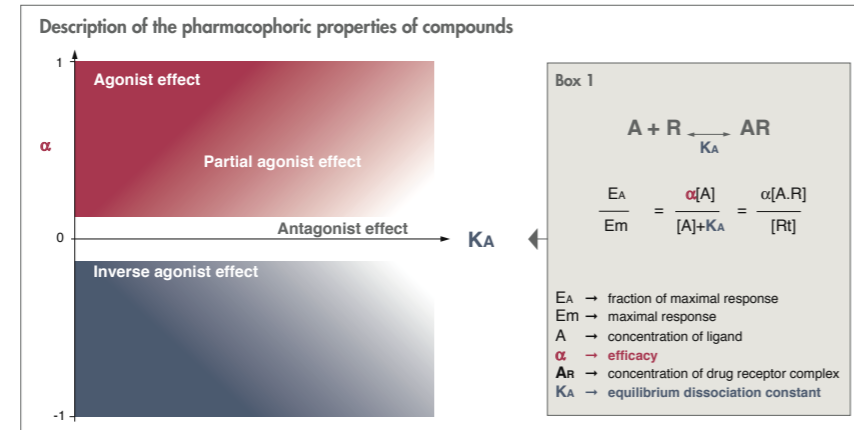


# Comparison of binding and cellular profiles of multi-target G protein-coupled receptors

B. Foucaq, S.R Wang, D. Piwnica, L. Coussy, T. Jolas, G. Néliat - Cerep - Le Bois l'Évêque, 86600 Celle l'Evescault, France - [www.cerep.com](http://www.cerep.com) - [b.foucaq@cerep.fr](mailto:b.foucaq@cerep.fr)

## A – Difference between agonist and antagonist

Using  $K_i$  calculations, Radioligand Binding Assays (RBA) are powerful tools to determine the affinity of one compound for specific targets. While binding affinity is important, the functional downstream effect is of greatest importance. A compound has several potential functional behaviors, such as agonist, antagonist, partial agonist, inverse agonist, etc. As is shown below, the functional effects of a compound can be described using affinity and efficacy property components.

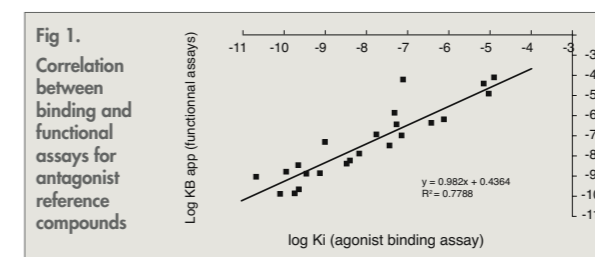


- For antagonist compounds, the efficacy constant ( $\alpha$  in Box 1) is very low and we can suppose that functional effects only depend on the affinity component of the equation (according to the law of mass action at the origin of occupancy theory). Using this approach, the comparison between binding (affinity component) and functional (affinity and efficacy component) can be performed by a comparison of the  $K_i$  and the  $K_{B\_app}$  of one compound (only for competitive antagonists).

- For agonist or inverse agonist compounds, the efficacy constant is not equal to 0 and direct comparison of binding ( $K_i$ ) and functional results ( $EC_{50}/IC_{50}$ ) is not possible.

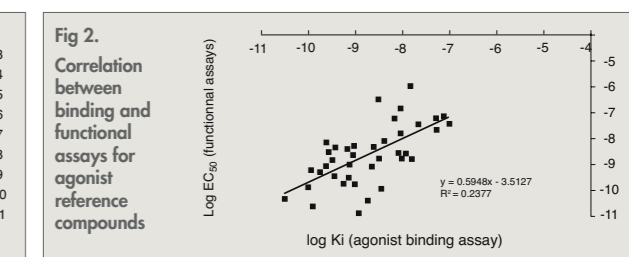
## C – Correlation between binding and functional assays

To analyze the correlation between functional and binding assays,  $\log K_i$  &  $\log K_B$  determined for reference antagonist compounds were compared on 56 different GPCR targets. As shown in figure 1, we obtain a very strong correlation between  $K_i$  and  $K_B$  with an  $R^2$  near 0.78 for the linear fit.



