Towards higher content for early drug discovery - target engagement analysis by NanoBret

B. Leuthner & J. Head & D. Zhang
March 7th, 2019
situation: High attrition rate
High Attrition rate due to unclear TE

Two major contributors to attrition

- Lack of efficacy
- Unacceptable safety (toxicity)

Reason: unclear mode of action (MOA)

- poor engagement with the primary target(s)
- undesired binding to off-target(s)
“Corpora non agunt nisi fixata”

„A drug will not work unless it is bound“

Paul Ehrlich

From: M. Faiza (2016). Bioinf review 5 (1)
Target Engagement of a drug is fundamental

TARGET ENGAGEMENT proofs direct binding of a drug molecule to its corresponding target in the physiologically relevant context independent of downstream functional consequences.

Idea: Monitoring TE allows early decision
TE is essential throughout whole drug discovery process

- Early concept/target validation:
  Tool cpd ID & characterisation/target ID

- SAR/Profiling/Cellular selectivity

- New target classes/PPI/MoA/target deconvolution

- Translational TE assays for in vivo/clinics

Enzyme binding studies with recombinant proteins

Cellular TE Assays

PET Imagine in Animal Model

Target Occupancy from Clinical Samples

- Target Concept
- Hit Discovery
- Hit Optimization
- Lead Optimization
- Clinical Studies
Features that are essential for TE assay in early stages

- Robust
- Broad applicable for different targets, different series, different MoA
- At least medium throughput
- Quantitative analysis to allow SAR
- Cellular system – e.g. live cell
- Bridges potency with efficacy
- Hints for selectivity
- Show direct modulation by drug molecules
- Do not involve complicated target biology
- No interference of off targets
Introduction to NanoBRET Technology from Promega

NanoLuc™ (Nluc) is a 19 kDa ATP dependent luciferase

Tracer captures unbound protein
High BRET signal

Tracer and compound competes for binding in cells
Diminished BRET signal

Analysis under dynamic equilibrium
- Unmodified compound affinity
- Real-time binding kinetics
- Independent of target abundance
- Independent of target stability
- Analysis under physiological temperatures
Assay set-up
Assay workflow

Culture Cells

Transfect with NanoLuc fusion target

Plate compounds and tracer
Serially diluted compounds in 100% DMSO, 96-well plate

- Dilute 10X (stamp plate in OPTI-MEM)
- Transfer 10x compound dilution to assay plate
- Add cells and tracer to 96-well plate
- Orbital Shake
- Incubate and Read

Direct compound to assay plating with Echo + media backfill

Add cells and tracer to 384-well plate
APPLICATION-examples
empowering early concepts -

identification & characterization of potential tool compounds

- Identify reference cpds for assay development of cellular functional assays
- Characterize tool cpds from literature & hits from virtual screens
- First hints for MoA
- Characterize cpds for target validation
- Set basics for soft-optimization
SAR application from HTS to HD correlation between NanoBret assay & biochemical assay

Very good correlation for two series - SAR confirmed
3rd series shows big IC50 shift in NanoBret assay & in SPR assay compared to biochemical assay

• NanoBRET was the first cellular assay in place to select cell active HTS hits
• Provided cellular kinase selectivity (unwanted kinase - close neighbor analysis)
• Allowed pre-selection for different MoA
• Supported decision to progress & prioritize chemical series
Adding complexity from biochemical/physical to NanoBret in live cells - characterisation of compounds

- full-length kinase
- **cell permeability**
- Protein complexes/activation status
- Intracellular metabolite conc esp [ATP]

• To investigate cell permeability issues the assay was performed with permeabilized cells,
• permeabilisation was controlled by microscopy with trypan blue

