RXFP1- Relaxin receptor 1 # PL068

Product specification

Acronym: RXFP1
Synonyms: LGR7
Origin species : Human
Protein reference : Q9HBX9 (UniProtKB)
NP_067647.2 (GenBank)
Family: GPCR class A

Expression system: E.coli based CFPS
Format: Proteoliposomes
Protein sequence: Met1 – Ser757
Tag : 6xHis tag (N-terminal)
Cleavage site: Factor Xa
Product MW: 89.1 kDa
Application: Drug screening & discovery, antibody development, structural biology

Product description

The relaxin/insulin-like family peptide receptor 1 is the cognate receptor for human relaxin-2 in humans. Human RXFP1 also known as LGR7, is located onto the chromosome 4q32.1 and is known to activate Erk1/2, tyrosine kinase(s), gene transcription, and nitric oxide (NO) signaling and can also interact with the glucocorticoid receptor (GC).
hRXFP1 is found in a wide range of reproductive tissues including ovary, uterus, placenta, mammary gland, prostate, and testis. The receptor is also present in the heart, kidney, lung, liver and blood cells as well as in several areas of the brain such as cortex, organum vasculosum of the lamina terminalis (OVLT), and subfornical organ (SFO). RXFP1 binds peptide hormones consequently, relaxin not only has autocrine and paracrine roles but also acts as a neuropeptide. It's a potent chronotropic with inotropic effects on the human heart. Relaxin peptide (RLN) has shown therapeutic effects in an acute heart failure clinical trial.

Recombinant protein sequence

His tag – factor X cleavage site –
MTSGSVFFYILIFGKYFSHGGGQDVKCSLGYFPCGNITKCLPQLLHCNGVDDCGNQADEDNCGDNNGWSLQFDKYFASYYKMTS
QYPFEAETPECLVGSVPVQCLCQGLELDCDETNLRAVPSVSSNVTAMSLQWNLIRKLPPDCFKNYHDLQKLYLQNNKITSISIYAFR
GLNSLTKLYLSHNRITFLKPGVFEDLHRLEWLIIEDNHLSRISPPTFYGLNSLILLVLMNNVLTRLPDKPLCQHMPRLHWLDLEGNHI
HNLRNLTFISCSNLTVLVMRKNKINHLNENTFAPLQKLDELDLGSNKIENLPPLIFKDLKELSQLNLSYNPIQKIQANQFDYLVKLKS
LSLEGIEISNIQQRMFRPLMNLSHIYFKKFQYCGYAPHVRSCKPNTDGISSLENLLASIIQRVFVWVVSAVTCFGNIFVICMRPYIRSE
NKLYAMSIISLCCADCLMGIYLFVIGGFDLKFRGEYNKHAQLWMESTHCQLVGSLAILSTEVSVLLLTFLTLEKYICIVYPFRCVRPGK
CRTITVLILIWITGFIVAFIPLSNKEFFKNYYGTNGVCFPLHSEDTESIGAQIYSVAIFLGINLAAFIIIVFSYGSMFYSVHQSAITATEIR
NQVKKEMILAKRFFFIVFTDALCWIPIFVVKFLSLLQVEIPGTITSWVVIFILPINSALNPILYTLTTRPFKEMIHRFWYNYRQRKSM
DSKGQKTYAPSFIWVEMWPLQEMPPELMKPDLFTYPCEMSLISQSTRLNSYS

Quality analysis

Purity: > 70% (determined by Coomassie Blue stained SDS-PAGE)

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).

Purification procedure: hRXFP1 proteoliposomes are purified on a sucrose gradient.

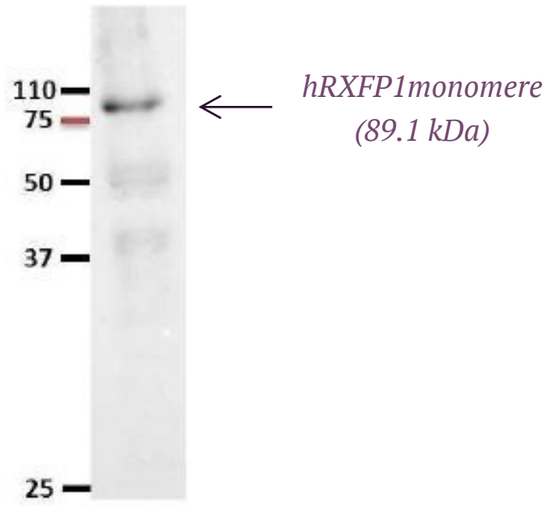


Fig.1: *Identification of hRXFP1 in proteoliposomes by Western Blot using an anti-6xHis antibody.*

Assessment of the receptor functionality

I. Introduction

The aim of this study was to develop a binding assay for hRXFP1 ligands on RXFP1 receptor proteoliposomes using SPRi. Indeed, we developed a membrane protein biosensor assay that offers the opportunity for high-throughput label-free screening that directly measures membrane protein-ligand interactions.
A specific signal was detected between hRXFP1 receptor and its natural ligand, the relaxin. This signal is depending on the expression condition.