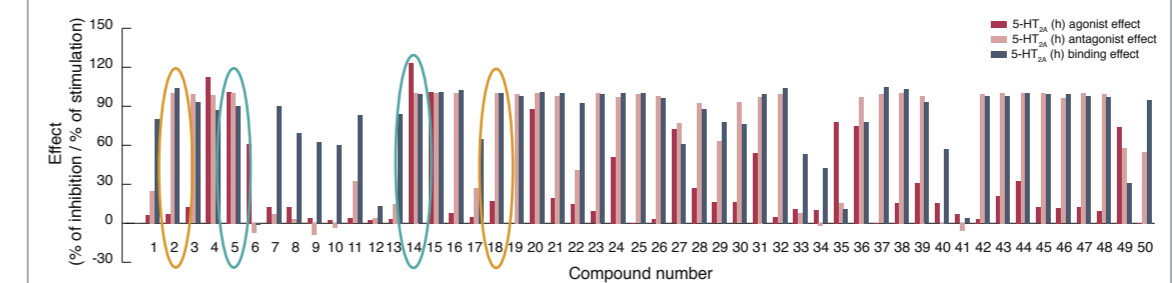
We confirm that application of occupancy theory (see part B) is a good model with which to analyze antagonist binding and functional effects. Moreover, the functional assay protocols, using only the frozen cells, produces the same quality of results as the corresponding binding assay.

In the figure 2, we observe a poor correlation between  $K_i$  and  $EC_{50}$  of agonist reference compounds tested on the same panel of assays. These results confirm that the cellular response to a pharmacological agonists depends on the efficacy of the agonist (which is a property of each individual agonist) and the sensitivity of the system (Kenakin, 2003).



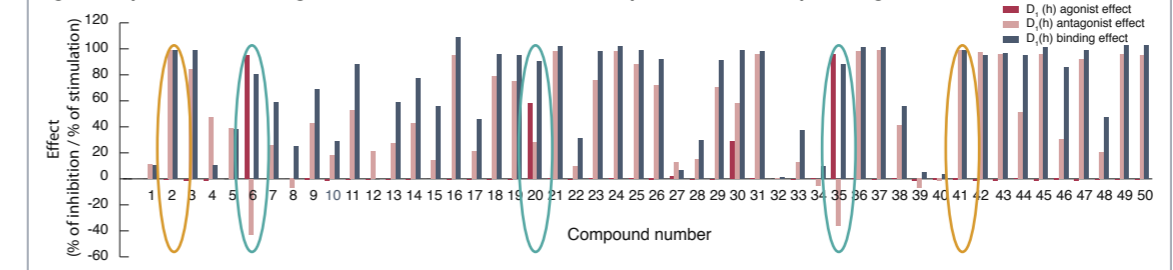
## D – Comparison of binding and functional profiles

**Fig 3.** Comparison of the binding and functional effect of 50 BioPrint® compounds in the 5HT<sub>2A</sub> receptor (using calcium fluorometric detection)



We performed binding and functional (agonist and antagonist mode) profiles of compounds selected for their high hit rates on GPCR targets. In figures 3 & 4, we show the profiles of 50 compounds on the 5HT<sub>2A</sub> and D<sub>1</sub> receptors. The typical effect of agonist or antagonist compounds are shown in blue and yellow circles respectively.

**Fig 4.** Comparison of the binding and functional effect of 50 BioPrint® compounds in the D<sub>1</sub> receptor (using cAMP HTRF® detection)

