Miniaturized Cyclic Nucleotide-Gated (CNG) Channel Assays to Discover Neuropeptide Y Receptor Modulators

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Presentation Outline

(1) Intro to Scripps Florida

(2) NPY-Y2/Y1 HTS Receptor Background

(3) Cyclic Nucleotide Gated (CNG) Assay Technology

(4) Developing uHTS CNG Assays on the FLIPR Tetra

(5) Results of the NPY-Y1 and NPY-Y2 Antagonist 1536 uHTS campaigns

About Scripps Florida

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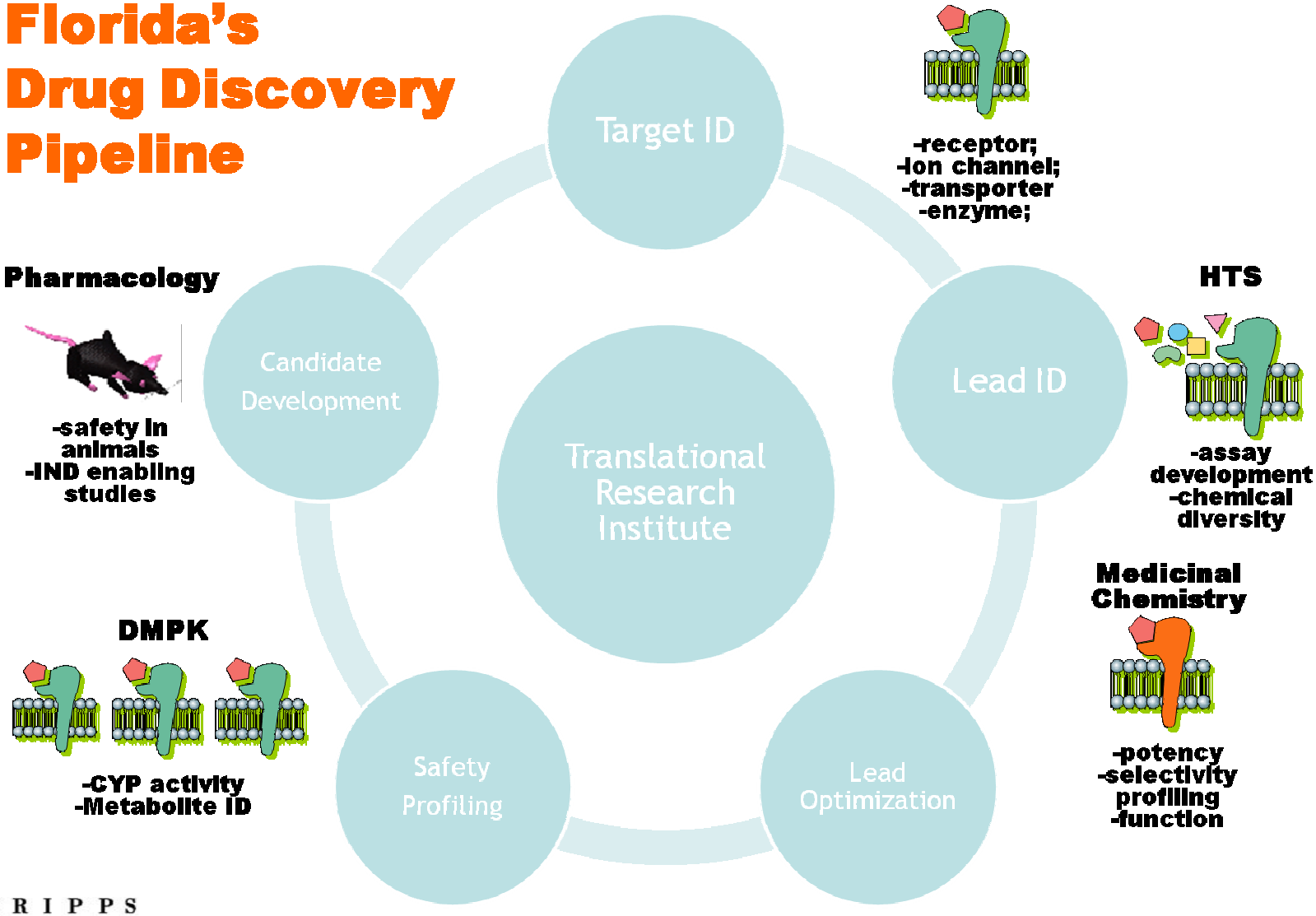
- Started activities in 2004
- Located in Jupiter, FL
- More than 300 employees
- New buildings in late 2008

Phase I & II (Feb 2005-Sep 2006): 75,000 sq ft lab space

Phase III (late 2008): 350,000 sq ft lab space

Lead ID in Scripps Florida's Drug Discovery Pipeline



The HTS/LeadID Department at Scripps

- Assay Development
- Assay miniaturization to 1536-well plate
- Ultra High-Throughput Screening
  - Integrated, 1536-HTS compatible MDS Tetra
- Compound management

Scripps Drug Discovery library >600K
MLSCN library: 196K & still growing

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The Molecular Libraries Screening Center Network (MLSCN)

Part of NIH's "roadmap" for medical research in the 21st century

Peer-reviewed process providing access to assay development or HTS technologies for academia, government, non-profit institutions and industry

Screening centers provide assay development, HTS, DMPK and Medicinal Chemistry support A/R

