Synthelis©

GPCR

Protein Catalogue

APJ/AR- Apelin receptor

Product specification

Acronym: APJ/AR
Class: GPCR Class A
Origin: Human
Molecular weight: 42,66 kDa
Application: Screening&Display Technologies, Structural Biology, Antibody development

Purity: >70%
Activity: Proven by Spri
Length: Full Length
TMD: 7
Biological function: Cardiovascular function, fluid homeostasis, gastrointestinal & immune-modulatory functions

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.

Protein Source: hAPJ wild type protein (Human Apelin receptor, uniprot ID: APJ_HUMAN):

Fig.1: AA sequence of hAP protein

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        10         20         30         40         50
MEEGGDFDNY YGADNQSECE YTDWKSSGAL IPAIYMLVFL LGTTGNGLVL
        60         70         80         90        100
WTVFRSSREK RRSADIFIAS LAVADLTFVV TLPLWATYTY RDYDWPFGTF
       110        120        130        140        150
[illegible]
       160        170        180        190        200
AVLWVLAALL AMPVMVLRTT GDLENTTKVQ CYMDYSMVAT VSSEWAWEVG
       210        220        230        240        250
LGVSSTTVGF VVPFTIMLTC YFFIAQTIAG HFRKERIEGL RKRRRLLSII
       260        270        280        290        300
VVLVVTFALC WMPYHLVKTL YMLGSLLHWP CDFDLFLMNI FPYCTCISYV
       310        320        330        340        350
NSCLNPFLYA FFDPRFRQAC TSMLCCGQSR CAGTSHSSSG EKSASYSSGH
       360        370        380
[illegible]
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Affinity Tag: Histidine tag fused to the N-terminal end and Streptavidin tag fused to the C-terminal end of the protein.

Production conditions: hAPJ 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 > 70% as determined by SDS-Page and Coomassie Blue staining.

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

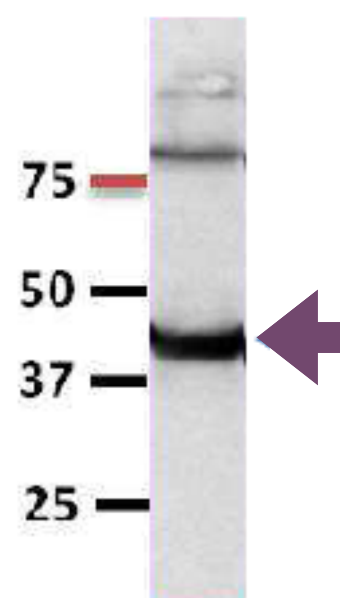


Fig.2: Western blot identification of hAPJ in proteoliposomes after purification.

After purification on a sucrose gradient, the protein appears at the right size on polyacrylamide gel. The dimer form is also present after purification (band at 84 KDa).

