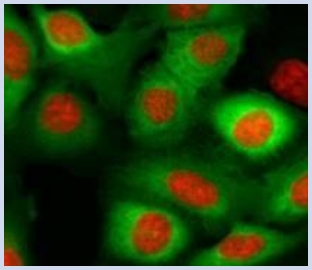
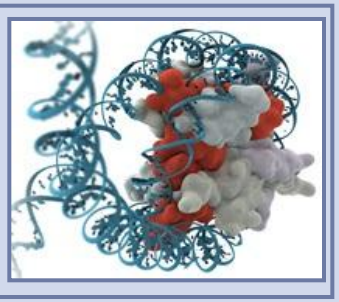




Science For A Better Life

Ultra HTS @ Bayer:

Use of IP-One and Tag-Lite assays in GPCR drug discovery

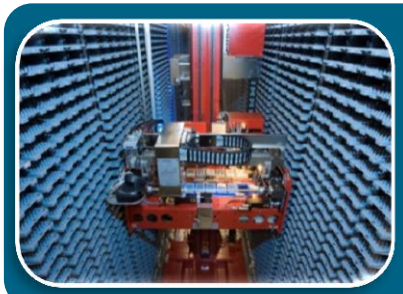
25th of April 2013

Katrin Nowak-Reppel, PhD, Lab Head

Agenda

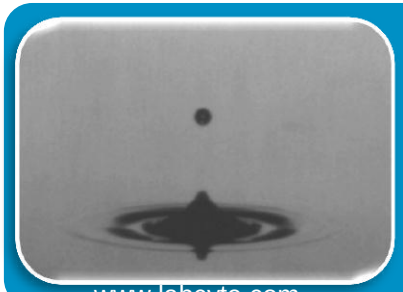
- Overview uHTS @ Bayer/Lead Discovery Berlin
- Highlights of cell-based screening
- 1536MTP format IP-One assay: development and screening
- Development of Tag-Lite binding assay in 1536MTP format
- Summary

Ultra HTS @ Bayer/Lead Discovery Berlin



Whole library screening

- ~3 million compounds for maximum of diversity
- Standard format is 1536MTP



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„Ready-to-assay“ plates

- Acoustic pre-dispension of compounds in assay plates
- Flexible use for different assay days

Fully automated & Benchtop uHTS

- Addition-only homogenous assays
- Non-homogenous and kinetic assays (e.g. High Content Imaging assays)

Assay technology preferences for uHTS @ Bayer/Lead Discovery Berlin

- Homogenous addition-only assay technologies
- Endpoint assays for higher flexibility in time
- Application for several target classes of different indications
- Miniaturization potential to fit to 1536MTP format and reduce costs
- Potential to use frozen cells and perform assay in suspension

→ HTRF assay is a preferred assay technology

Cell-based uHTS @Bayer/LD Berlin

Use of frozen cells

- Reduce inter assay variance → Quality
- Thaw only these cells required for daily screening run → Flexibility
- Scale experiment corresponding to requirements → Efficiency

Evaluation of cell lines

- Physiological relevant cell lines (e.g. oncology – tumor type addressed)
- Endogenous expression of targets
- Avoid artificial expression of target

Phenotypical screening – High content analysis screening

- Whole pathway can be adressed
- Multiple parameter accessed at once
- High physiological relevance

GPCR screening project background

Background:

- Gq protein coupled receptor
- Member of ß-subgroup of rhodopsin receptor subfamily
- Target in family with 2 relative receptors

Project goal:

Identification of specific antagonists interfering with agonist-induced GPCR activation