All results are publicly available (www.pubchem.gov)

Scripps is one of 10 MLSCN Screening Centers Located in the US

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Case study: MLSCN screens for NPY Receptor Modulators

Focus on two NPY receptors: NPY-Y1 & NPY-Y2
•Both are Gi/Go-coupled GPCRS

Neuropeptide Y (NPY) is a 36 residue peptide neurotransmitter in the brain & autonomic nervous system

NPY Signals through 5 related G-protein coupled receptors (Y1, Y2, Y4, Y5, Y6)

NPY-Y1 selective antagonists may treat obesity

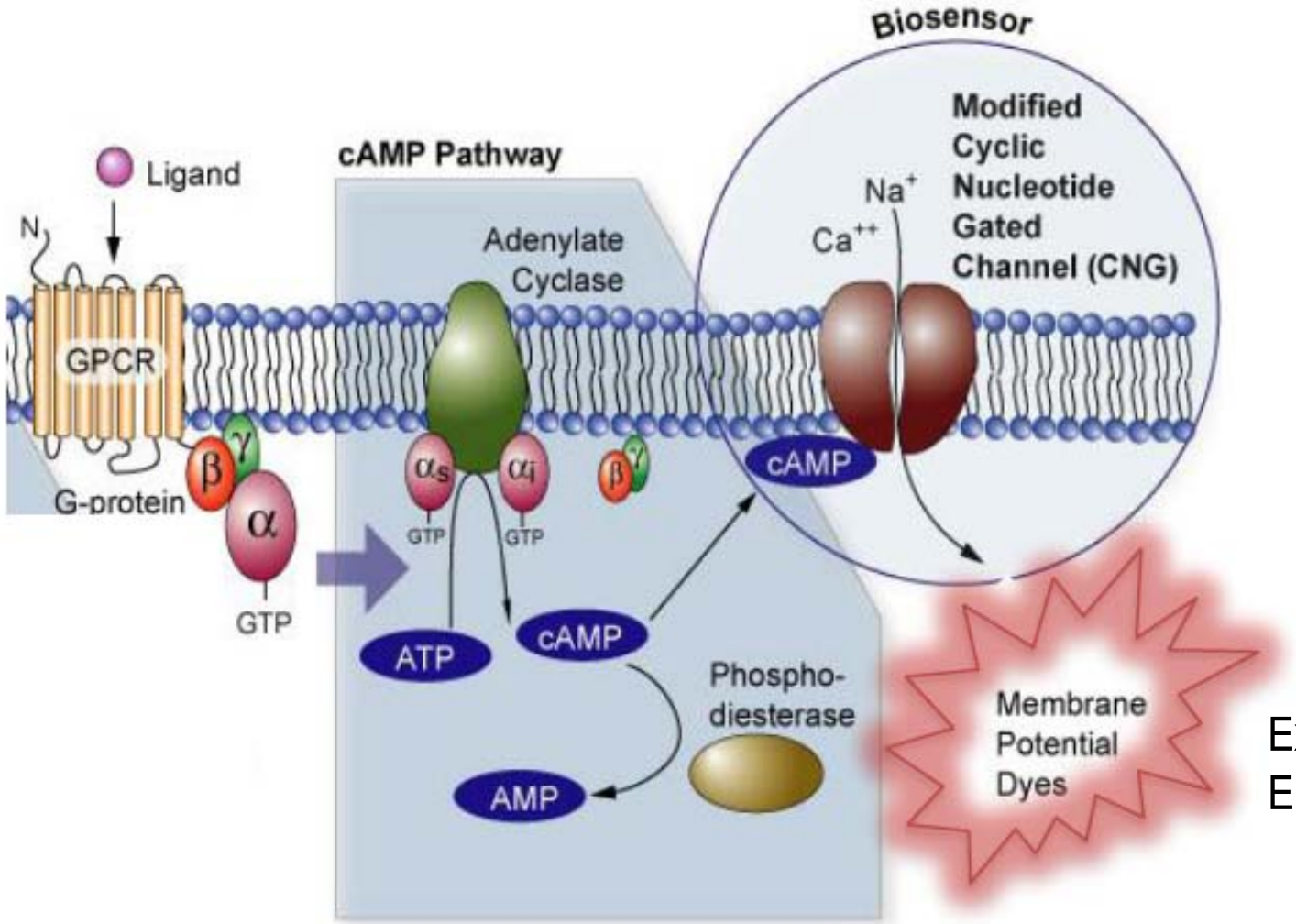
NPY-Y1 antagonists: **BIBP3226**, SR120819A, PD160170, LY357897 & J-11581411 have poor oral pharmacokinetic properties that limit CNS exposure

NPY-Y2 selective antagonists may treat alcoholism and anxiety

NPY-Y2 antagonists: **BIIE0246**, JNJ-5207787

Prof. Claes Wahlestedt, Scripps Florida, Grant Number: R21 NS056950-01 PubChem AIDs: 793 & 1040

ASSAY TECHNOLOGY: ACT*One* Live cell cAMP Biosensor Assay



Exc. @ 530 nm
Em. @ 590 nm

ACT*One* (BD Biosciences) uses a fluorescent membrane potential dye to detect changes in intracellular cAMP levels

Tetra-compatible assay format!

