Synthelis

GPCR

Protein Catalogue

GLP1R- **Glucagon-like-peptide 1 receptor**

Product specification

Acronym: GLP1R
Class: GPCR Class B
Origin: Human
Molecular weight: 53 kDa
Application: Screening & display technologies, Structural biology, Antibody development

Purity: >50%
Activity: Proven by SPRi
Length: Full Length
TMD: 7
Biological function: Glucose-dependent insulin secretion

Product description

The glucagon-like peptide-1 receptor (GLP-1R) is a class B GPCR that is a major therapeutic target for the treatment of type 2 diabetes. The receptor is activated by the incretin peptide GLP-1 (Glucagon Like Peptide) promoting a broad range of physiological effects including glucose-dependent insulin secretion and biosynthesis, improved insulin sensitivity of peripheral tissues, preservation of β-cell mass and weight loss, all of which are beneficial in the treatment of type 2 diabetes.

Protein Source: hGLP1R wild type protein (Human hGLP1R).

Fig.1: AA sequence of GLP1R protein

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        10         20         30         40         50
MAGAPGPLRL ALLLLGMVGR AGPRPQGATV SLWETVQKWR EYRRQCQRSL
        60         70         80         90        100
TEDPPPATDL FCNRTFDEYA CWPDGEPGSF VNVSCPWYLP WASSVPQGHV
       110        120        130        140        150
YRFCTAEGLW LQKDNSSLPW RDLSECEESK RGERSSPEEQ LLFLYIIYTV
       160        170        180        190        200
GYALSFSALV IASAILLGFR HLHCTRNYIH LNLFASFILR ALSVFIKDAA
       210        220        230        240        250
LKWMYSTAAQ QHQWDGLLSY QDSLSCRLVF LLMQYCVAAN YYWLLVEGVY
       260        270        280        290        300
LYTLLAFSVL SEQWIFRLYV SIGWGVPLLF VVPWGIVKYL YEDEGCWTRN
       310        320        330        340        350
SNMNYWLIIR LPILFAIGVN FLIFVRVICI VVSKLKANLM CKTDIKCRLA
       360        370        380        390        400
KSTLTLIPLL GTHEVIFAFV MDEHARGTLR FIKLFTELSF TSFQGLMVAI
       410        420        430        440        450
LYCFVNNEVQ LEFRKSWERW RLEHLHIQRD SSMKPLKCPT SSLSSGATAG
       460
SSMYTATCQA SCS
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Affinity Tag: Histidine tag fused to the N-terminal end of the protein.

Production conditions: hGLP1R is expressed in a cell-free expression system in the presence of lipid vesicles. 100 µg can be produced and qualified in about 1 week.

Quality analysis

Purity: Typically > 50% as determined by SDS-Page and Coomassie Blue staining.

Purification procedure: As standard, hGLP1R proteoliposomes are purified on a sucrose gradient. Further purification steps can be added if required.

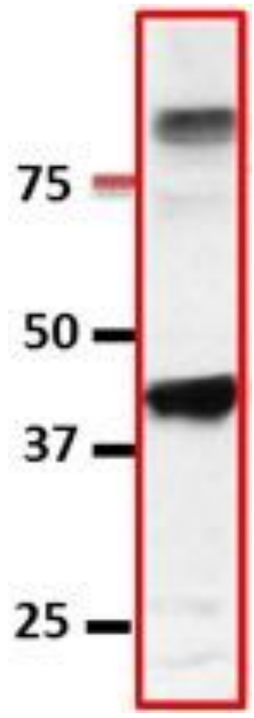


Fig.2: Proteoliposome hGLP1R after purification (Western blot identification).