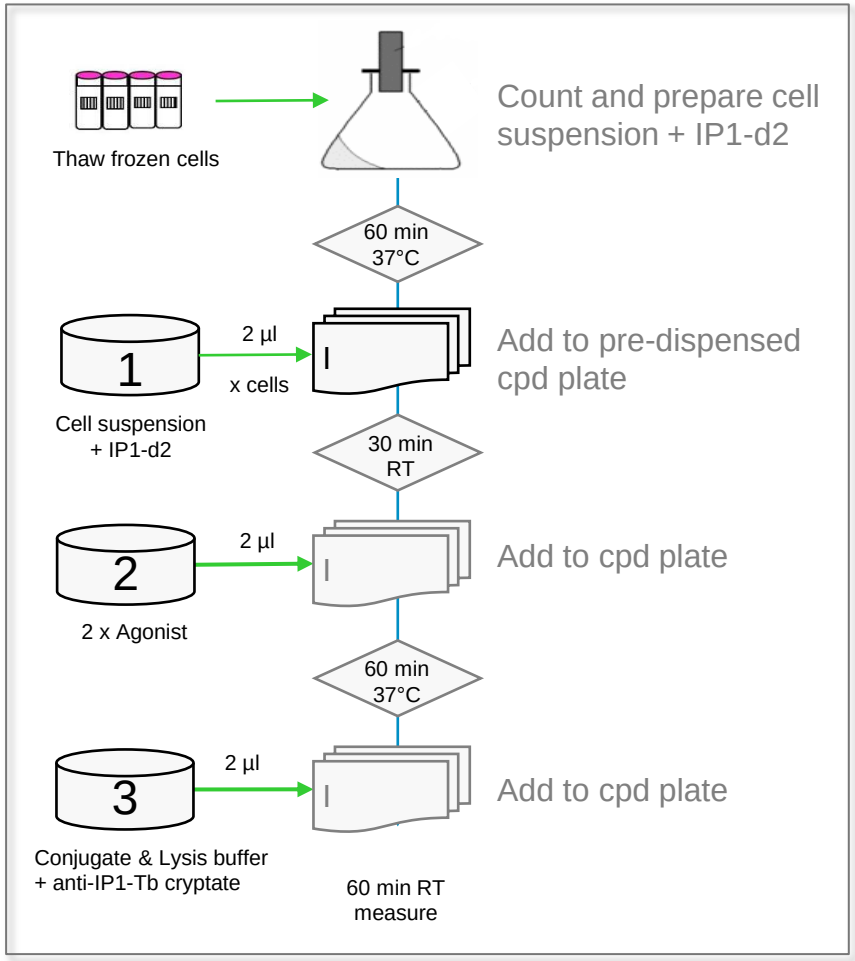
Development of an uHTS compatible IP-One Assay

Start of assay development with standard IP-One HTRF Assay protocol.

Parameters tested during AD:

- Different cell lines and ligands
- Cell number/well
- Incubation time cells with compounds before ligand addition
- Incubation time and temperature of cells, compounds and agonist
- Volumina of addition steps
- Plate colour
- Dilution of IP1-d2 and anti-IP1-Tb cryptate
- …