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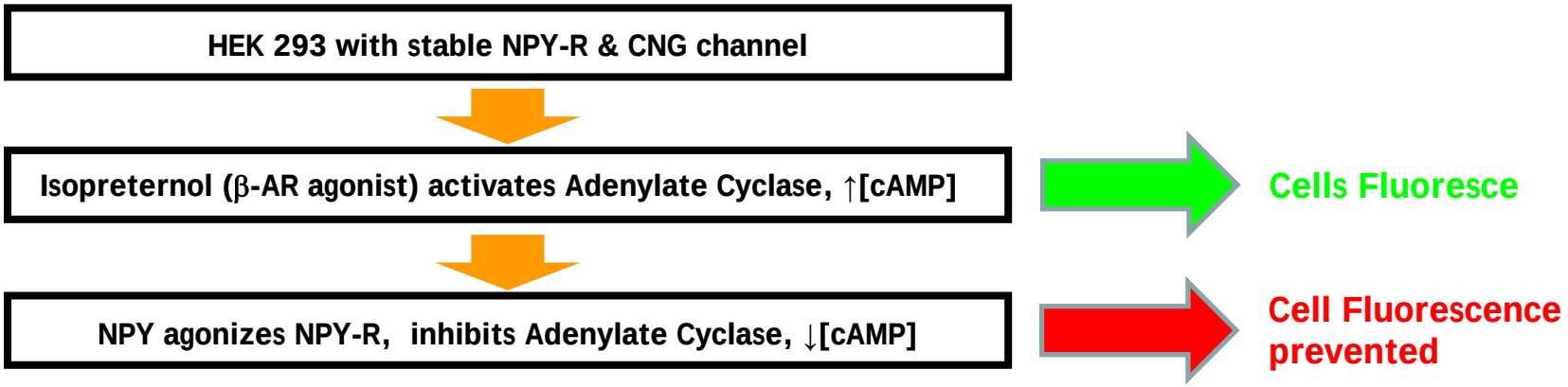
Measuring NPY Pharmacology with ACTOne



