GLP1R- Glucagon-like-peptide 1 receptor # PL067

Product specification

Acronym: GLP1R
Synonyms: GLP-1-R, GLP1 receptor
Origin species : Human
Protein reference : P43220 (UniProtKB)
NP002053.3 (GenBank)
Family: GPCR class B

Expression system: E.coli based CFPS
Format: Proteoliposomes
Protein sequence: Met1 – Ser463
Tag : 6xHis tag (N-ter)
Cleavage site: Factor Xa
Product MW: 55.5 kDa
Application: Drug screening & discovery, antibody development, structural biology

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.

Recombinant protein sequence

His tag – factor X cleavage site –
MAGAPGPLRLALLLLGMVGRAGPRPQGATVSLWETVQKWREYRRQCQRSLTEDPPPATDLFCNRTFDEYACWPDGEPGSFVNVS
CPWYLPWASSVPQGHVYRFCTAEGLWLQKDNSSLPWRDLSECEESKRGERSSPEEQLLFLYIIYTVGYALSFSALVIASAILLGFRHL
HCTRNYIHLNLFASFILRALSVFIKDAALKWMYSTAAQQHQWDGLLSYQDSLSCRLVFLLMQYCVAANYYWLLVEGVYLYTLLAF
SVLSEQWIFRLYVSIGWGVPLLFVVPWGIVKYLYEDEGCWTRNSNMNYWLIIRLPILFAIGVNFLIFVRVICIVVSKLKANLMCKTD
IKCRLAKSTLTLIPLLGTHEVIFAFVMDEHARGTLRFIKLFTELSFTSFQGLMVAILYCFVNNEVQLEFRKSWERWRLEHLHIQRDSS
MKPLKCPTSSLSSGATAGSSMYTATCQASCS

Quality analysis

Purity:
Liposomes are directly incorporated into the Cell-Free reaction, thus, some impurities from the *E.coli* lysate might be present in the proteoliposomes.
A negative control (proteoliposomes without the protein of interest) can be provided (useful for screening, immunization…).

The purity can be improved by protein expression in detergent and relipidation after purification step(s).

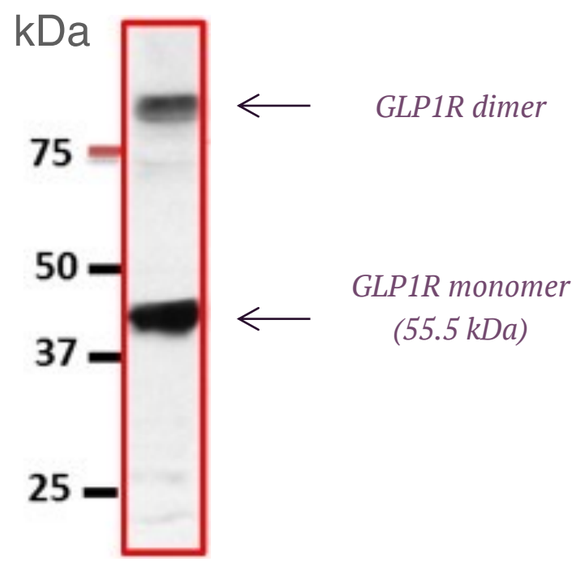


Fig.1: *Identification of hGLP1R in the proteoliposomes by Western blot (using an anti-6xHis antibody).*

Purification procedure: GLP1R proteoliposomes are purified on a sucrose gradient.

NB 1: Migration of membrane proteins on SDS-PAGE can result in « gel shifting » due to the presence of hairpins (helix-loop-helix)[1-3].

NB 2: a Strep-tag added to the C-terminus end of GPL1R confirmed the full length expression of this protein.