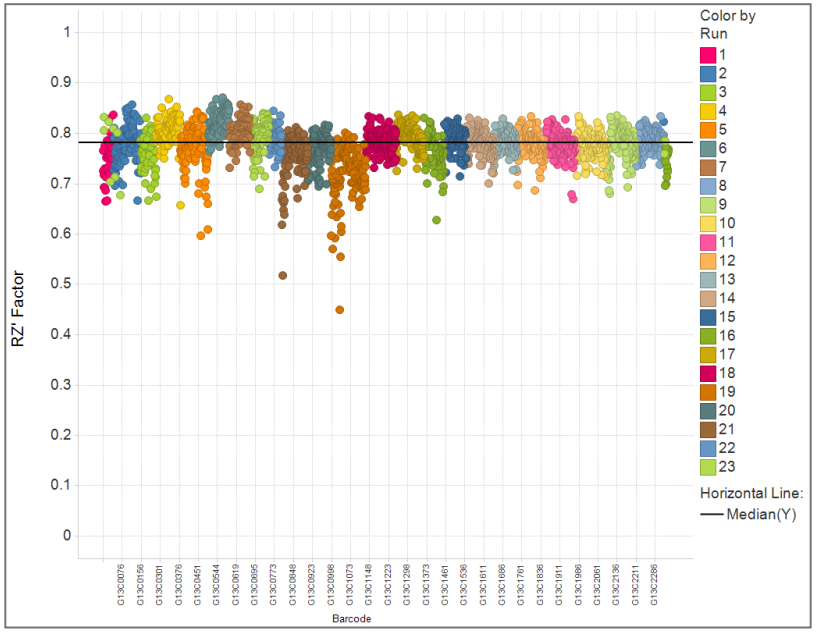
Setup of uHTS compatible IP-One assay

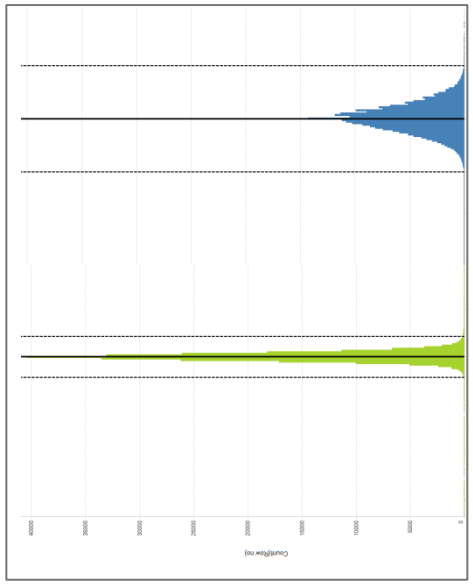


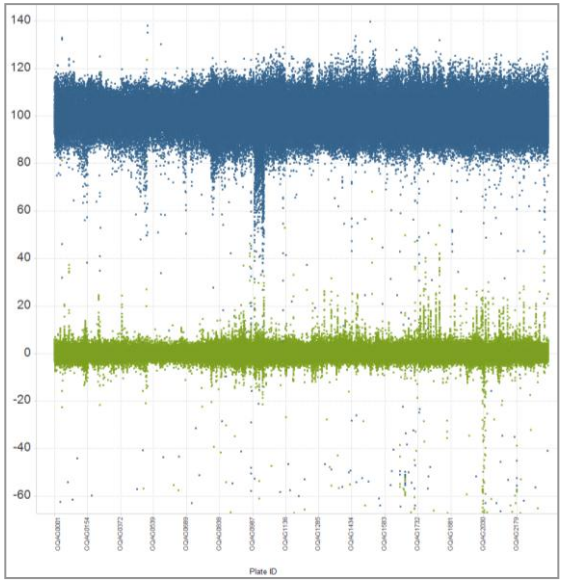
- Assay volume: 4µl
- Detection volume: 6µl
- Final compound concentration: 10µM

- Flexibility by use of frozen cells
- Accuracy by use of ready-to-assay plates
- Efficiency by reduction to 4µl volume and only three addition steps