NPY Agonists: Isopreternol + Compound → **Cell Fluorescence prevented**

NPY Antagonists: Isopreternol + NPY + Compound → **Cells Fluoresce**

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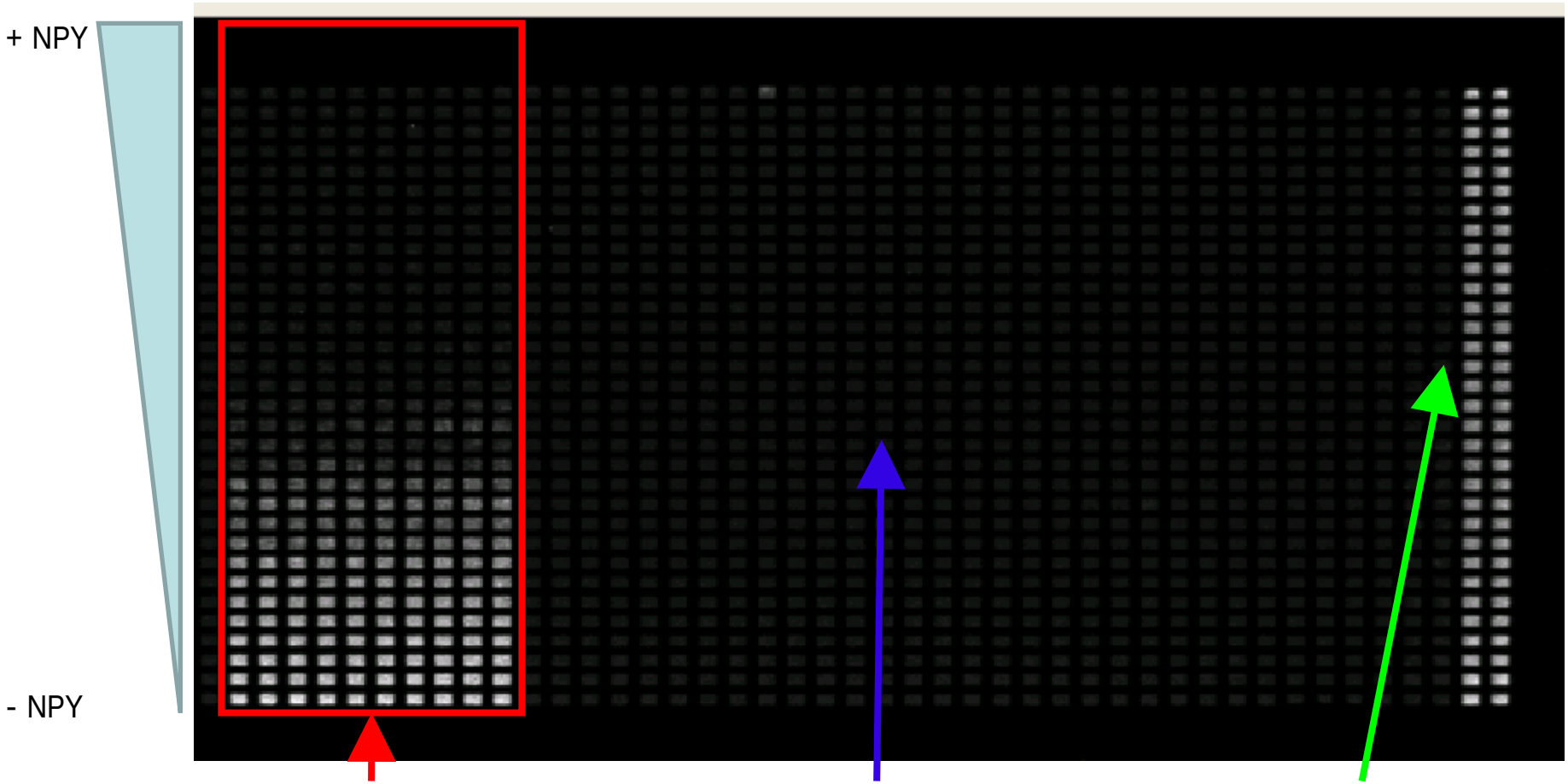
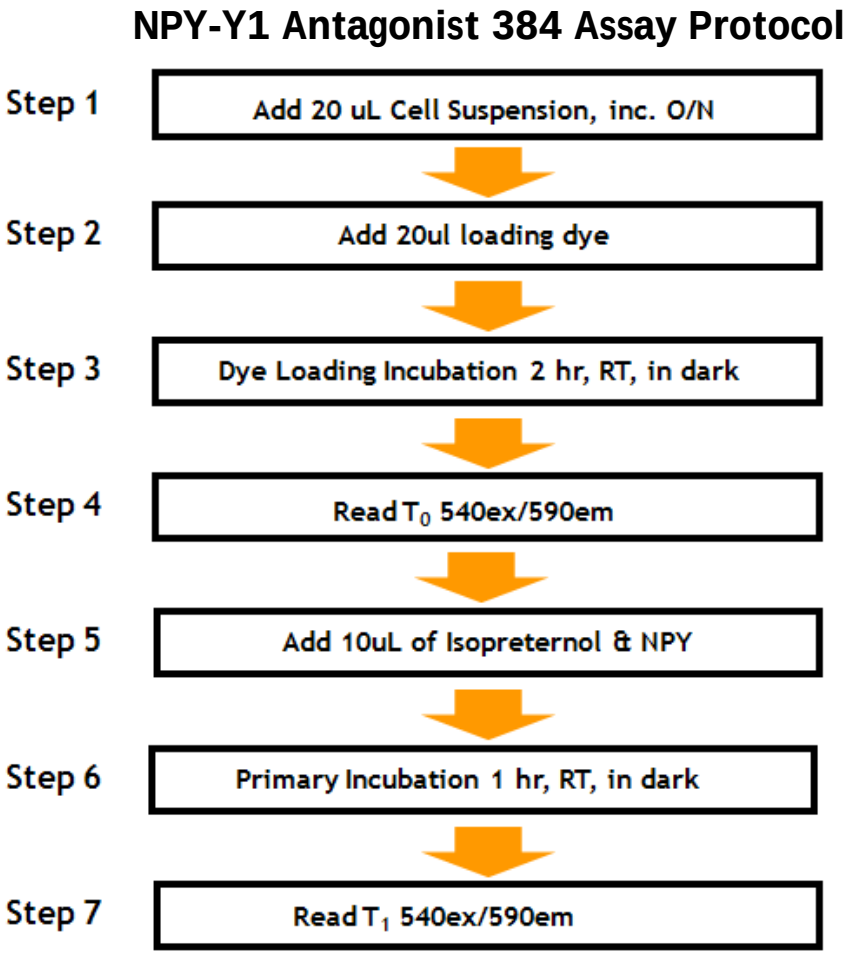
How does the assay look on the FLIPR TETRA?



Miniaturizing the NPY-Y1 Antagonist assay to 1536



384 vs.1536

(1) Total Dye Incubation time (4 hours)

(2) NPY and Isopreternol additions are separated: NPY is preincubated for 1 hour

(3) Reagent dispensing (prevents dislodging of cells)

5.5-fold reagent savings between 384 (50 µ L) & 1536 (9 µL)
Membrane Potential Dye cost : 1 cents/well in 1536