II. Materials

1. Relaxin ligand

Expression by cell-free and activity binding tests of hRXFP1 receptor were performed using the specifc ligand (the relaxin), soluble in the running buffer.

2. Proteoliposomes

We have immobilized three different RXFP1 receptors on the biochip:
– RXFP1 receptor expressed without Calcium
– RXFP1 receptor expressed with 0.5 µM of Calcium
– RXFP1 receptor expressed with 1 mM of Calcium

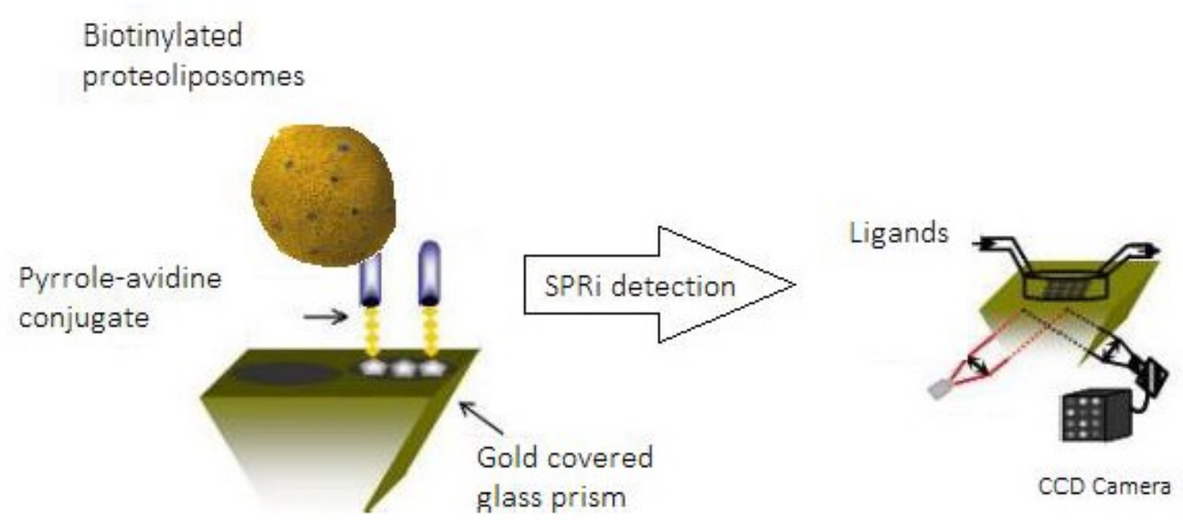
3. Assay design

3.1 SPRi Detection

– Immobilization 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 hRXFP1 receptor and biotinylated proteoliposomes without this protein (negative control) were deposited on pyrrole-avidin spots. Each proteoliposome 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. Avidin pyrroled was immobilized on the gold surface, biotinylated proteoliposomes were deposited on the avidin spots and the ligands were injected on all spots.*



3.2 Capture of ligands on biochip

The capture of ligands was performed at 25°C using SPRiPlexII (manual instrument). The hRXFP1receptors were immobilized at 5 µg/mL. The relaxin 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 the proteoliposomes on the surface. The data were analyzed using SPRi1000 software.

3.3 Experimental conditions

The ligand time injection (correlating to flow-rate) and the saturation condition were optimized in order to reduce the non-specific binding to the sensor surface (Table 1).

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
Relaxin concentration	5 µg/mL
Report time	Before end of sample injection
Regeneration solution	Not needed

Table 1 : *experimental conditions*

III. Results

1.Validation of the expression :

The full length expression of RXFP1 in presence of different concentrations of calcium has been validated (Figure 3). For this, we have used the His- RXFP1 -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 ≈ 70kDa corresponding to the full length of RXFP1.