IP-One screen proves excellent assay quality



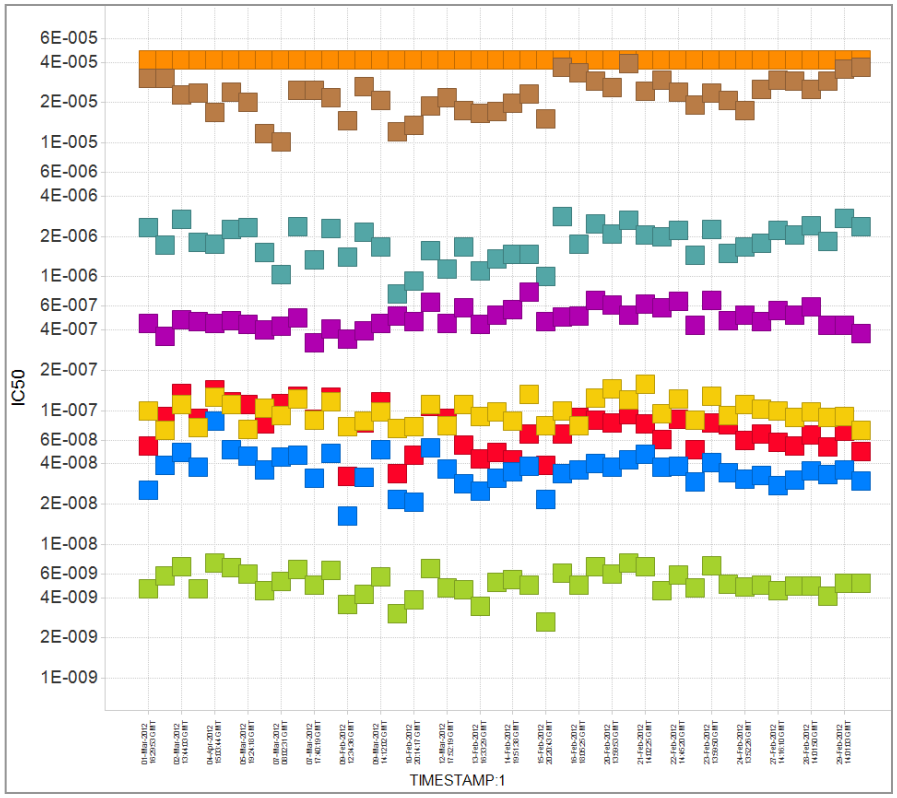


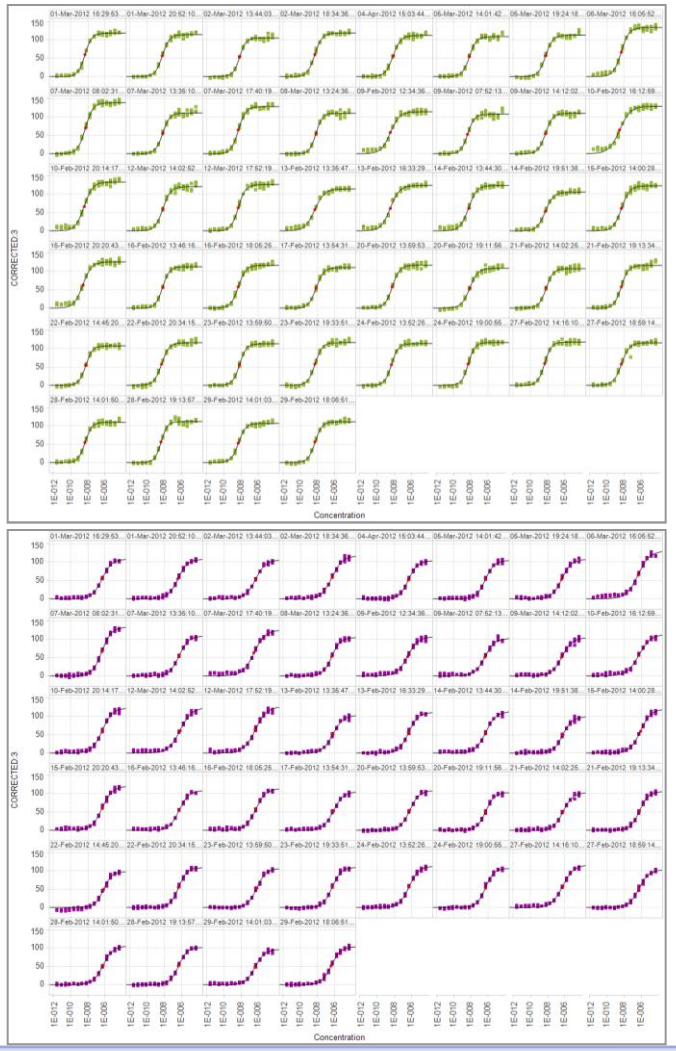


High robust Z‘ factors in every run

Broad separation of controls

IP-One screen proves excellent assay quality





Constant IC50 values of reference compounds over whole screening

Screening cascade

3 000 000	Compounds screened at 10µM at target with IP-One HTRF assay
13 000	Selection of primary hits with sufficient activity Deselection of ugly compounds
11 600	Primary hits retested at target
9 200	Confirmed hits – confirmation rate of about 80% Deselection of ugly compounds Filtering of confirmed hits (cluster representatives)
3 000	Confirmed hits for dose response testing

Requested assay options:

Determine IC50 values of confirmed hits → Potency

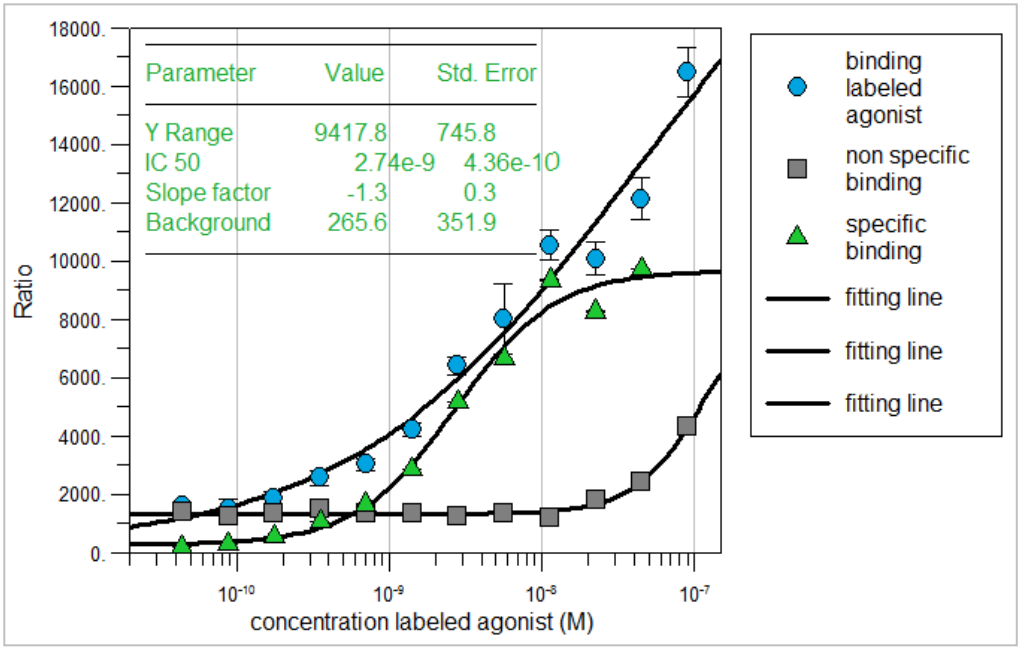
Analyse binding of confirmed hits → Mode-of-Action

Transfer and establishment of available Tag-Lite binding assay

Starting point: Tag-Lite assay for target available as kit

(384MTP format, 20µl volume, scheduled for medium throughput)

First inhouse experiment:

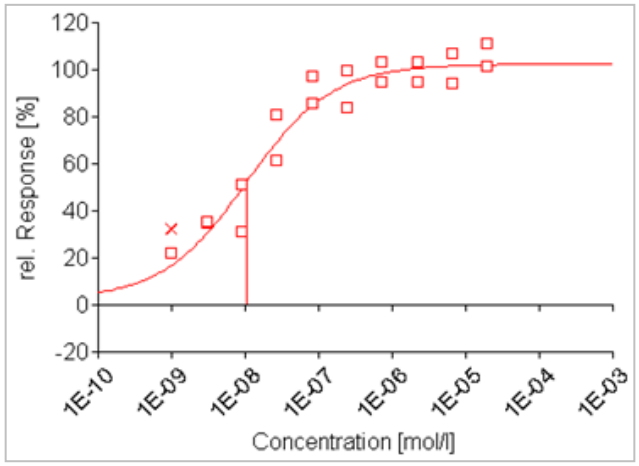


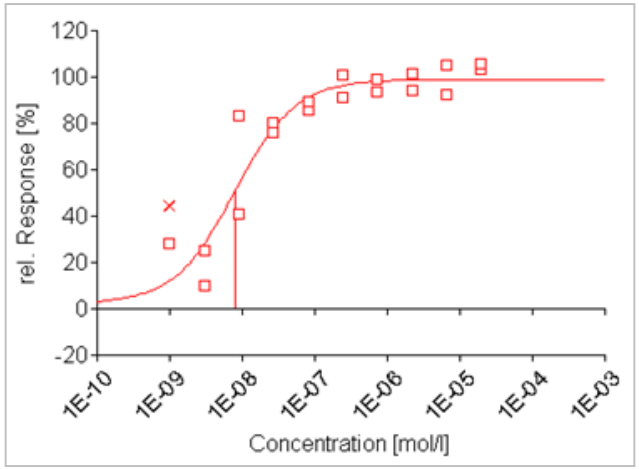
- 384MTP format
- Assay volume: 20µl
- Frozen cells
- Compound transfer

➢ Assay transfered successfully

➢ IC50 of labeled agonist confirmed

Tag-Lite competition experiment with pre-dispensed compounds

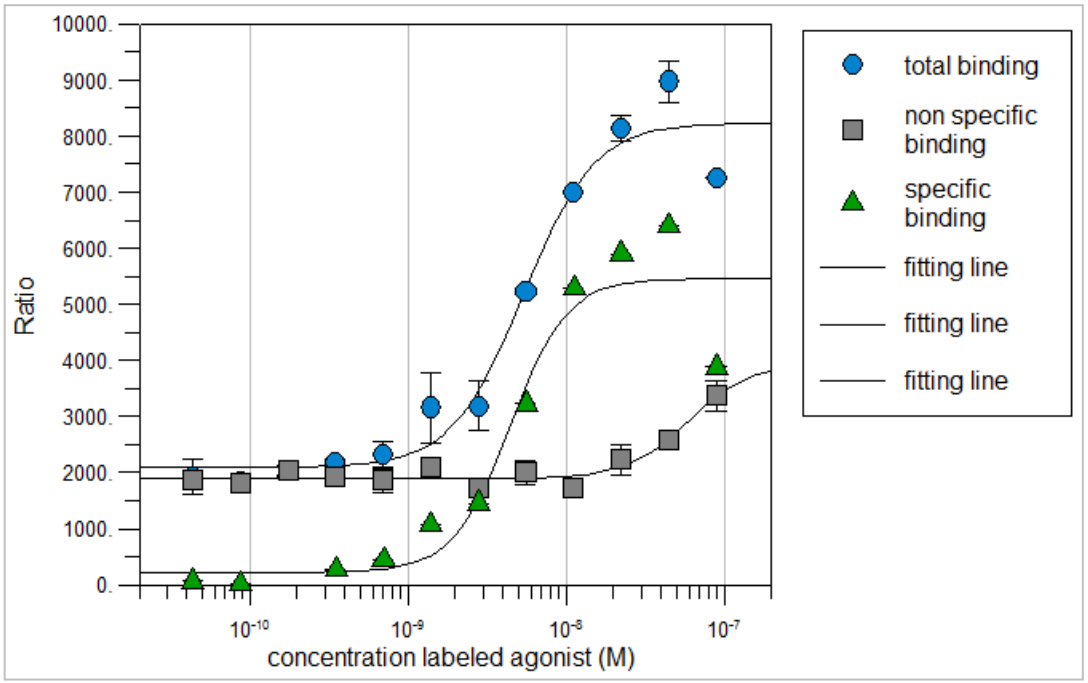




- 384MTP format
- Assay volume: 20µl
- Frozen cells
- 100nl compounds pre-dispensed
- Transfer of labeled agonist

- ➢ Excellent quality of competition experiment
- ➢ Use of pre-dispensed plates successful
- ➢ Data of orthosteric antagonists known from literature confirmed