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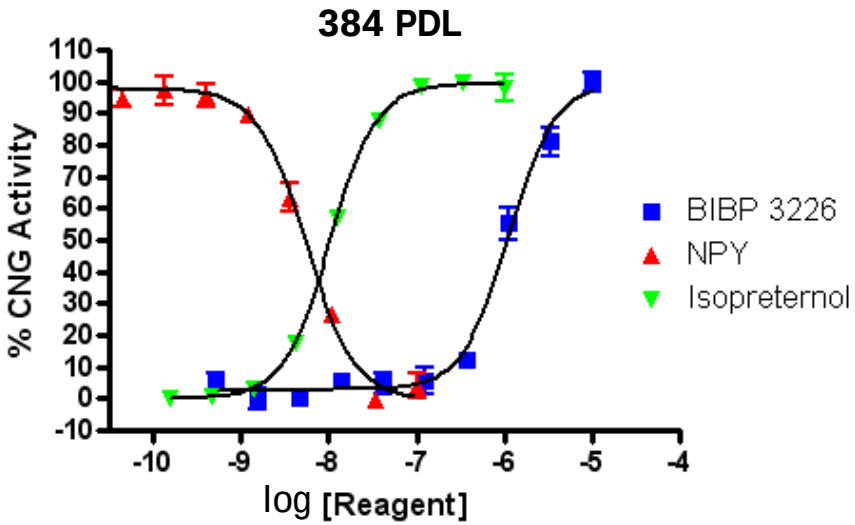
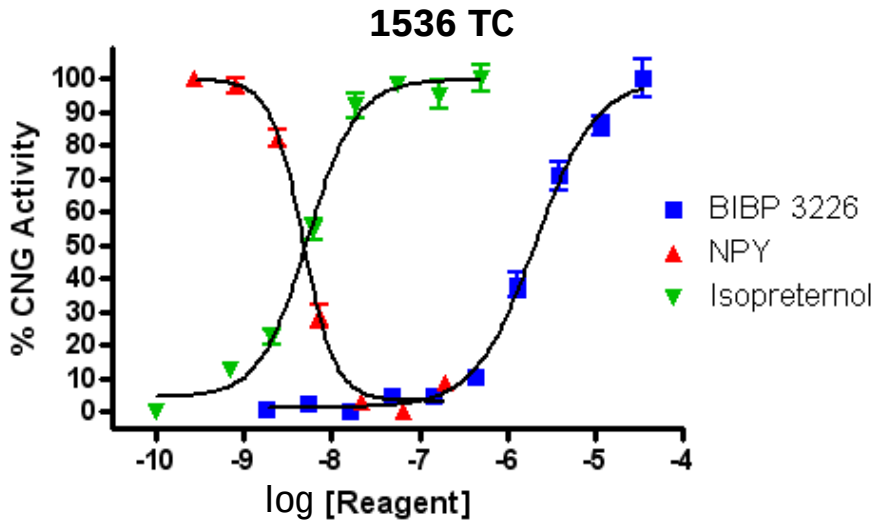
Miniaturizing the NPY-Y1 Antagonist assay to 1536





	BIBP 3226		NPY		Isopreternol	
Plate Format	384	1536	384	1536	384	1536
EC50	1.1 µM	2.1 µM	5.6 nM	4.7 nM	10 nM	5.3 nM
Hill Slope	1.6	1.3	-1.6	-2.4	1.7	1.6

384 PDL-treated vs. 1536 TC-treated plates: NPY-Y1 receptor pharmacology comparable

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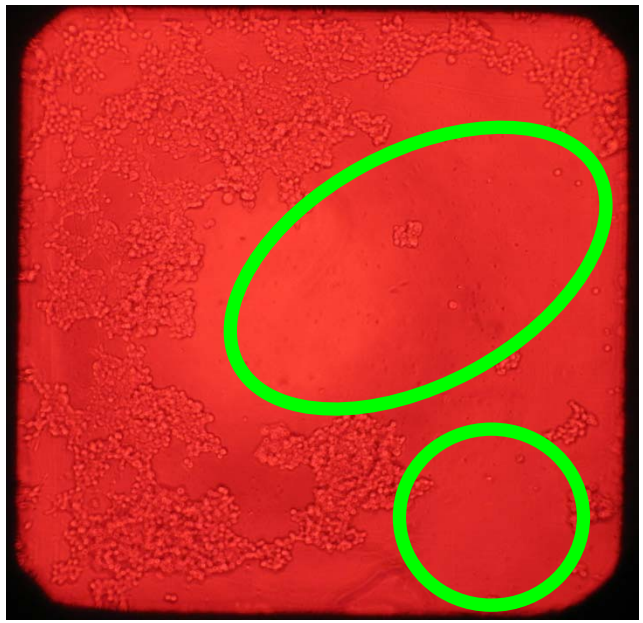
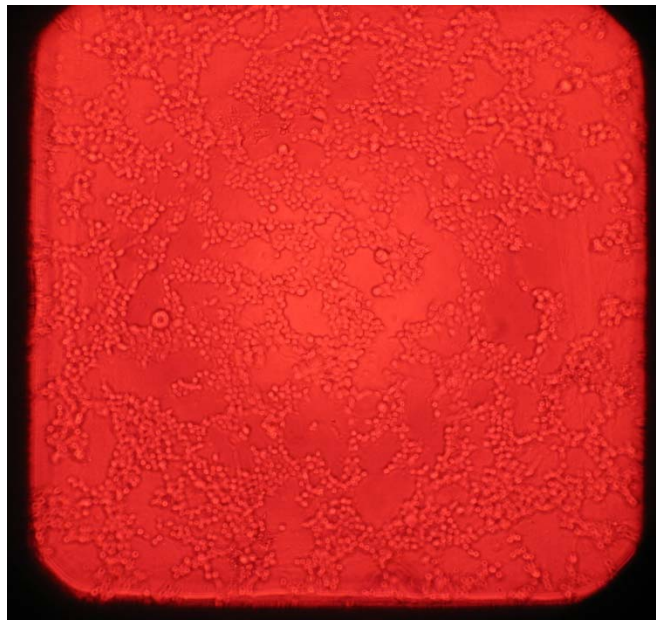
Miniaturizing the NPY-Y1 Antagonist assay to 1536

Standard 1536 Liquid Reagent Dispensing



Optimized 1536 Liquid Reagent Dispensing



Standard reagent dispensing *dislodges* HEK 293 cells, *compromising* quality of FLIPR Tetra data