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>permeabilised cells</th>
<th>intact cells</th>
<th>factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSC1</td>
<td>4,1E-06</td>
<td>3,0E-05</td>
<td>0,1</td>
</tr>
<tr>
<td>MSC2</td>
<td>5,4E-06</td>
<td>3,0E-05</td>
<td>0,2</td>
</tr>
<tr>
<td>MSC3</td>
<td>8,4E-07</td>
<td>6,4E-07</td>
<td>1,3</td>
</tr>
<tr>
<td>MSC4</td>
<td>2,1E-06</td>
<td>7,7E-06</td>
<td>0,3</td>
</tr>
<tr>
<td>MSC5</td>
<td>2,5E-06</td>
<td>2,0E-06</td>
<td>1,3</td>
</tr>
<tr>
<td>MSC6</td>
<td>2,4E-07</td>
<td>2,1E-07</td>
<td>1,1</td>
</tr>
<tr>
<td>MSC7</td>
<td>1,8E-06</td>
<td>2,3E-06</td>
<td>0,8</td>
</tr>
<tr>
<td>MSC8</td>
<td>1,6E-06</td>
<td>2,1E-05</td>
<td>0,1</td>
</tr>
<tr>
<td>MSC9</td>
<td>9,3E-06</td>
<td>6,2E-06</td>
<td>1,5</td>
</tr>
<tr>
<td>MSC10</td>
<td>2,3E-07</td>
<td>1,8E-07</td>
<td>1,3</td>
</tr>
</tbody>
</table>

• Even better correlation between SPR and NanoBret perm cell data
• **Potential different MoA of series with different splits** -> Sort by mode of action
Demonstrated first time that in-house compounds bind to kinase in living cells

Provided compound cellular activity while other cellular assays were under development

Potentials to determine selectivity in cells with other kinases
SAR studies – three different kinase projects

HEK293 kinase 1 NanoLuc with Kinase Tracer-05

HEK293 kinase 2 NanoLuc with Kinase Tracer-05

HEK293 kinase 3 NanoLuc with Kinase Tracer-05

Confirmed cellular functional modulation driven by kinase binding

- Reagent available for >250 kinases
Interrogate selectivity by measuring target engagement in a cell context, which could be better predictive than biochemical approaches.

- Permeability
- Full-length kinase in presence of intracellular regulatory elements
- Test binding under physiologically relevant [ATP].

Tested at a clinically relevant dose of 100 nM against kinase diversity set

- Out of >120 kinases tested, a handful show occupancy over 50%
- Strong engagement observed with ABL and SRC family kinases
Dasatinib selectivity
Validating 384W Profiling With Promega’s Published Data

Live cell data (Promega)
Each mark represents a target engaged at or above a minimum 50% occupancy threshold.

Live cell internal data
Each mark represents a target engaged at or above a minimum 50% occupancy threshold.

Elrig symposium B. Leuthner_7th March 2019
Profiling Bosutinib

- Different selectivity profile observed in cell setting.
- Caveat: Reaction Biology panel more expansive than NanoBRET vector library

Reaction Biology at 1 uM

NanoBRET at 1 uM

Bosutinib Fractional Occupancy

% Occupancy
Further opportunities: residence time analysis

- Kinetic reads to examine compound residence time in live cells

- Homogeneous Immunoassays
- Screening of Antibody Biologics
- Intracellular PPI
- Cytokine Binding
- Target Engagement
- Target Identification
1. Target engagement is a fundamental pre-requisite for drug discovery-to analyze TE, several supplementary approaches are necessary

2. Monitoring TE in early drug discovery can accelerate the process, reduce attrition and save resources

3. NanoBret® Technology from Promega allows determination of cellular binding of the compound to its target in early cellular assay in assay cascades

4. The NanoBret kinase panel allows kinome wide profiling of compounds in live cell system as the next step towards physiological conditions including permeability, ATP concentration, protein complexes

5. The technology not only provides insight into target occupancy but also allows for drug/target residence time measures across the human kinome
NANOBRET
bridging the gap
between biochemical and cellular functional assays
Acknowledgment

• Hao Lu
• Mirek Jurzak
• Ingo Kober
• Jan-Carsten Pieck
• Oliver Poeschke
• All colleagues @ Disco Pharm
• All project team members

Collaborators from Promega
Special thanks to Matt Robers for his great continuous support

Dagmar Wolf  Ulrike Bauer  Evelyne Barrey
Ansgar Wegener  Beatrix Blume  Sarah Wallrodt
Frank Fischer
Nina Großmann  David Fischer  Daniel Schwarz