APJ/AR- Apelin receptor # PL061

Product specification

Acronym: APJ/AR
Origin species : Human
Protein reference : P35414 (UniProtKB)
NP_005152.1 (GenBank)
Family: GPCR class A, capside protein, enzyme

Expression system: E.coli based CFPS
Format: Proteoliposomes
Protein sequence: Met1 – Asp380
Tag : 6xHis tag (N-ter)
Cleavage site: Factor Xa
Product MW: 44.7 kDa
Application: Drug screening & discovery, antibody development, structural biology

Product description

The apelin receptor (AR or APJ) is a class A GPCR involved in numerous physiological processes. The apelin receptor is implicated in angiogenesis during tumour formation and as a CD4 co-receptor for entry of human immunodeficiency virus type 1 (HIV-1) to cells.
APJ and apelin peptides have been found to be involved in the regulation of cardiovascular function and fluid homeostasis. Broad roles of apelin system has been established in lowering blood pressure, as a potent cardiac inotrope, in modulating pituitary hormone release and water intake. The apelin system is also involved in stress activation, and as a novel adipokine that is excreted from fat cells and regulates insulin.

Recombinant protein sequence

His tag – factor X cleavage site –
MEEGGDFDNYYGADNQSECEYTDWKSSGALIPAIYMLVFLLGTTGNGLVLWTVFRSSREKRRSADIFIASLAVADLTFVVTLPLWA
TYTYRDYDWPFGTFFCKLSSYLIFVNMYASVFCLTGLSFDRYLAIVRPVANARLRLRVSGAVATAVLWVLAALLAMPVMVLRTTG
DLENTTKVQCYMDYSMVATVSSEWAWEVGLGVSSTTVGFVVPFTIMLTCYFFIAQTIAGHFRKERIEGLRKRRRLLSIIVVLVVTFA
LCWMPYHLVKTLYMLGSLLHWPCDFDLFLMNIFPYCTCISYVNSCLNPFLYAFFDPRFRQACTSMLCCGQSRCAGTSHSSSGEKSA
SYSSGHSQGPGPNMGKGGEQMHEKSIPYSQETLVVD

Quality analysis

Purity: > 70 % (determined by SDS-Page and Coomasie Blue staining)
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: hAPJ proteoliposomes are purified on a sucrose gradient.

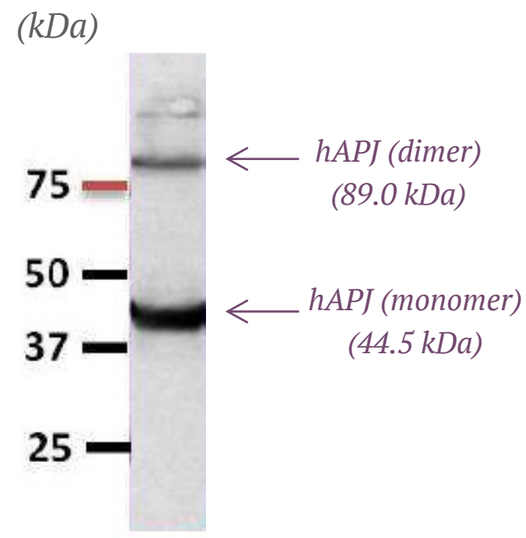


Fig.1: *Identification of hAPJ in the proteoliposomes by Western blot using an anti-6xHis antibody*

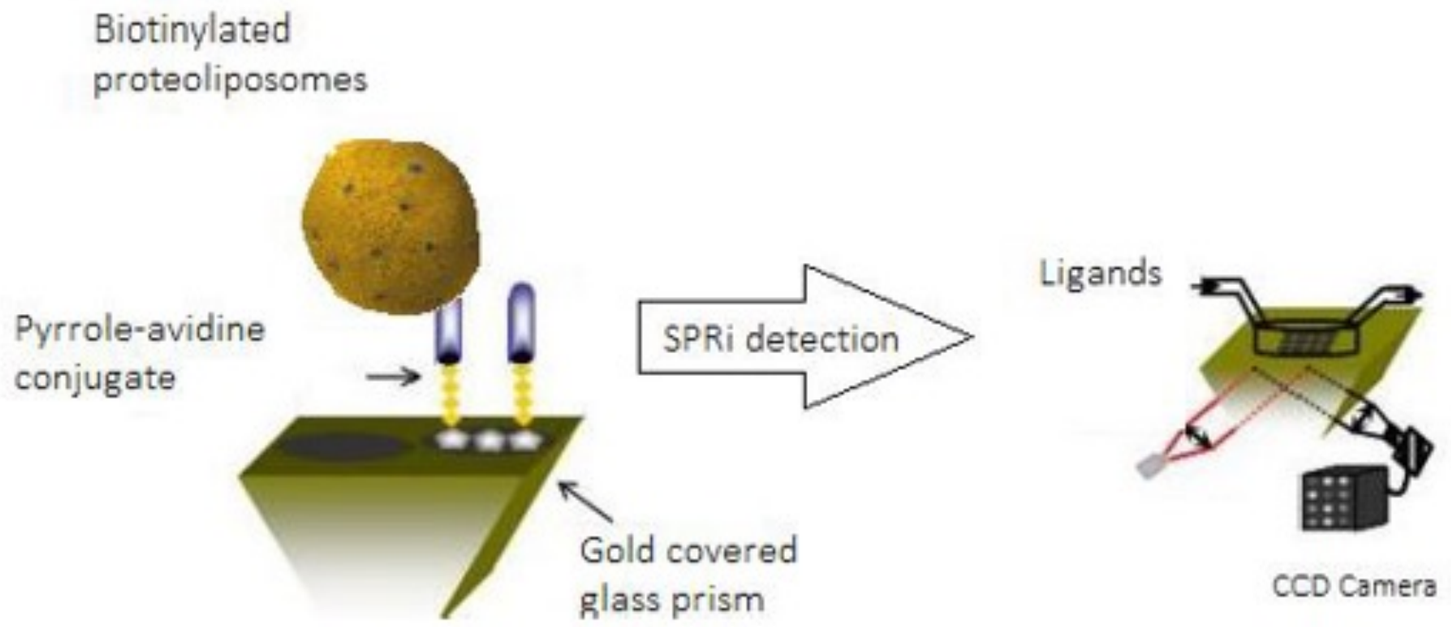
Assessment of functionality

1- SPRi Detection

A. 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 the apelin 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.*