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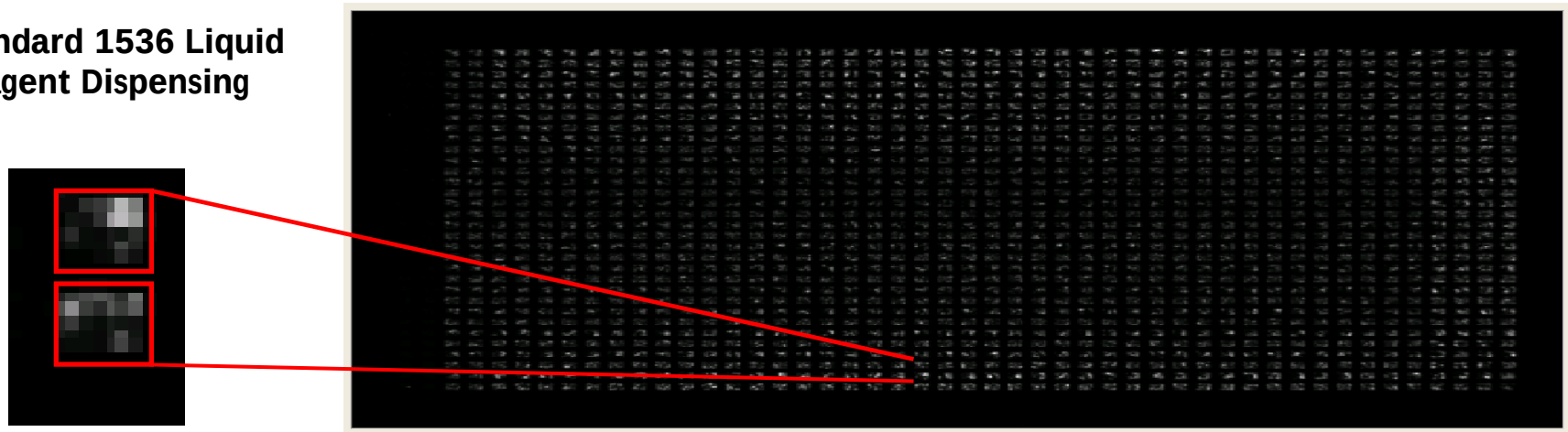
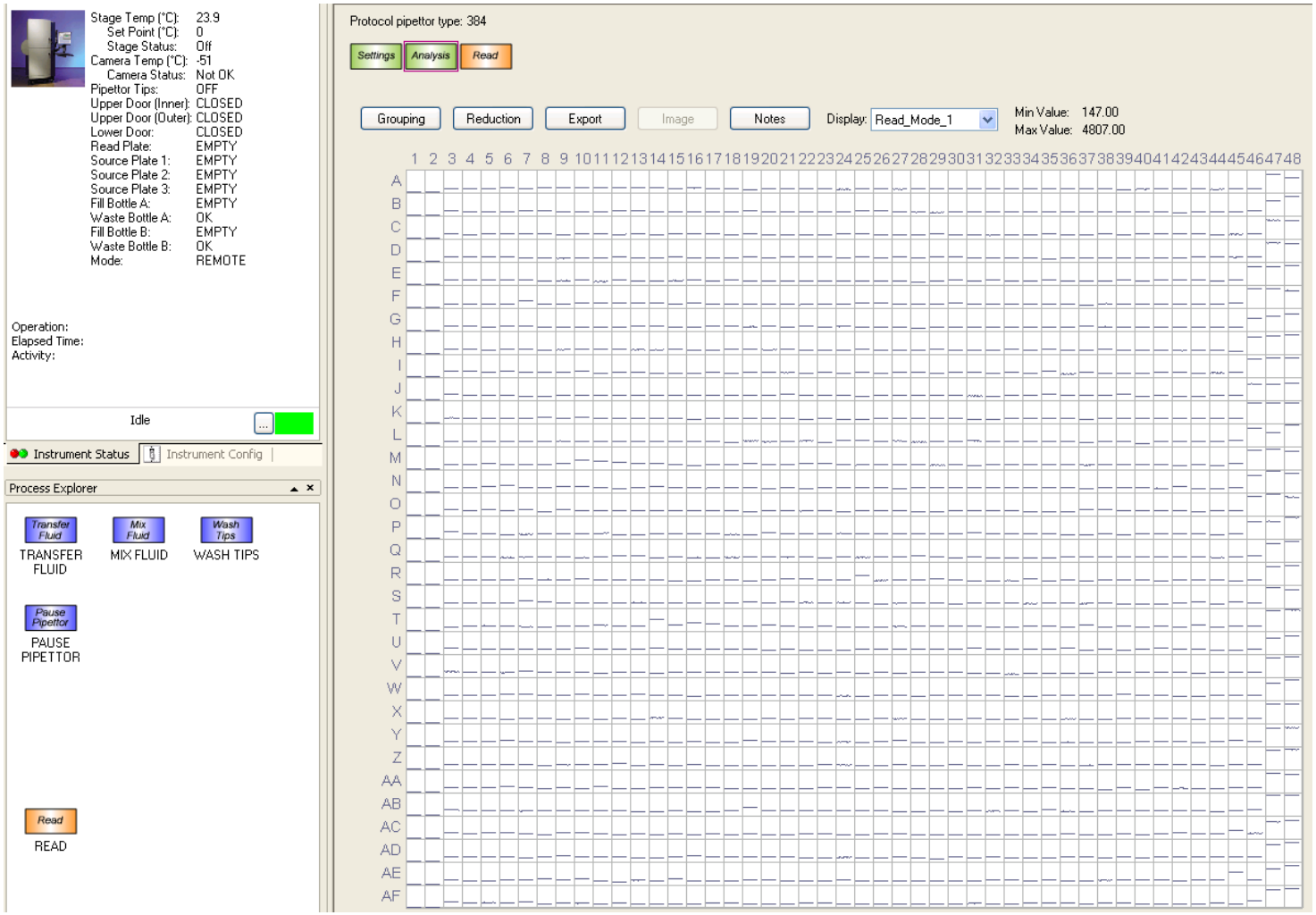
Miniaturizing the NPY-Y1 Antagonist assay to 1536