References :
1 – Rath A., et al., Detergent binding explains anomalous SD-PAGE migration of membrane proteins PNAS, 2009 Feb 10, vol. 106
2 – Rath A., et al., Acrylamide concentration determines the direction and magnitude of helical membrane protein gel shifts, PNAS, 2013 Sep 24, 110(39)
3 – Rath A., et al., Correction factors for membrane protein molecular weight readouts on sodium dodecyl sulfate-polyacrilamide gel electrophoresis, Anal. Biochem., 2013 Mar 1, 434(1)

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

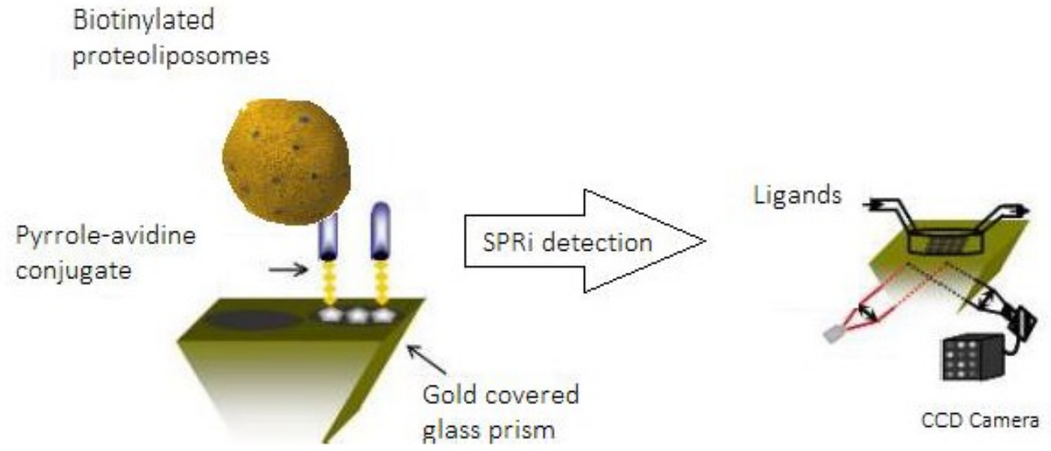
GLP1 ligand
Expression of GLP1 receptor by cell-free and activity binding tests were performed using the specifc ligand (Glucagon like peptide), soluble in the running buffer.

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 discontinuous sucrose gradient to separate proteoliposomes from precipitated protein and empty liposomes. After ultracentrifugation 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 SDS-PAGE.
A production of 20 µg of GLP1 receptor +/- ligands and a non-relevant protein as negative control into biotinylated liposomes were required for SPRi studies.

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 proteoliposomes condition was spotted in five copies using an Omnigrid robotic arrayer (HORIBA Scientific) (**Figure 2**).

Fig.2*: *Diagram of proteoliposome immobilization and ligand capture method.
Pyrrole-Avidin 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 5 µg/mL. The GLP1 ligand was injected over the immobilized receptor at a flow rate of 50 µL/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)**.