Western blot using anti-His-tag monoclonal antibody proved that the GLP1 receptor was expressed. As shown in Figure 2, a band about 40 kD was visualized. The molecular weight of GLP1 receptor protein seems to be lower than the calculated one (53 kDa) but a survey of membrane proteins applied to SDS–PAGE showed that observed MW may diverge by as much as approximately ±50% from formula values .
Migration on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) that does not correlate with formula molecular weights, termed “gel shifting,” appears to be common for membrane proteins. The protein adopts equivalent shapes after SDS treatment, unfolding conformation. The presence of hairpins (helix-loop-helix) could be an explanation of this gel shift. The literature concerning membrane proteins mentions a differential solvation by SDS (replacing protein-detergent contacts with protein-protein contacts, implying that detergent binding and folding are intimately linked). The apparent MWs among this group deviate widely from formula MW with gel shifts (migration on PAGE that does not correspond to formula MW) ranging from -46% to + 48%.

References:

– Proc. Natl. Acad. Sci., February 10, 2009, vol. 106
Detergent binding explains anomalous SDS-PAGE migration of membrane proteins.
– Proc. Natl. Acad. Sci. September 24, 2013, vol. 39
Acrylamide concentration determines the direction and magnitude of helical membrane protein gel shifts
– Analytical Biochemistry 434 (2013) 67–72
Correction factors for membrane protein molecular weight readouts on sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

Assessment of functionality

Introduction

The aim of this study was to optimize the cell-free expression of GLP1 receptor proteoliposomes by adding, for example, the specific ligand directly in the reaction mix. We have constructed a new plasmid containing Strep-tag at the C-terminus of the receptor which allows validation of full length of GLP1 receptor. After that, we have developed a binding assay for GLP1 ligands using SPRi. A specific signal was detected between GLP1 ligand and GLP1 receptor. This signal is depending on the expression condition.

Materials

GLP1 ligand
Expression of GLP1 receptor by cell-free and activity binding tests were performed using the specifc ligand (Glucagon like peptide), soluble in the running buffer.

Proteoliposomes

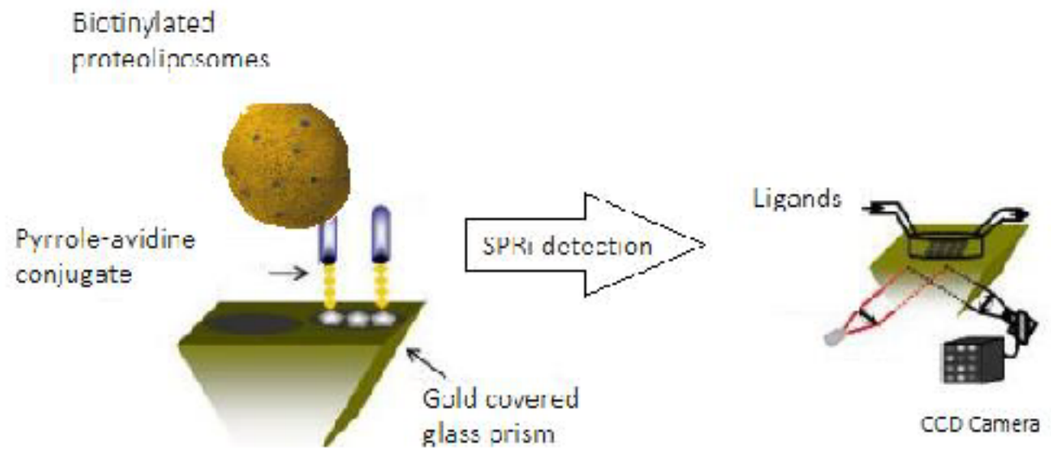
A first feasibility study allows the determination of the specific parameters for the expression of GLP1 receptor.
We have used these conditions to produce GLP1 receptor proteoliposomes in absence or in presence of its ligand. We have screened a range of concentrations of this ligand in order to select the best concentration for the SPRi assay. A construction containing a strep-Tag at the C-terminus was included in this study.
After the proteoliposome production, the mixtures were directly loaded onto a 3-step discontinuous sucrose gradient (60%, 30% and 5%) to separate proteoliposomes from precipitated protein and empty liposomes. After ultracentrifugation (1h at 200,000xg at 4°C), the different fractions were collected for analysis by Western blotting.
The final concentration
of the protein of interest and its degree of purity were estimated with Coomassie blue staining.
A production of 20µg of GLP1 receptor +/- ligands and a non-relevant protein as negative control into biotinylated liposomes were required for SPRi studies.

