fluorescent techniques applied for HTS in terms of artifact responses?

False positive problems associated with screening:
NADH readout at 460nm- yellow colored compounds may cause problems since many compounds have fluorescent properties. One individual commented he ran kinetic assays so that he could eliminate the effect of the compound since one could monitor the activity of the assay over time.

Another researcher commented his lab ran interference assays that mimicked the screening assay as closely as possible. If the compound still produced a signal it was considered a false positive. Other researchers run orthogonal assay formats to determine if a compound is a false positive. One example given was a cell based proliferation assay

using a luminescent readout. In this example, an orthogonal alamar Blue assay was performed confirming 92% of the hits, basically producing the same hit rate as the original assay. It was also mentioned that there are different kinds of luciferase and an inhibitor of one type of luciferase may not inhibit a mutant form of luciferase.

It was also mentioned that if one discards all fluorescent compounds in the wavelength of interested you might discard a potential hit since although the compound may be fluorescent it may still have activity against the target of interest.

It was discussed that keeping a record of false positive is a good practice. However, it was noted that 25% of these compounds hit only in one assay so one needs to be careful not to exclude compounds. In addition, a false positive result in one assay may not make the same compound a false positive in another assay.

4. New technologies for Ab-free HTS.

Can Development of Ab-free detection assays using molecular recognition tools such as aptamers, binding proteins and synethic ligands greatly improve HTS?

There was little to no discussion surrounding this topic.

5. Does label free technology have a role in plate based screening and if so, is it to understand mechanism of action or HTS?

Label free technology

Label free technology was discussed but it was quickly noted that it was not yet considered to be high throughput. Researchers noted that the surface needs to have the ability to preserve the conformation of the target protein. Precipitation was also problematic in these assays and that performing a compound solubility assay before running an assay was helpful.

The use label free technology was said to be beneficial for conformation of hits.

6. Why do people choose a specific technology for their assay development? How much understanding of the signaling pathway and desired outcomes is considered when making a choice?

Deciding upon best format for new assays

Many researchers agreed that most frequently the assay technology that is used widely in the lab is the one chosen for the assay development. At times new techniques are explored in order to determine which methodology provides the most information about the target. Usually researchers use what they are familiar with and work within the constraints of the technology.

Individuals commented that sometimes they assayed with the format with which they were familiar and at other times determined the technology at the beginning of the assay.

One factor was whether the assay needed to be pharmacologically relevant or physiologically relevant.

Also mentioned systems biology

Natural coupling/multiple pathways in cells

Cell assays verses target specific assays.

7. Compare and contrast different functional methods for measuring GPCR ligand engagement and their ability to identify relevant antagonists and agonists. Which is the most pharmacologically meaningful and predictive of activity of compounds *in vivo*?

There was little to none open discussion regarding this topic.