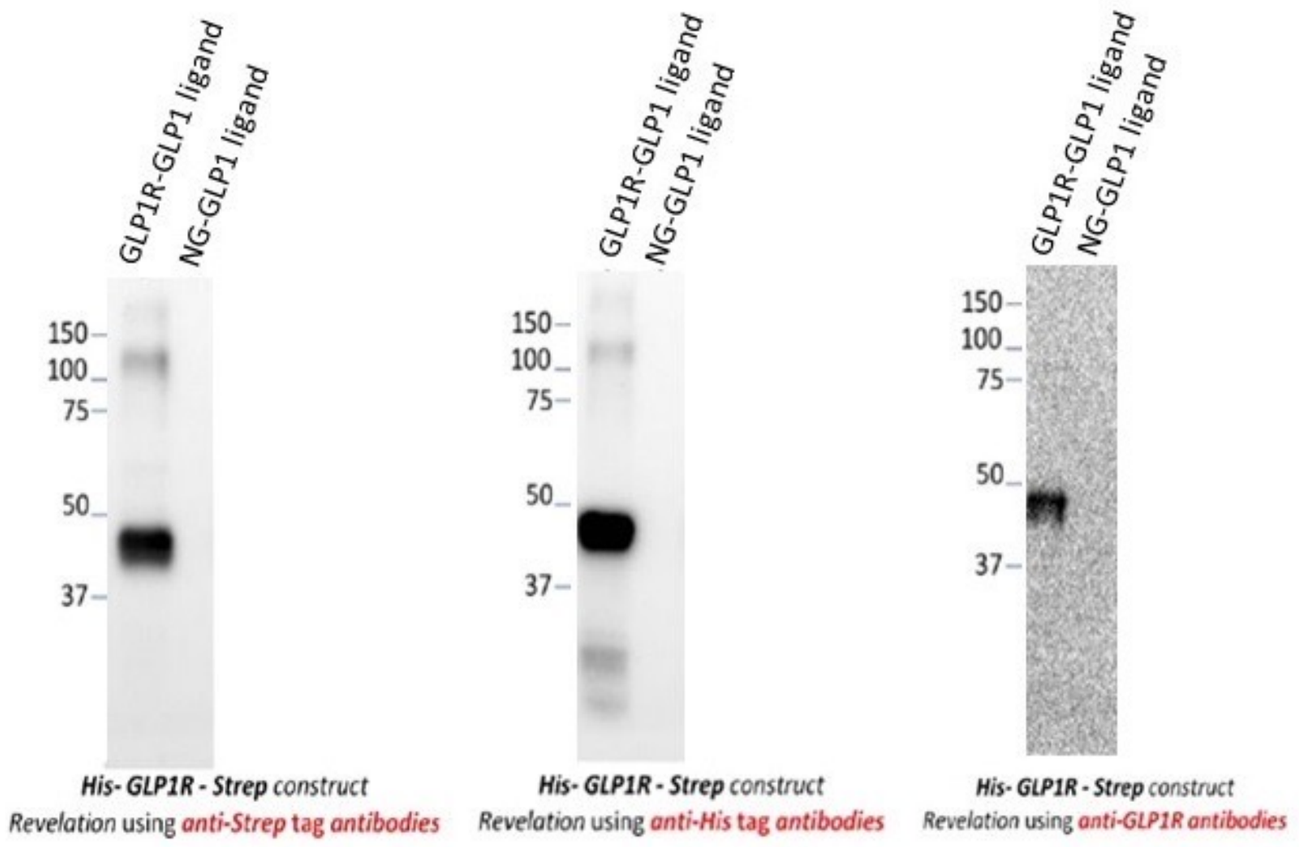
Table 1 : Experimental conditions

Running buffer	Phosphate, MgCl2, 0.2 mg/mL BSA
Blocking solution	2 % BSA
Flow rate	50 µL/min
Kinetics angle	57,89°
Sample loop volume	200 µL
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 ≈ 45 kDa 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 3**).

Fig.3: 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 fraction containing proteoliposomes was diluted and centrifuged. The resulting pellet containing the GLP1R proteoliposomes 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 was injected sequentially in the same cycle.
GLP1 ligand was diluted in running buffer and injected in a concentration range between 0.5 µM and 400 µM. 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.