Assay design

SPRi Detection
Immobilisation by electro-polymerization on gold surface

Avidin was immobilized on a gold-coated glass prism by electrochemical copolymerization of pyrrole-avidin conjugates. Biotinylated proteoliposomes containing GLP1 receptor and biotinylated proteoliposomes without this protein (negative control) were deposited on pyrrole-avidin spots (without electro-polymerization).
Each proteoliposome condition was spotted in five copies using an Omnigrid robotic arrayer (HORIBA Scientific) (**Figure 3**).

Fig.3: ***Diagram of proteoliposome immobilization and ligand capture method.*** *Avidin pyrroled was immobilized on the gold surface, biotinylated proteoliposomes were deposited on the avidin spots and the ligands were injected on all spots.*



We have immobilized two different GLP1 receptors on the biochip:
– GLP1 receptor expressed without additive
– GLP1 receptor expressed in presence of GLP1 ligand

Capture of ligands on biochip

The capture of ligands was performed at 25°C using SPRiPlexII (manual instrument). The GLP1 receptors were immobilized at 5µg/mL. The GLP1 ligand was injected over the immobilized receptor at a flow rate of 50µL/min. Association was monitored for 4 min, and dissociation was monitored for 5 or 10 min.
For each experiment, all injections were carried out successively. The dissociation was completed and no regeneration was performed between ligand injections. After the injection of the compounds, the chip was rinsed with the running buffer to remove unbound compounds. All compounds were diluted in the running buffer.
The binding responses of the ligand were normalized to the density of proteoliposomes on the surface. The data were analyzed using SPRi1000 software.

Experimental conditions

We have optimized the ligand time injection (correlating to flow-rate) and saturation condition in order to reduce the non-specific binding to the sensor surface **(Table 1)**.
Table 1 : Experimental conditions

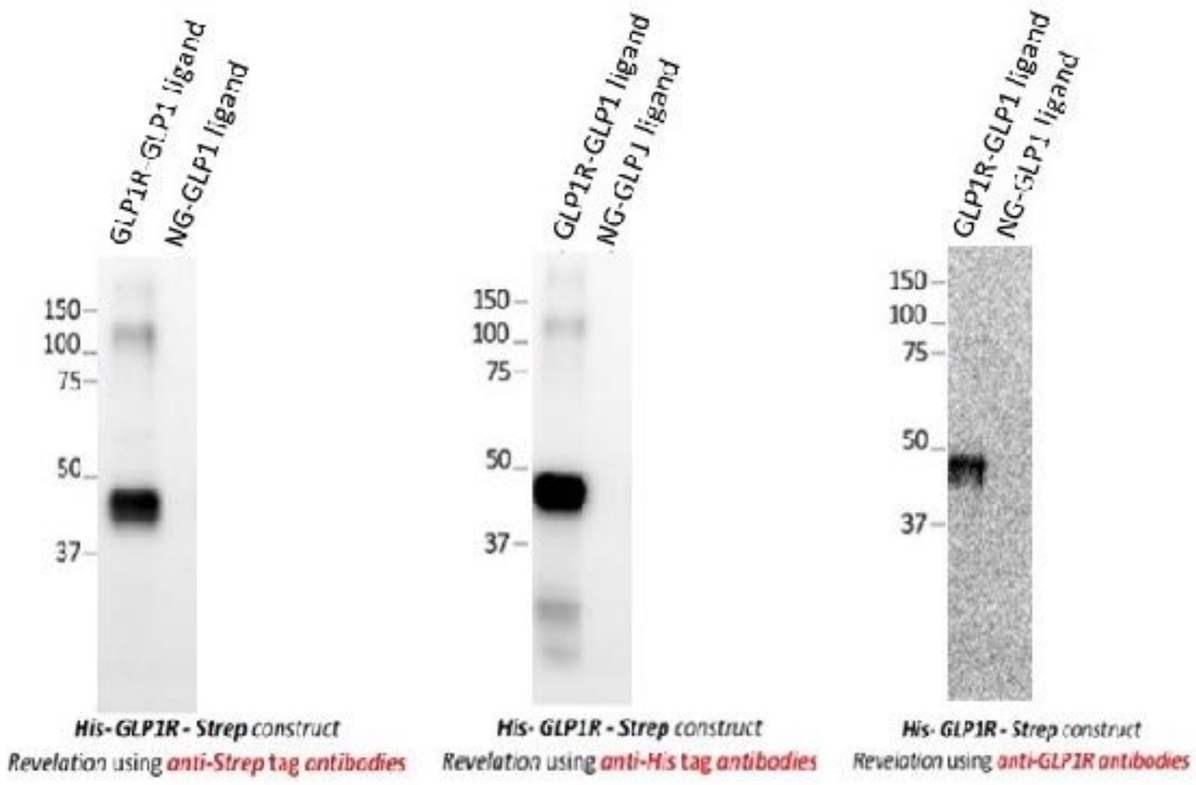
Running buffer	Phosphate, $MgCl_2$,0.2mg/mL BSA
Blocking solution	2 % BSA
Flow rate	50 µL/min
Kinetics angle	57,89°
Sample loop volume	200µL
Contact time	4 min
GLP1 Receptor Concentration	5 µg/mL
Report time	Before end of sample injection
Regeneration solution	Not needed

