A Novel Screening Platform to Monitor GPCR-G Proteins Complex Formation

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Abstract

The generation of stable G-protein coupled receptors (GPCR)-G proteins complex is one of the bottlenecks in cryoEM structure determination to support structure-based drug design.
Here, we present a homogeneous time-resolved fluorescence (HTRF) assay to detect the complex formation between glucagon-like peptide receptor (GLP-1R) and G proteins. We have demonstrated the platform can be applied to determine optimal GPCR-G proteins complex formation such as construct optimization, expression ratio, buffer and detergent screen and purification optimization.

Introduction

- The advancement in cryoEM structure determination provides more opportunities for structure-based drug design with membrane protein targets.
- The GPCR-G protein complex formation is important for identify agonist ligand and several groups have demonstrated strategies to trap the complex in this desired active state[1].
- Here, we have developed a HTRF assay with GLP1R as a model to monitor complex formation with G proteins in the presence of agonist to facilitate reagent generation for cryoEM study.

Schematic of HTRF system design

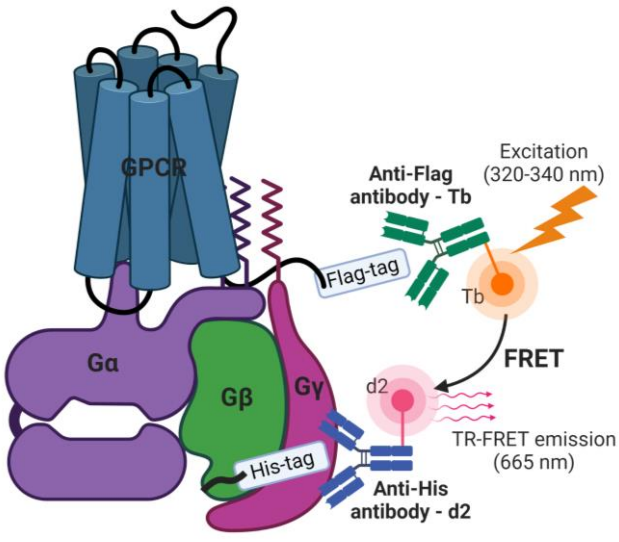

Figure 1. **Schematic of HTRF system design.** The HTRF system is displaying energy transfer between GPCR – G protein complex. Specific antibodies bind to protein tags and allow signal transfer from Tb (donor) fluorophore to d2 (accepter) creating a FRET signal.

Methods

- The GLP1-R and G proteins are engineered to have unique and specific tag (His and Flag tag) to enable binding to the specific antibody.
- The specific antibody are labelled with either d2 or Tb and upon excitation the FRET signal can be transferred and detected.
- With optimization, this platform is suitable to monitor complex formation with purified protein reagent and crude lysate.
- In addition, this HTRF assay is a plate-based format to increase throughput for the need of large screening (Fig. 2).

Plate-based HTRF platform

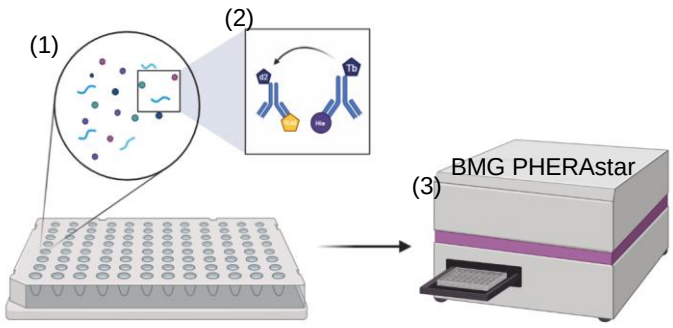

Figure 2. **Plate-based HTRF Platform**. (1) Protein reagent is prepared and incubated with ligand. (2) Both protein reagent mix and specific antibody with fluorophore tag are added to each well for further incubation. (3) The plate is scanned and data recorded via BMG PHERAstar detector.

Results

HTRF platform development

Dangulipron is a known agonist to GLP1R with the potency in nM range[2]. We have utilized this knowledge to optimize our HTRF platform. This includes fluorophore selection, tag position, linker length, and protein reagent quantity to achieve > 3 signal to background (S/B) ration and Z' value of > 0.5.