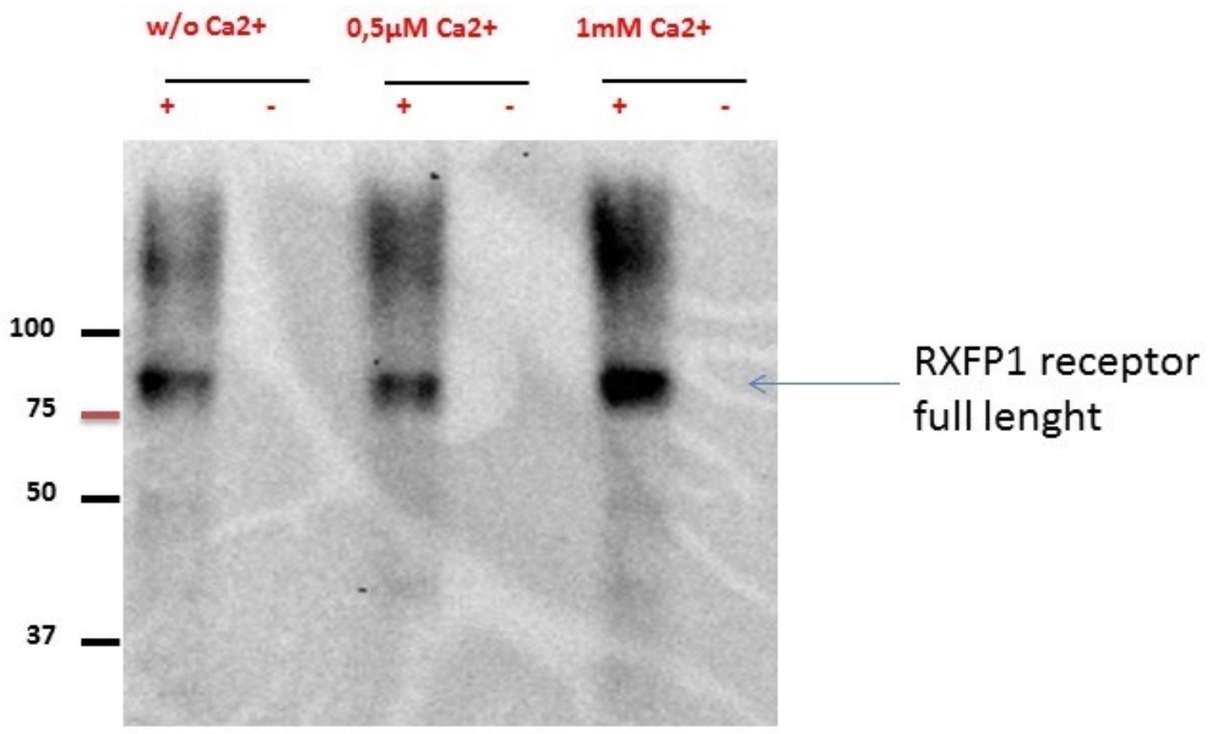


Fig.3: *RXFP1 expression in presence of different concentrations of calcium. Detection of RXFP1 receptor full length by Western Blot with anti-STREP antibody*

2.SPRi results :

