# FLIPR 1536-WELL: ARE YOU UP TO IT?

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## Abstract and Introduction

To measure the activity of Gq-coupled GPCRs in drug discovery and compound screening campaigns, intracellular calcium flux assays using fluorescent calcium binding dyes play an important role. The FLIPR Tetra® system offers real-time kinetic measurement of calcium flux within cells and is traditionally performed in 384-well assay plates. However, as high-throughput screening campaigns have been increasing in size over the past years, the substantial number of cells and reagents required, and the overall duration of the campaigns have become increasingly important restricting factors.

To circumvent these limitations, we have miniaturized a cellular calcium flux assay to 1536-well format. Using a FLIPR Tetra® equipped with a 1536-pipettor head, we performed a mini-screen of 2,816 compounds from the Evotec-lead like compound library. We identified agonists and allosteric modulators of endogenously expressed purinergic receptors in wild type CHO cells (WT CHO) showing dose-dependent activation, demonstrating suitability of the 1536-well format for calcium flux assays in screening mode.

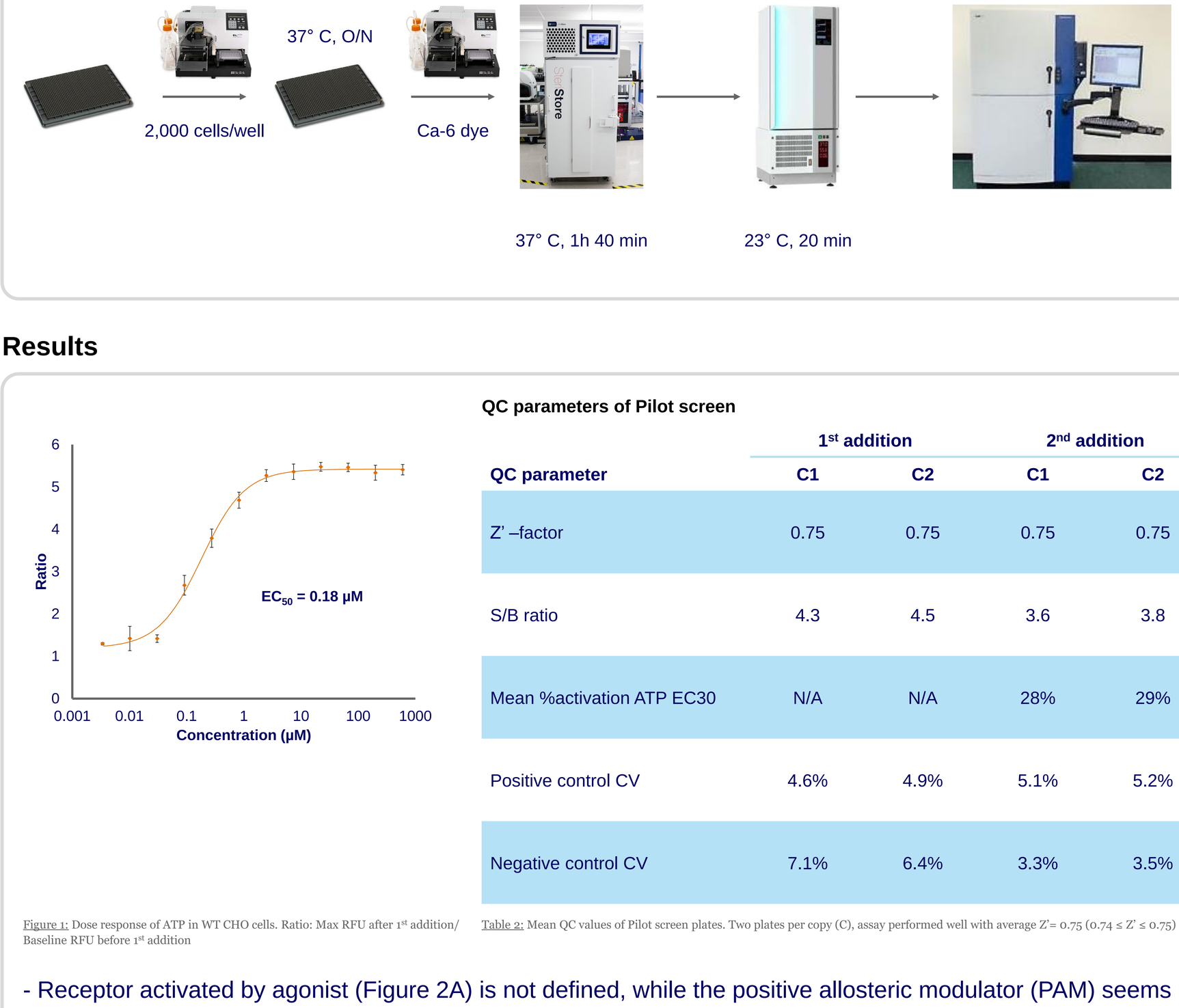
Our study demonstrates the advantages of miniaturization of a cell-based calcium flux assay into a 1536-well format using the FLIPR Tetra®, by gathering real time data in high-throughput mode using much less cells, smaller reagent volumes and a significantly increased throughput time, thereby enabling time- and cost-efficient screening of libraries with >250,000 compounds.