Standard 1536 Liquid Reagent Dispensing



Optimized 1536 Liquid Reagent Dispensing

Optimized Tetra Assay Parameters



FLIPR Parameters

Exc.	510_545
Em.	565_625
Gain	80
Exp Time	0.2
Exc. Intensity	40
No. Read	20
Read Type	Average

Some other important considerations

(1) Ratio Metric read T0/T45 improves Z' score:

NPY-Y1 Antagonist Assay

Before:

S/B	Z'
3.6	0.41

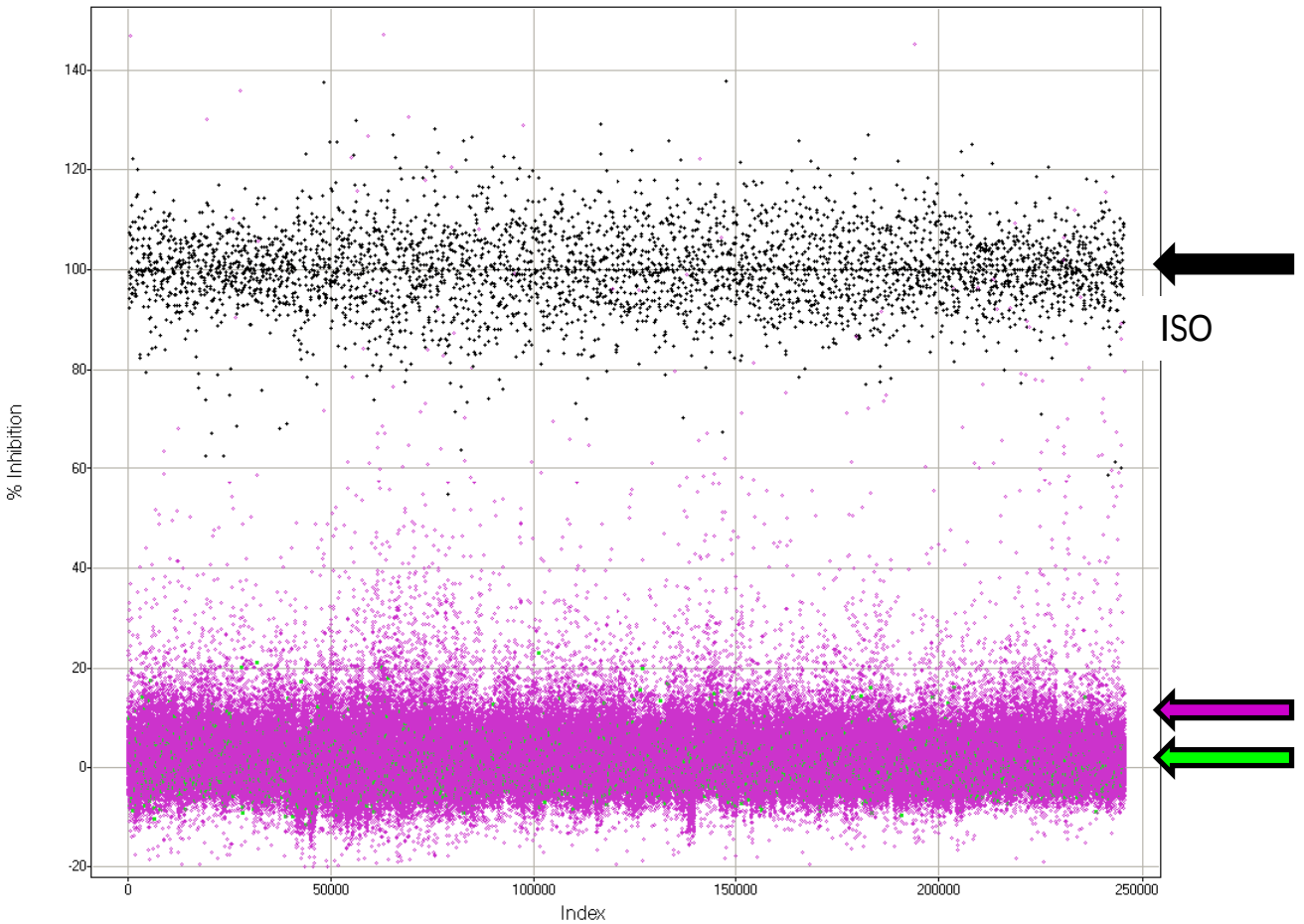
After:

S/B	Z'
3.7	0.62

(2) Tissue culture anecdotes (batch –to-batch reproducibility):
(A) Assay quality sensitive to culture time before assay (ideally 3 days)
(B) Confluency of cells before assay (less than 80%)
(C) Growth time of cells in 1536 plates before assay (ideally 24 hours)

All 3 factors probably promote NPY receptor number and improve/stabilize assay window (i.e. S/B)