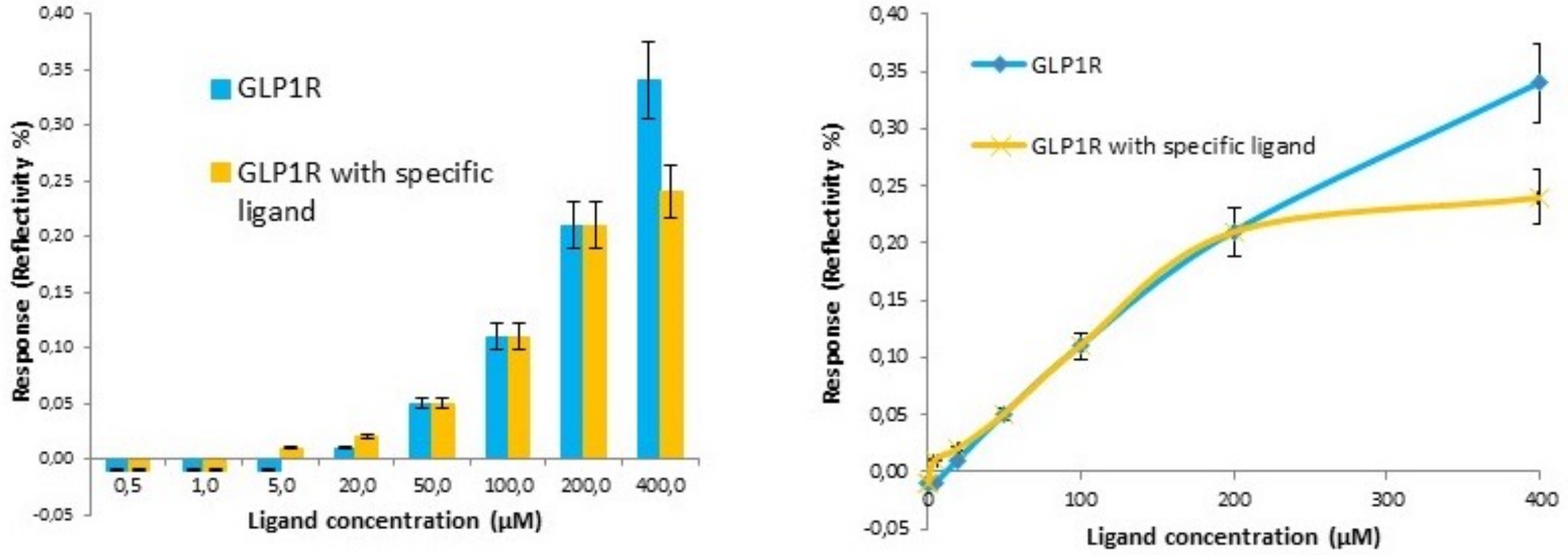


Fig.4*: *Variations of reflectivity obtained at steady state for each sample immobilized and for each injection of GLP1 ligand. *These variations were calculated between two points: Report point 1 was the baseline level before the injection of the compound and point 2 the binding level in the end of 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 is specific because the response levels tend to a saturation plateau. The response is concentration dependent **(Figure 4). Expression in presence of specific ligand or without additive seems to be 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

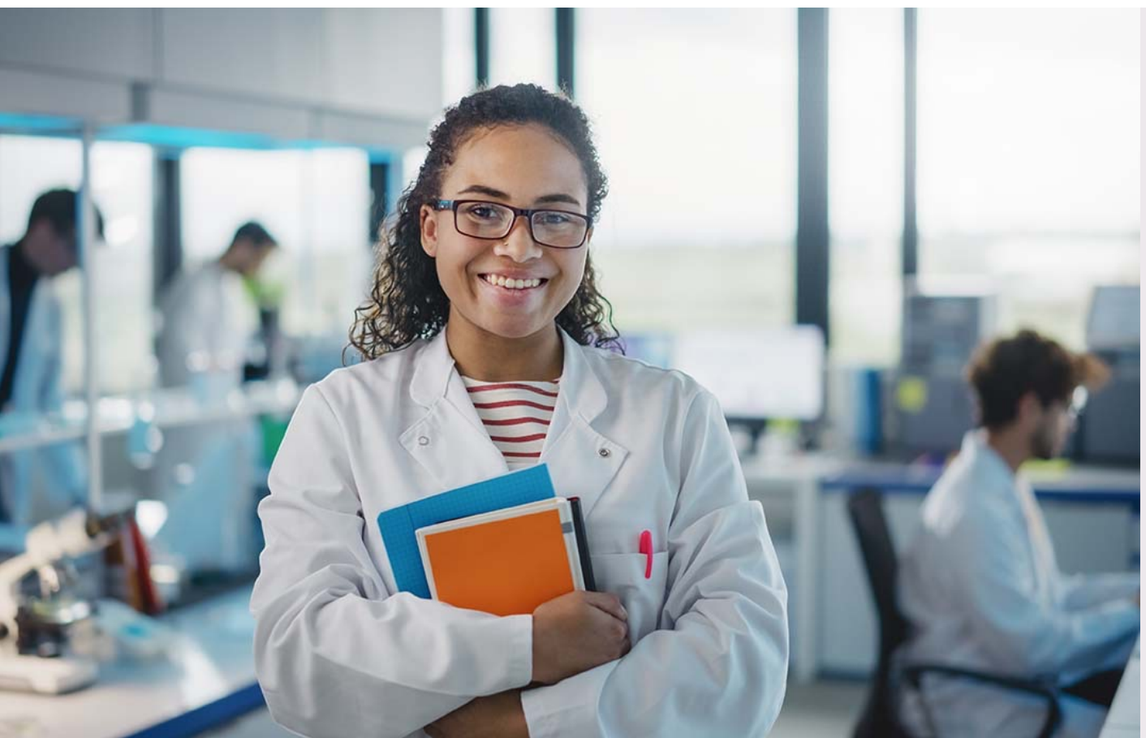
Buffer: Available in Hepes 50mM, pH 7.5 with cryoprotectants. Others buffers or customized formulation can be provided upon request.

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: 10 µg, 50 µg, 100 µg, customized quantity on request.



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