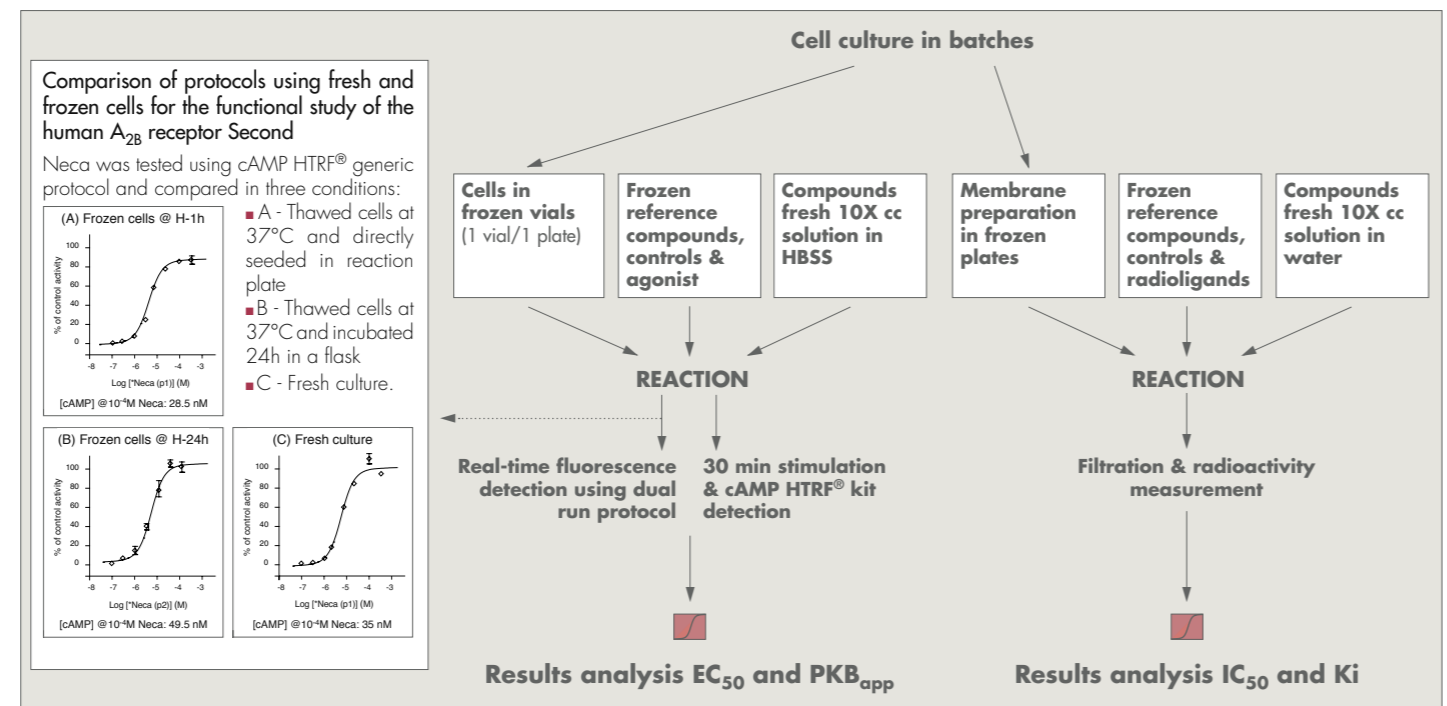


**Remark:** Profiles of agonist compounds differ between detection technologies. In fact, using calcium fluorimetry, desensitization of the calcium system (G<sub>q</sub> coupling receptors) induces a false antagonist effect. Conversely, for G<sub>s</sub> coupled receptors, overproduction of cAMP induced by agonist induces negative inhibition.

## B – Material and methods

To control for the quality of our results in terms of homogeneity and reproducibility, we produce large batches of cells which express GPCR receptors. From these batches prepared membranes and whole cells are frozen and stored for binding assays and functional assays respectively.

Two technologies were selected for use in this platform: HTRF® to determine cAMP concentration and real time fluorescence to monitor calcium concentration both in living cells. Measuring these second messengers allows us to develop functional assays for receptors coupled to G<sub>i</sub>, G<sub>s</sub>, and G<sub>q</sub>.



## Introduction

In designing leads, it is of interest to consider that GPCRs can couple to a large variety of cellular effects. A new approach based on functional profiling of compounds can provide high density information necessary to optimize compounds during the development of successful drugs.

Using generalized and uniform conditions, Cerep is currently developing a complete high-throughput functional profiling GPCR platform adapted to monitor G<sub>s</sub>, G<sub>i</sub> and G<sub>q</sub>-coupled receptors. To understand the correlation between receptor binding and intracellular signalling (Calcium flux and cAMP production) we have compared results obtained in GPCR receptor radioligand binding and functional assays.



## Bibliography

- Ariën, E. J. et al. (1979), *The receptor, a comprehensive treatise*. Edited by R. O'Brien, p 33-91. Plenum Press, NY
- Kenakin, T. et al. (2003), *Predicting therapeutic value in the lead optimization phase of drug discovery*. *Nature reviews*, 2: 429-38.
- Hill, S. J. (2006), *G-protein-coupled receptors: past, present and future*. *British journal of pharmacology*, 147 (S1): S27-37

## Acknowledgements

We acknowledge Mr Jacques Migeon for his assistance, Mr Olivier Godet for IT part of this work. We thank Mrs Catherine Moreau and Mr Loïc Dorgeret for poster preparation.

## Conclusion

To understand the correlation between receptor binding data and intracellular signalling data (Calcium flux and cAMP production) we have compared results obtained in GPCR receptor binding and functional assays using reference compounds. For the antagonist compounds, we obtained a good correlation between binding and functional effects due to the single affinity component of functional effect. For the agonist compounds, both efficacy and affinity drive the functional effects of the compound. In fact, the affinity, an intrinsic property of the molecule, can easily be determined by binding assays, but the efficacy depends on the environment of the receptor, expression level of receptors & coupling proteins and the method of detection. New advances in pharmacology have introduced new descriptions of molecules. Inverse agonists, allosteric modulators and proteans can be described in many systems (Hill, 2006). In the case of the work presented here, we use a simple approach to functionality. Inverse agonists appear as antagonists and we are unable to detect allosteric potentiators. Nevertheless profiling molecules using this new approach provides information about their abilities to simulate or to block a signal via a receptor. During the lead optimization phase, functional information combined with binding results now opens a new dimension in pharmacological profiling which allows us to more efficiently approach on and off target biological effects.