Results

Validation of the expression :

We have validated the full length of GLP1R on the final expression (**Figure 2**). For this, we have used the His-GLP1R-Strep construct and we have revealed the expression with an antibody against Strep-tag. The Western blot under reducing conditions revealed the presence of a band at ≈ 45kDa corresponds to the full length of GLP1 receptor. Also, we have observed the same band when we revealed with a specific antibody for GLP1R (**Figure 4**).

*Fig.4: **GLP1R expression in presence of GLP1 ligand.***



GLP1R was expressed in the reaction mixture using optimal condition in presence or absence of GLP1 ligand. To separate proteoliposomes from liposomes and aggregated protein, a discontinuous sucrose gradient was used. The sample was loaded at the bottom of a discontinuous 5–60% (w/v) sucrose gradient (3 ml) and centrifuged for 2 h, at 100,000 ×g at 4 °C. Liposomes and proteoliposomes resolved separately, while unincorporated protein remained in the lower 60% sucrose layer. The fraction containing proteoliposome was diluted and centrifuged for 30 minutes at 30,000×g at 4 °C. The resulting pellet containing the GLP1R proteoliposome was resuspended in buffer and analyzed by Western Blot. Detection was done by using an anti-Strep antibody; using anti-His antibody or using anti-GLP1R antibody. Negative control (NC) corresponds to a reaction sample without DNA template.

SPRi results :

Image Biochip

We have immobilized three different receptors on the biochip:
– GLP1 receptor expressed without additive (GLP1R)
– GLP1 receptor expressed in presence of GLP1 ligand (GLP1R with GLP1 ligand)
– Receptor X expressed without additive (Negative control : NC)

GLP1 Ligand Injection

GLP1 ligand used for the expression of GLP1 receptor was also injected over the biochip. We have monitored the specific interaction between GLP1 receptor and GLP1 ligand by SPRi. In this present experiment, the kinetic analysis was based on single-cycle kinetics. The GLP1 ligand is injected sequentially in the same cycle.
GLP1 ligand was diluted in running buffer and injected in a concentration range between 0.5µM and 400µM. A sensorgram corresponding to a plot of response against time, showing the progress of the interaction, was recorded (**Figure 5**) and from sensorgram of the analyte interactions, the SPR signal (% reflectivity) at steady state (equilibrium binding) is determined and described in the Figure 5. **These data correspond to the subtraction of the SPR signal on the negative control.**
The compound concentration and injection time were correct since the steady state was reached and the dissociation is complete. After the injection of the compounds, the chip was rinsed with running buffer to remove unbound compounds.

