INTRODUCTION

PPARs

- Ligand-activated nuclear receptors\(^1\);
- Three human isoforms are known (PPARα, PPARβ and PPARγ)\(^2\);
- Involved in several metabolic and cellular processes, such as:
  - fatty acids oxidation
  - inflammation
  - lipids storage\(^2\);
- Regulated by a spectrum of endogenous and exogenous compounds;
- Natural ligands include SFA, MUFA and PUFA, and fatty acids' derivatives\(^3\).

RESULTS

Sensor Characterization

- Activation of cells transfected with mpFN26A[PPARγ] individually (A) or mpFN26A[PPARγ] – β and γ simultaneously (B), and mpGL4.35[Nluc], and exposed to the respective agonist of each PPAR (10 µM) or to the solvent control (DMSO). Data are represented as mean ± SEM (n=12).

Table 1: Values of \(K_D\), EC\(_{50}\), LOD, LOQ and LRD of cells transfected with mpFN26A[PPARγ] individually (uniplex mode) or with mpFN26A[PPARα], β and γ simultaneously (triplex mode), and mpGL4.35[Nluc] and exposed to rosiglitazone (n=3).

<table>
<thead>
<tr>
<th>PPAR Ligand</th>
<th>(K_D) (µM)</th>
<th>EC(_{50}) (nM)</th>
<th>LOD (nM)</th>
<th>LOQ (nM)</th>
<th>LRD (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARγ</td>
<td></td>
<td>16.74</td>
<td>210.43</td>
<td>1.27</td>
<td>34.1</td>
</tr>
<tr>
<td>PPARα</td>
<td>34.1</td>
<td>24.83</td>
<td>1.27</td>
<td>34.1</td>
<td>[6.4;100]</td>
</tr>
<tr>
<td>PPARγ + β</td>
<td>10.23</td>
<td>43.38</td>
<td>1.19</td>
<td>144.59</td>
<td>[6;250]</td>
</tr>
<tr>
<td>PPARγ + γ</td>
<td>34.1</td>
<td>144.59</td>
<td>1.19</td>
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</tr>
</tbody>
</table>

Screening of Cyanobacteria fractions

- Illustrative scheme of cell assays
- Optimization of a cell-based sensor for PPAR ligands detection
- Screening of a cyanobacteria extracts library
- The cell-based sensor system consisted of:
  - Two dependent modified reporter vectors
  - Fluc/Nluc\(^\circledast\) luciferases
  - Glow reporter assay system kit (long lasting signal)
  - Homogeneous quantification
  - Uniplex or Triplex mode
  - 96-well plates

FIGURE 3. Transactivation activity of cells transfected with mpFN26A[PPARγ] individually (A) or mpFN26A[PPARγ] – β and γ simultaneously (B), and mpGL4.35[Nluc], and exposed to the respective agonist of each PPAR (10 µM) or to the solvent control (DMSO). Data are represented as mean ± SEM (n=12).

FIGURE 4. Dose-response curve of transactivation activity of cells transfected with mpFN26A[PPARγ] individually (A) or mpFN26A[PPARα] + β + γ simultaneously (B), and mpGL4.35[Nluc] and exposed to rosiglitazone (n=3).

DISCUSSION

- Uniplex mode presented a † sensitivity and affinity to rosiglitazone;
- † in sensitivity is consistent with a † of the amount of mpFN26A[PPARγ] in the triplex mode;
- 3x more information is obtained in triplex mode per assay → less time and lower costs.