NPY-Y1 Antagonist uHTS



Run statistics:

- **Ave Z = 0.58 ± 0.07**
- **Ave Z' = 0.65 ± 0.06**
- **Ave S/B = 3.35 ± 0.28**

- **196,318 compounds**
- **Hit-cutoff: 20.11%**
- **1989 hits**
- **Hit rate: 1.01%**

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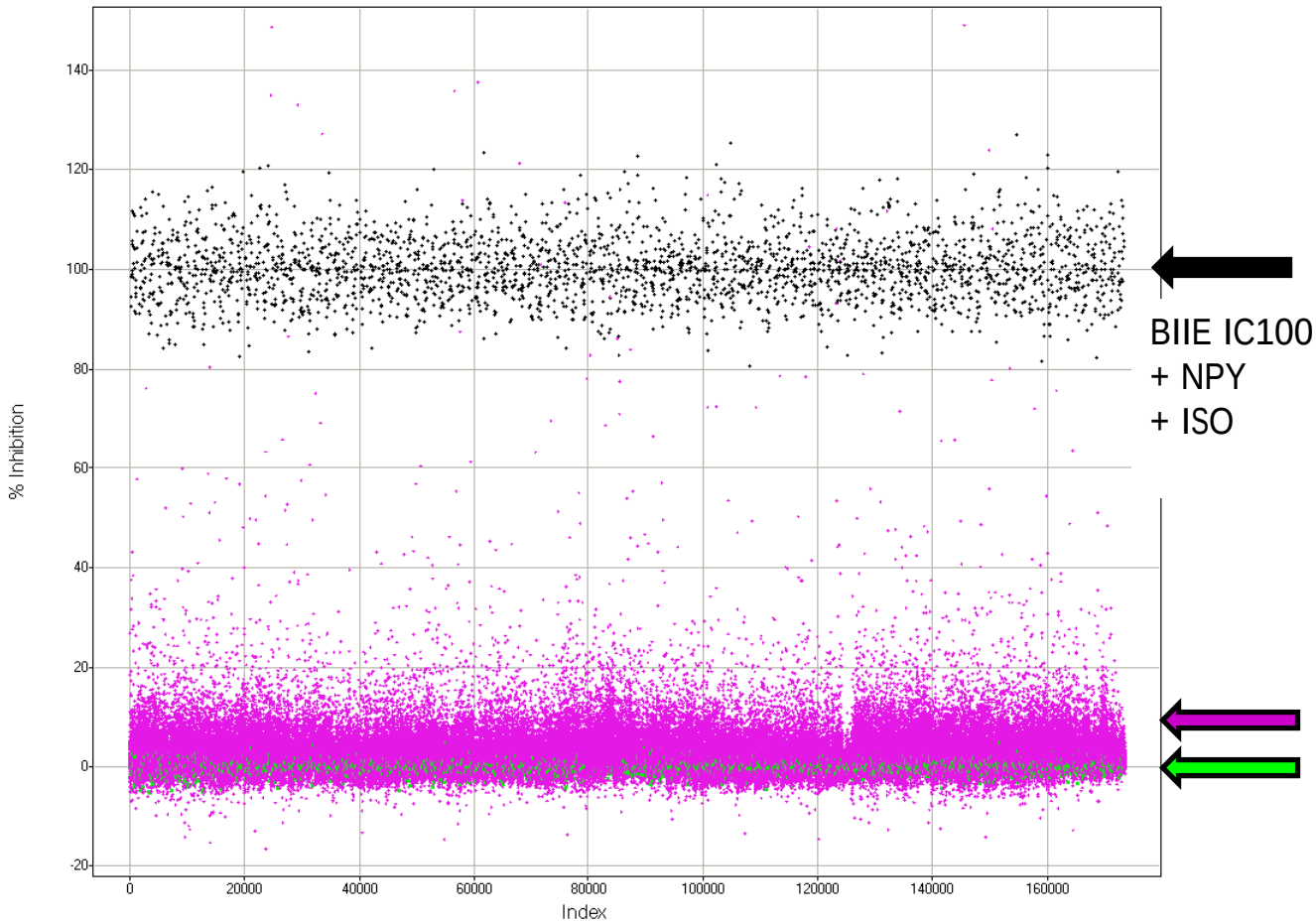
NPY-Y2 Antagonist uHTS



BIIE IC100
+ NPY
+ ISO

Compounds

NPY + ISO

Run statistics:

- **Ave Z = 0.67 ± 0.07**
- **Ave Z' = 0.78 ± 0.04**
- **Ave S/B = 5.09 ± 0.33**

- **140,111 compounds**
- **Hit-cutoff: 17.08%**
- **1384 hits**
- **Hit rate: 0.98%**

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(1) Data for NPY-Y1 & NPY-Y2 campaigns are viewable in PubChem

(2) Titration Assays in Progress: 125 compounds for NPY-Y2
63 compounds for NPY-Y1

Conclusions

(1) The Act*ONE* CNG assay in 1536-format is compatible with the FLIPR Tetra

(2) NPY receptor biology is unaffected by miniaturizing to 1536

(3) Assay quality in 1536 is dependent on (1) Cell culture (2) Reagent dispensing & (3) Ratio metric reading

(4) Consequently 1536 NPY / Act*ONE* uHTS assays on the FLIPR Tetra are cost-effective, robust and consequently feasible!

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