Ultra HTS @ Bayer: Use of IP-One and Tag-Lite assays in GPCR drug discovery
25th of April 2013
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Whole library screening
- ~3 million compounds for maximum of diversity
- Standard format is 1536MTP

„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)

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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
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 addressed
- Multiple parameter accessed at once
- High physiological relevance
Background:

- Gq protein coupled receptor
- Member of β-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

1. **Thaw frozen cells**
   - Count and prepare cell suspension + IP1-d2
   - 60 min at 37°C

2. **Cell suspension + IP1-d2**
   - 2 µl x cells
   - Add to pre-dispensed cpd plate
   - 30 min at RT

3. **2 x Agonist**
   - 2 µl
   - Add to cpd plate
   - 60 min at 37°C

4. **Conjugate & Lysis buffer + anti-IP1-Tb cryptate**
   - 2 µl
   - Add to cpd plate
   - 60 min at RT
   - Measure

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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

<table>
<thead>
<tr>
<th>Compounds screened at 10µM at target with IP-One HTRF assay</th>
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<tr>
<td>Selection of primary hits with sufficient activity</td>
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<tr>
<td>Deselection of ugly compounds</td>
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<tr>
<td>Primary hits retested at target</td>
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<tr>
<td>Confirmed hits – confirmation rate of about 80%</td>
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<td>Filtering of confirmed hits (cluster representatives)</td>
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<td>Confirmed hits for dose response testing</td>
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<td>Requested assay options:</td>
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<tr>
<td>Determine IC50 values of confirmed hits → Potency</td>
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<tr>
<td>Analyse binding of confirmed hits → Mode-of-Action</td>
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</table>
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 transferred 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 curve 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 dispersion on compounds
- Dispensation 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
Compounds which could be shifted with increase of agonist display potent binding activity in Tag-Lite assay
Screening cascade

- Compounds screened at 10µM at target with IP-One HTRF assay
  - Selection of primary hits with sufficient activity
  - Deselection of ugly compounds
- Primary hits retested at target
- Confirmed hits – confirmation rate of about 80%
  - Deselection of ugly compounds
  - Filtering of confirmed hits (cluster representatives)
- Confirmed hits for dose response testing
- Confirmed, specific hits with functional activity and orthosteric mode of binding
  - Hit to lead process
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.
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