Volume reduction of Tag-Lite assay



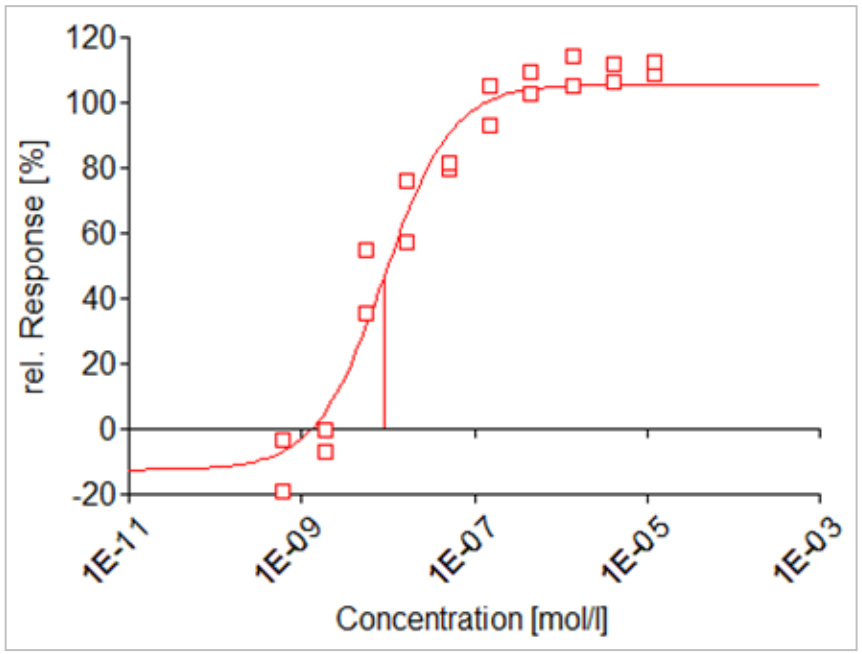
- 384MTP format
- Assay volume: 8µl
- Frozen cells

- Binding curve of labeled agonist reproduced in lower volume
- Reduction of assay volume successfully
- Assay performance in 1536MTP should be possible