B. Capture of ligands on biochip

The capture of ligands was performed at 25°C using SPRiPlexII (manual instrument). The apelin receptors were immobilized at 25 µg/mL. The Apelin-17 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.

C. Experimental conditions

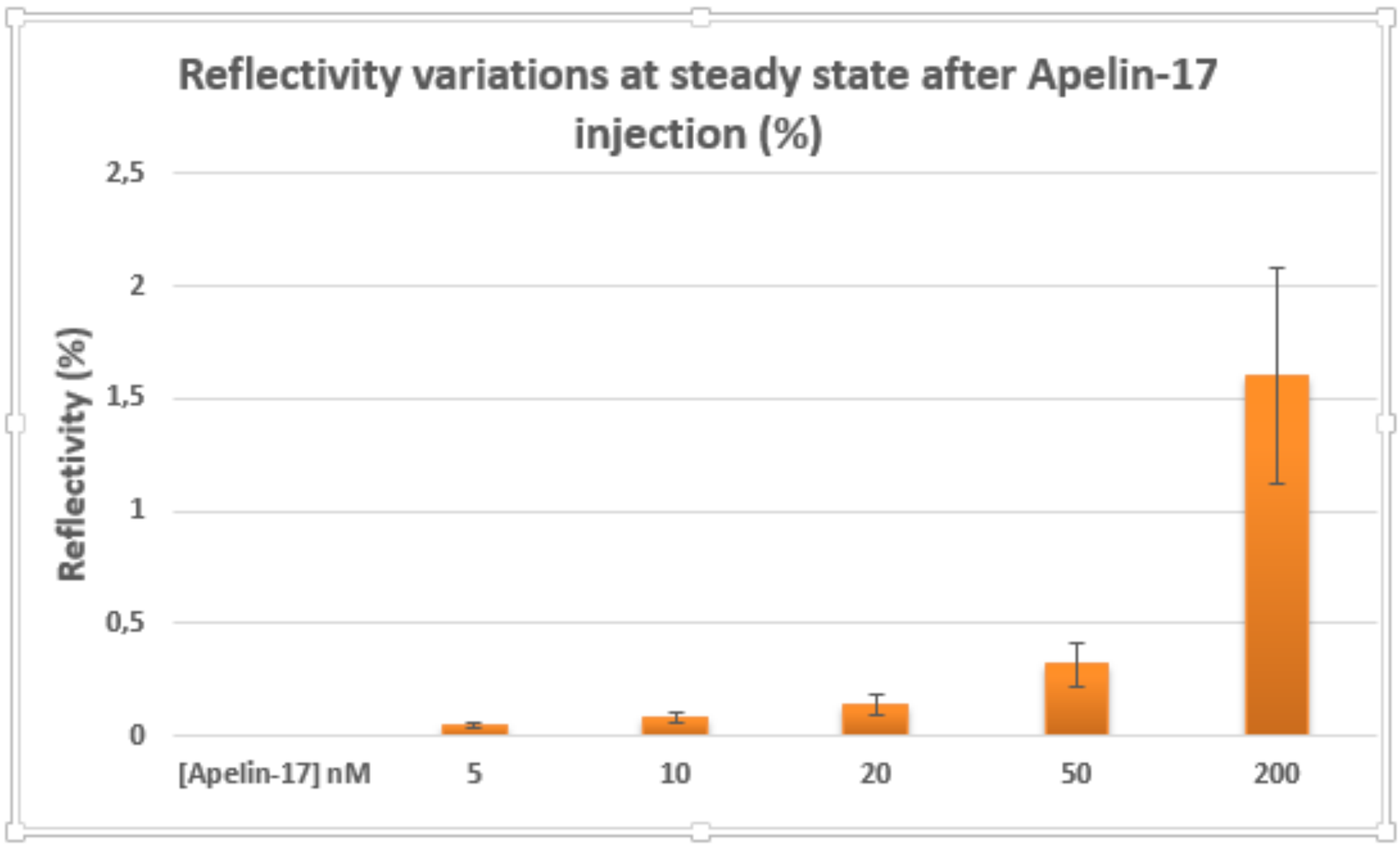
The ligand time injection (correlating to flow-rate) and the saturation condition were optimized in order to reduce the non-specific binding to the sensor surface (Table 1).

Buffer	**Phosphate, $MgCl_2$**
Flow rate	50 µL/min
Contact time	4 min
Apelin Receptor Concentration	25 µg/mL
Report time	Before end of sample injection
Regeneration	Not needed

2- Results

After injection of Apelin-17 at different concentrations, a specific interaction between Apelin receptor and its ligand was observed. The binding of Apelin-17 to the captured Apelin receptor proteoliposomes was reproducible and concentration dependent (Figure4). This type of signal is characteristic of specific binding.

Fig.4*: Validation of specific interaction between Apelin receptor and Apelin-17 ligand: Variations of reflectivity obtained at steady state for each sample immobilized and for each injection of Apelin-17 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.*



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.

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