Dose response of Dangulipron

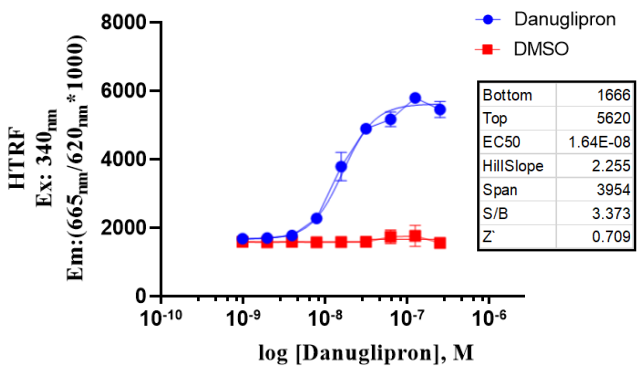

Figure 3. **Dose response curve of Dangulipron**. In our dose response experiment, a 16nM EC50 is determined for Dangulipron in relationship of GLP1R-G protein complex formation.

Screening tool for optimal condition

Buffer optimization such as salt concentration and detergent selection (Fig. 4) have also been investigated to identify optimal condition for complex formation.

Detergent screen

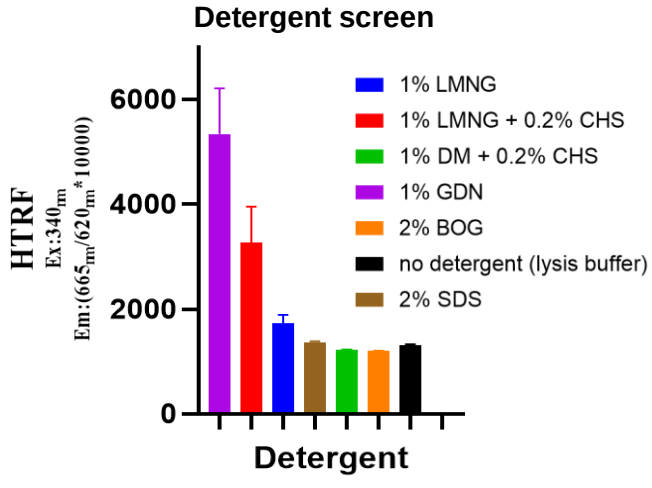

Figure 4. **Detergent screen**. The GLP1R-G protein complex formation is monitored by HTRF assay to investigate the effect of detergent in complex formation.

Optimizing expression ratio to improve yield

In addition to buffer optimization, protein expression of each component (GLP1-R, Gα,Gβ,Gγ) is also important factor to increase the final yield of complex reagent.
We have applied HDE to investigate the co-expression ratio to improve protein complex generation (Fig 5).

HDE design to optimize co-expression ratio

(1)

Sample Nr.	Receptor	G alpha	G beta/gamma
1	5.5	10	10
2	1	1	5.5
3	1	10	5.5
4	10	5.5	1
5	5.5	5.5	5.5
6	10	1	5.5
7	5.5	1	10
8	10	10	5.5
9	5.5	10	1
10	5.5	5.5	5.5
11	1	5.5	10
12	5.5	1	1
13	5.5	5.5	5.5
14	10	5.5	10
15	1	5.5	1

(2)

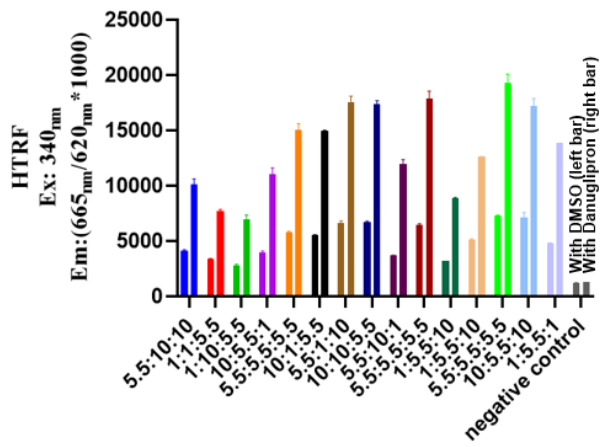

Figure 5. **HDE design to optimize co-expression ratio**. (1) The range of GLP1-R, Gα, Gβ, and Gγ is inputted to JMP to design the minimum required co-expression combination to perform. (2) Co-expressed cultures with different ratio are prepared for HTRF assay with and without the presence of Dangulipron. The level of complex formation is then feedback to JMP to calculate the optimal co-expression ratio.

Conclusions

- With GLP1-R as a model, we have successfully developed a plate-based HTRF platform to monitor the complex formation of GLP1-R with G proteins.
- Comparable EC50 measurement to known agonist compound, Dangulipron, indicating potential application for hit validation as well as compound screening.
- Optimization of buffer condition and expression system can be achieved with our HTRF platform.

References
1. Liang *et al.*, Nature 555 121-125 (2018)
2. Griffith *et al.*, J. Med. Chem. 65. 8208-8826 (2022)

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