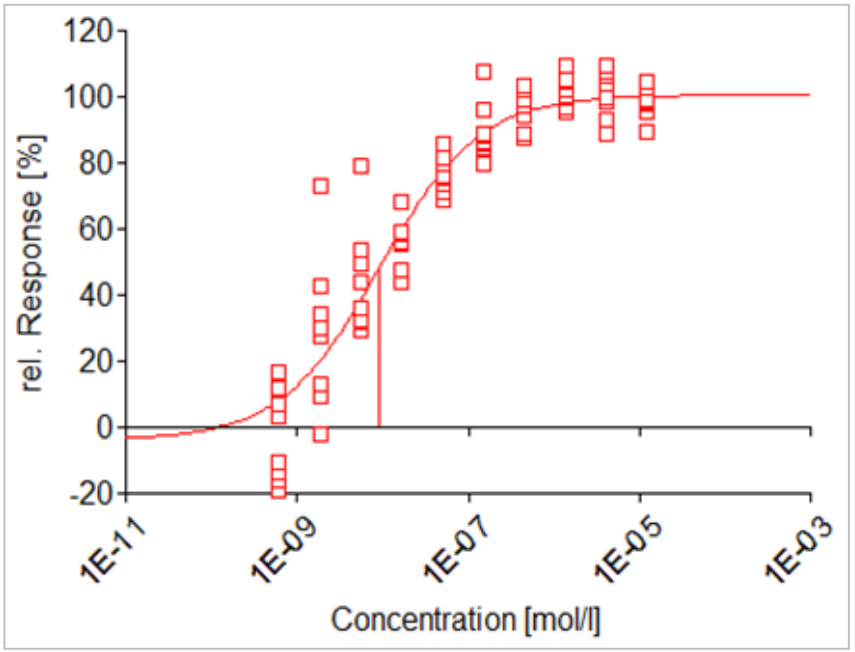
Miniaturization of Tag-Lite assay

Competition dose response curvey of known antagonist in

384MTP



1536MTP



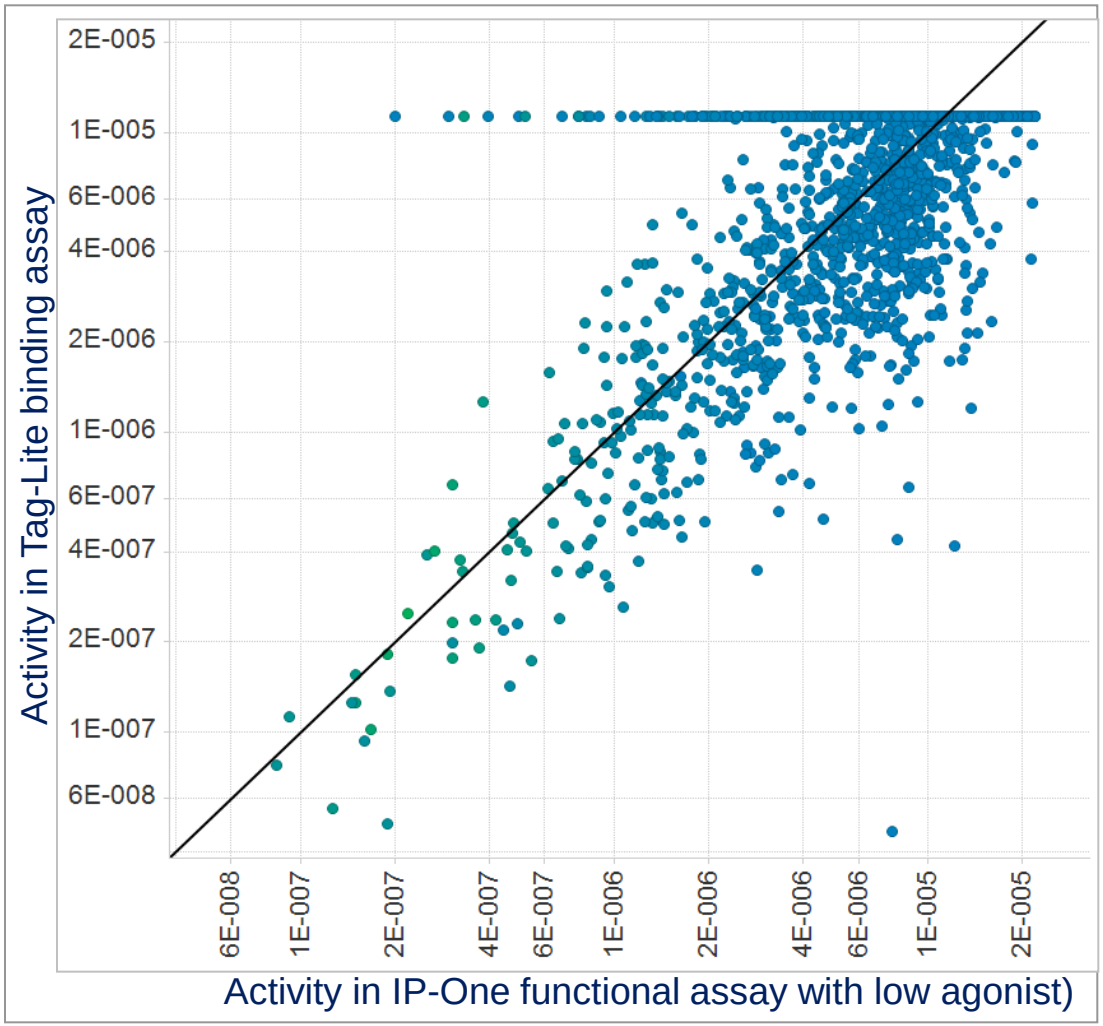
➢ Transfer of miniaturized Tag-Lite assay to 1536MTP format successful

Automatization of miniaturized 1536MTP format Tag-Lite assay

Additional parameters tested and optimized:

- Automatized cell dispension on compounds
- Dispension of solution with labeled agonist
- Incubation before measurement
- Signal stability over time
- Screen batches of cells and labeled agonist

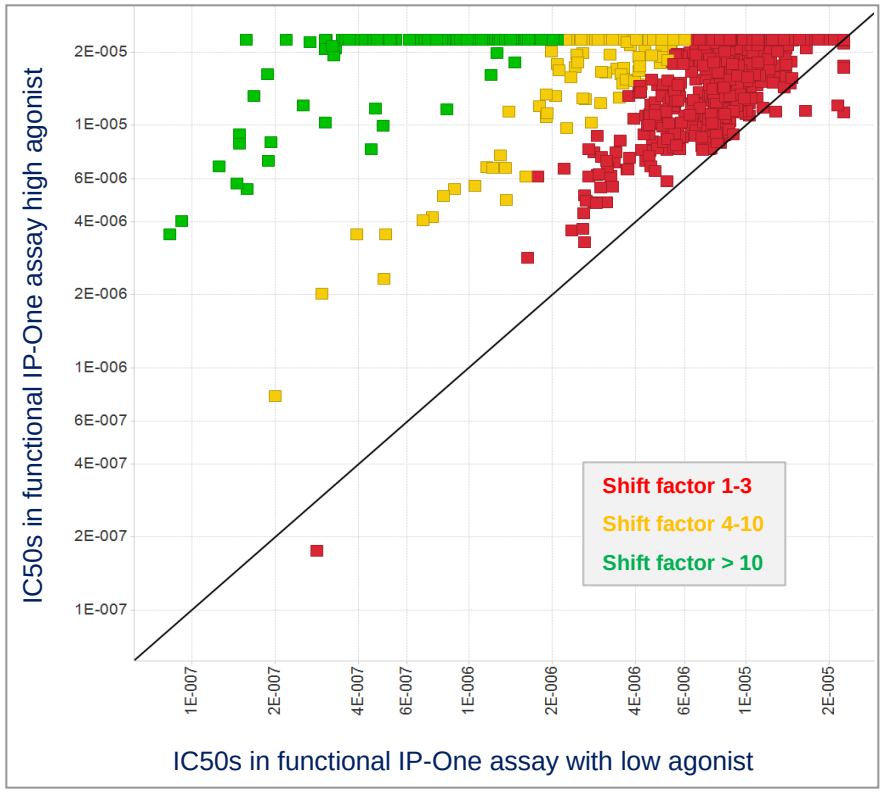
Correlation of data in binding and functional cell-based HTRF assays