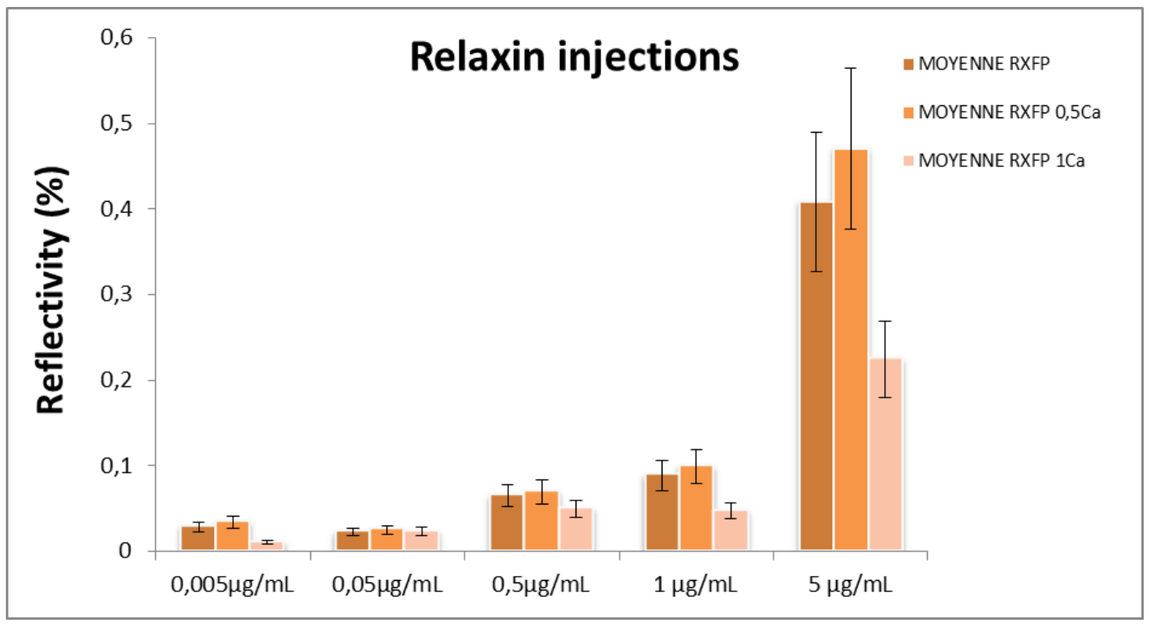
2.1 Relaxin ligand injection

The relaxin ligand was injected over the biochip. We have monitored the specific interaction between RXFP1 and relaxin ligand by SPRi. In this present experiment, the kinetic analysis was based on single-cycle kinetics. The relaxin ligand was injected sequentially in the same cycle.

The relaxin ligand was diluted in running buffer and injected in a concentration range between 0.5 µM to 400 µM. A sensorgram corresponding to a plot of response against time, showing the progress of the interaction, was recorded and from sensorgram of the analyte interactions, the SPR signal (% reflectivity) at steady state (equilibrium binding) is determined and described in the Figure 4. 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: *Validation of interaction RXFP1 / Relaxin ligand: Variations of reflectivity obtained at steady state for each sample immobilized and for each injection of relaxin ligand. These variations were calculated between two points: report point 1 was the baseline level before the injection of the compound and point 2 was the binding level at the end of the association phase (corresponding to the steady state). The reflectivity corresponds to an average of 5 spots.*



After injection of relaxin at different concentrations, we have observed a specific interaction between RXFP1 receptor and relaxin ligand. The expression condition is very critical and has a real impact to keep the receptor activity.

Formulation

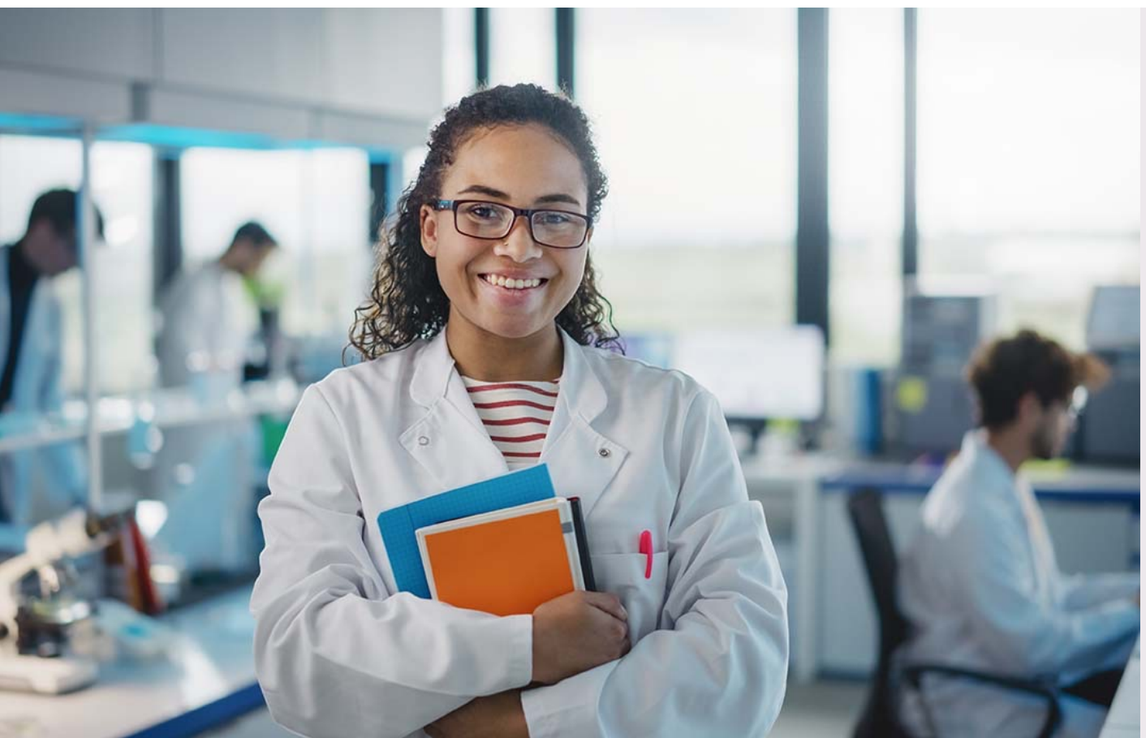
Buffer: Available in Hepes 50mM, pH 7.5 with cryoprotectants. Other buffers or customized formulation can be provided upon request.

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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