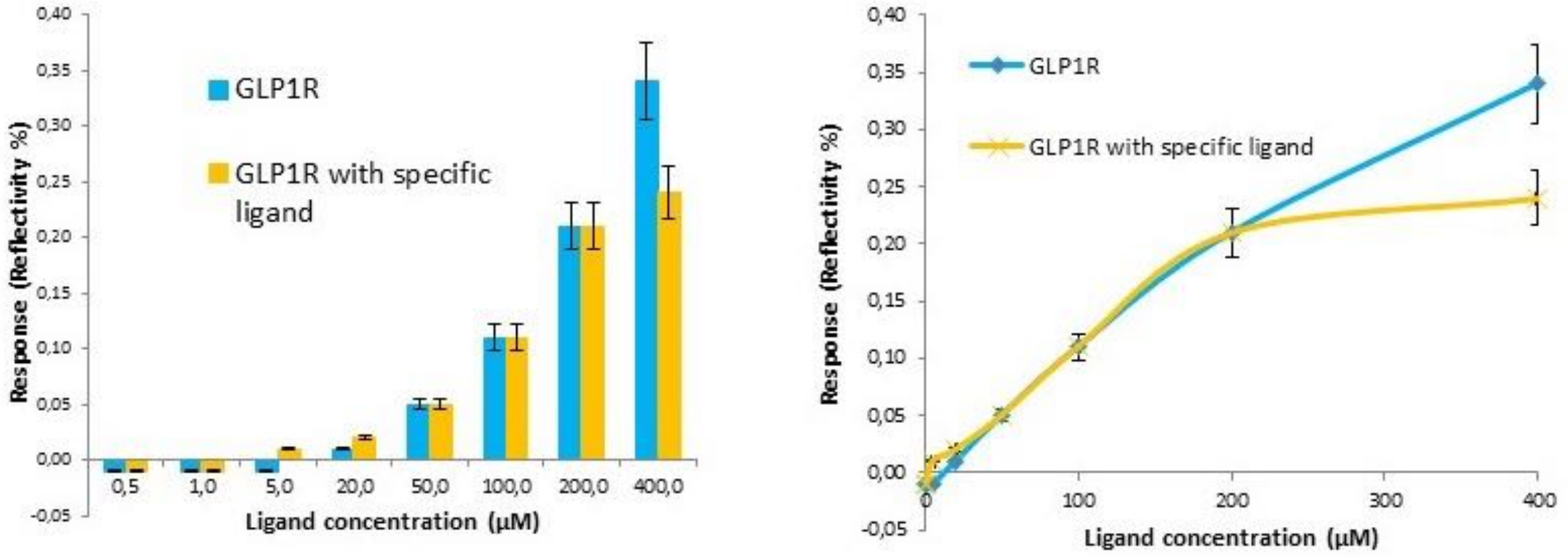


Fig.5: ***Variations of reflectivity obtained at steady state for each sample immobilized and for each injection of GLP1 ligand.*** *These variations were calculated between two points: Report point 1 was the baseline level before the injection of the compound and point 2 the binding level in the end of the association phase (corresponding to the steady state). GLP1R: expression of GLP1R without additive; GLP1R with specific ligand: expression of GLP1R in presence of GLP1 ligand. The reflectivity corresponds to an average of 5 spots.*

The response obtained on GLP1R expressed with specific ligand or on GLP1R expressed without additive are specific binding because the response levels tend to a saturation plateau. The response is concentration dependent **(Figure 5). Expression in presence of specific ligand or without additive seems to be the most adapted to preserve the functionality and integrity of this receptor.** The expression condition is very critical and has a real impact to keep the receptor activity.

Formulation

Buffer: Available in Tris 50mM, pH 7.5. Others buffers or customized formulation can be provided upon request.

Customized Hydrophobic matrix: Customized formulation with specific lipids like PEGylated or biotinylated lipids can be used upon request, as well as targeting molecules.

Storage/Stability: Store at +4°C for up to one week or several months at -80°C. Aliquot for storage. Do not freeze-thaw after aliquoting.

Use restrictions: For life science research use only.

Available sizes:10µg, 20µg ,100 µg , 200 µg, 500 µg,bulk

Need a specific amount, a quote or any additional information?
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