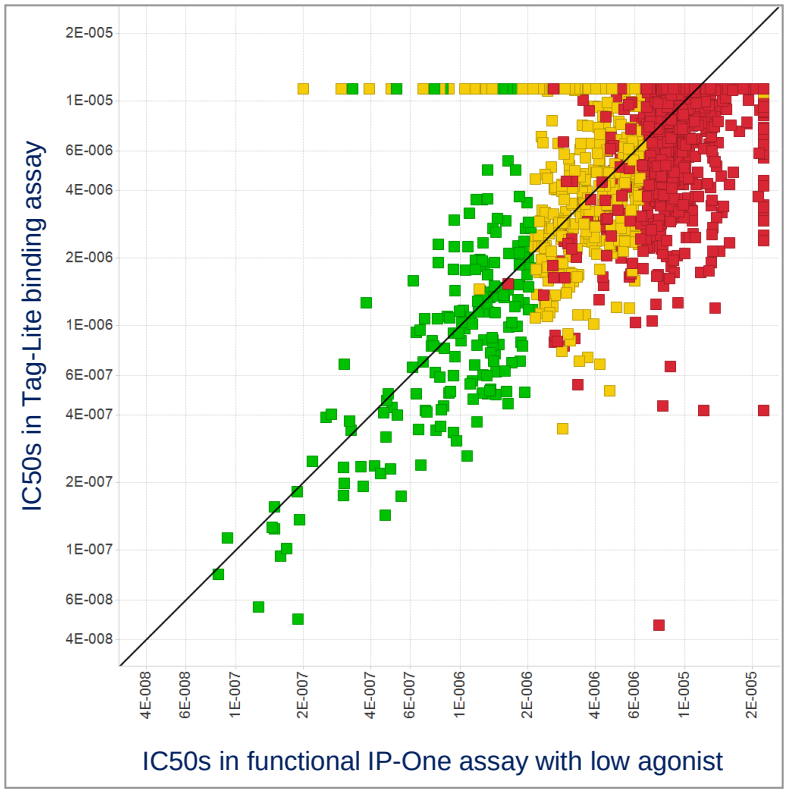


- Dose response curves of ~3000 compounds
- IP-One functional assay
- Tag-Lite binding assay
- 1536MTP format
- Frozen cells

➢ Good correlation of functional and binding data

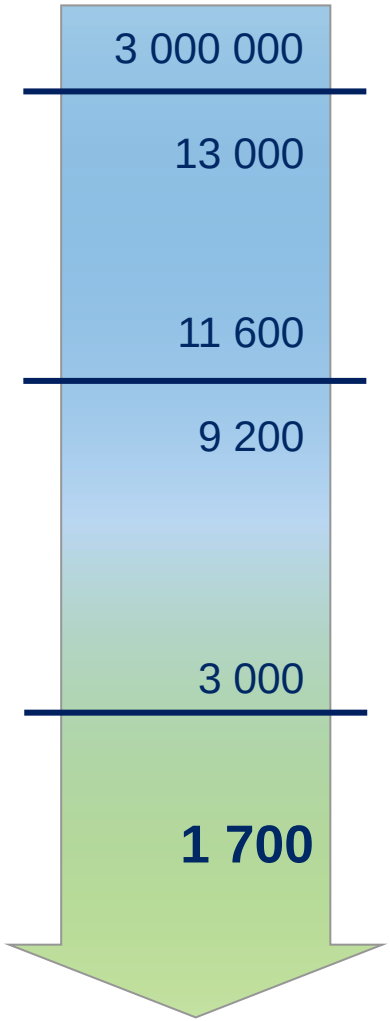
Comparison of results with different Mode-of-Action assay settings





- Compounds which could be shifted with increase of agonist display potent binding activity in Tag-Lite assay

Screening cascade



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3 000	Confirmed hits for dose response testing
1 700	Confirmed, specific hits with functional activity and orthosterical mode of binding

→ Hit to lead process

Summary

An IP-One HTRF assay in 1536MTP format and by using frozen cells was developed.

The uHTS campaign with this IP-One assay yielded in excellent quality and promising hits.

To address binding an available Tag-Lite assay was further optimized and miniaturized for performance in 1536MTP format.

Dose response curves of ~3000 compounds were performed in this Tag-Lite assay and displayed good correlation to functional data.

IP-One and Tag-Lite HTRF assays are suitable technologies for high-throughput cell-based assays and amenable for further automatization.

Acknowledgements

Jessica Schulze

Sabrina Reimann

Benjamin Bader

Karsten Parczyk

Marielle Mazille, Cisbio Bioassays

Reiner Hein, Cisbio Bioassays

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Thank you