## Methods

*Compound selection:* From CHEMBLv24 any compound shown to activate human P2Y receptors in a dose-dependent manner was retrieved. These compounds were clustered and three maximally diverse representatives per cluster were used as seeds for close analogue search against the Evotec-lead like compound collection.

*Assay plate preparation:* WT CHO cells were seeded at a density of 2,000 cells/well into 1536-well (Greiner 783092) plates, in a volume of 4 µl, plates were incubated overnight at 37° C in 5% $CO_2$.

*FLIPR Calcium kit protocol:* 2 µl/well of Ca-6 loading dye were dispensed into assay plates. Plates were then processed as displayed below. Plates were read in a FLIPR Tetra using the settings shown in Table 1. To detect allosteric modulators, ATP was added to the cells in the 2nd addition at a concentration corresponding to $EC_{30}$. All assay steps were performed on a HighRes Biosolutions fully automated screening system.




| Parameter | Setting |
|---|---|
| Excitation | 470-495 nm LED Module |
| LED intensity | 70% |
| Filter | 515-575 nm |
| Camera gain | 100 |
| Exposure | 0.4 sec |
| Pipetting volume | 2 µl |
| Height | 2 µl |
| Speed | 5 µl/sec |
| Tip removal speed | 10 mm/sec |

Table 1: Setup parameters for FLIPR Tetra equipped with a 1536-well pipettor head

## Results




Figure 1: Dose response of ATP in WT CHO cells. Ratio: Max RFU after 1st addition/ Baseline RFU before 1st addition

**QC parameters of Pilot screen**

| | 1st addition | | 2nd addition | |
|---|---|---|---|---|
| QC parameter | C1 | C2 | C1 | C2 |
| Z' –factor | 0.75 | 0.75 | 0.75 | 0.75 |
| S/B ratio | 4.3 | 4.5 | 3.6 | 3.8 |
| Mean %activation ATP EC30 | N/A | N/A | 28% | 29% |
| Positive control CV | 4.6% | 4.9% | 5.1% | 5.2% |
| Negative control CV | 7.1% | 6.4% | 3.3% | 3.5% |

Table 2: Mean QC values of Pilot screen plates. Two plates per copy (C), assay performed well with average Z'= 0.75 (0.74 ≤ Z' ≤ 0.75)

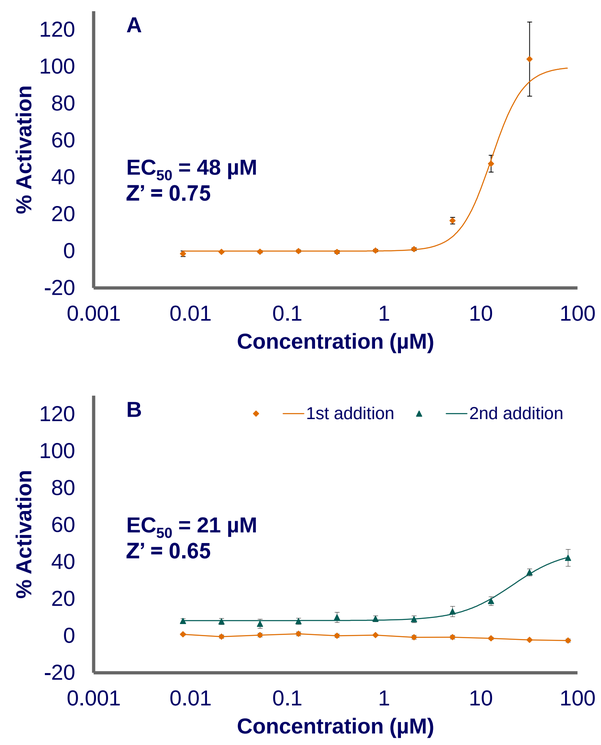



Figure 2: Example of an identified agonist (A) & an allosteric modulator (B) showing a dose-dependent activity. The allosteric modulator exhibits activity only after addition of ATP at $EC_{30}$. Orange line: 1st addition, Green line: 2nd addition.

- Receptor activated by agonist (Figure 2A) is not defined, while the positive allosteric modulator (PAM) seems related to purinergic receptors (Figure 2B)

## Conclusion

- We successfully miniaturized a cell-based calcium flux assay into 1536-well format
- The assay behaved robustly as indicated by the S/B ratio and Z' derived from controls on assay plates
- We performed a Pilot screen for modulators of purinergic receptors in CHO cells and could identify compounds with dose-dependent activity
- Using a 1536-pipettor head on FLIPR Tetra significantly improves the throughput capacity (~4-fold reduction in time needed for a screening campaign)