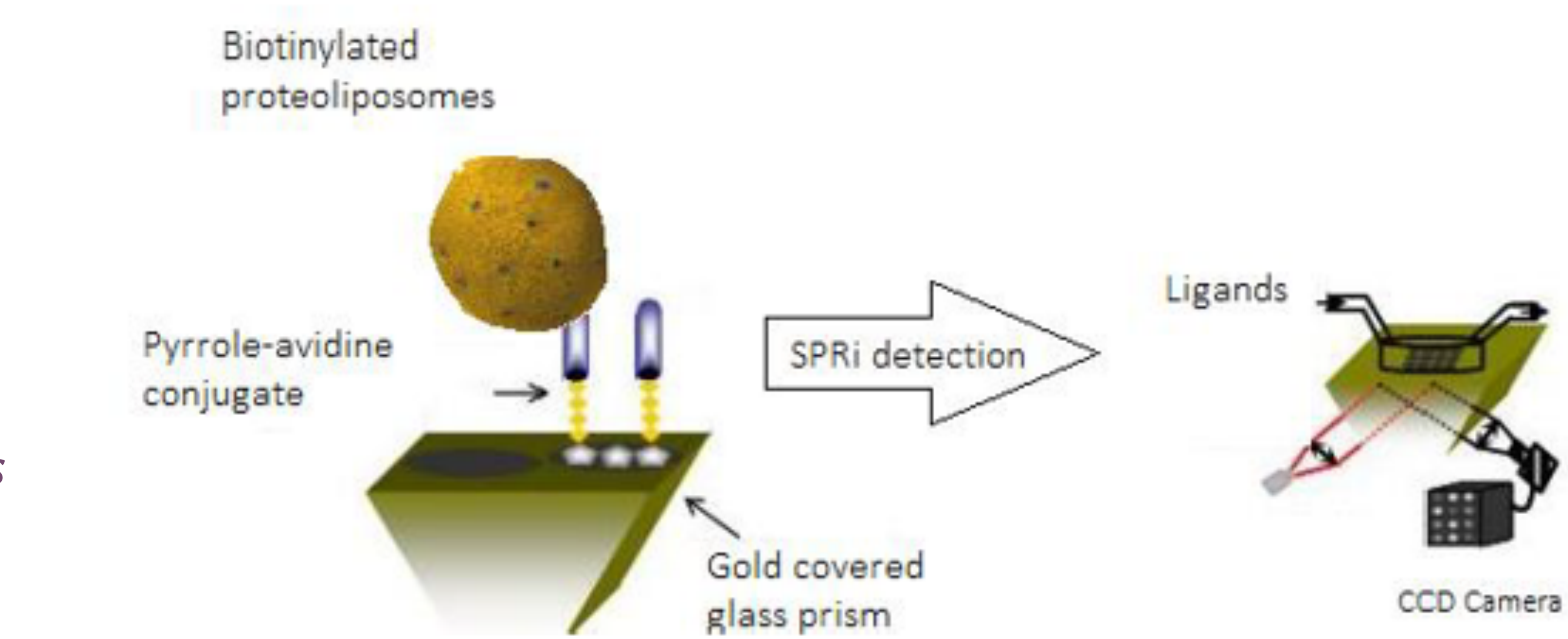
Assessment of functionality

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 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.



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 25µg/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).

Assessment of functionality

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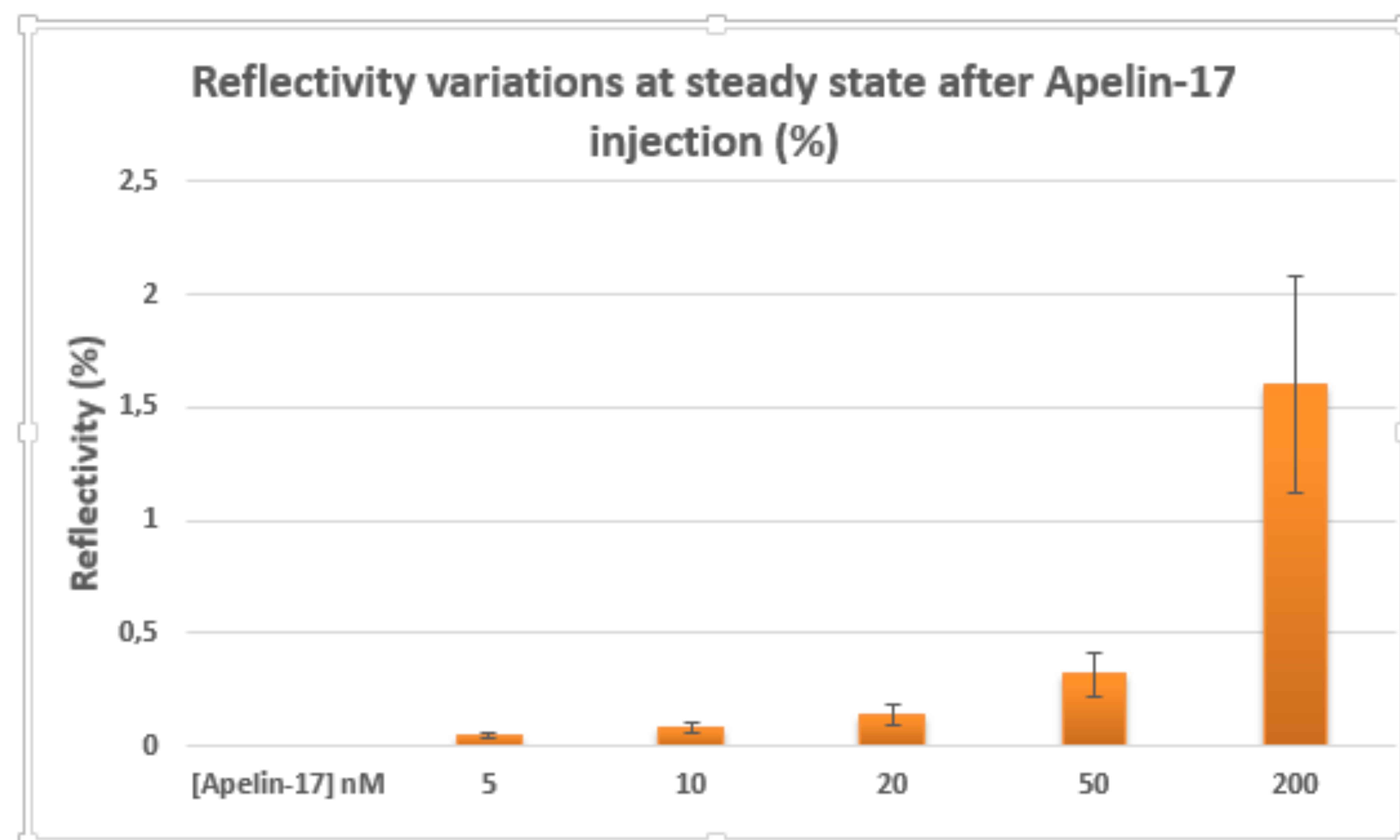
Fig.3: Diagram of proteoliposome immobilization and ligand capture method. Avidin pyrroled was immobilized on the gold surface, biotinylated proteoliposomes were deposited on the avidin spots and the ligands were injected on all spots.

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

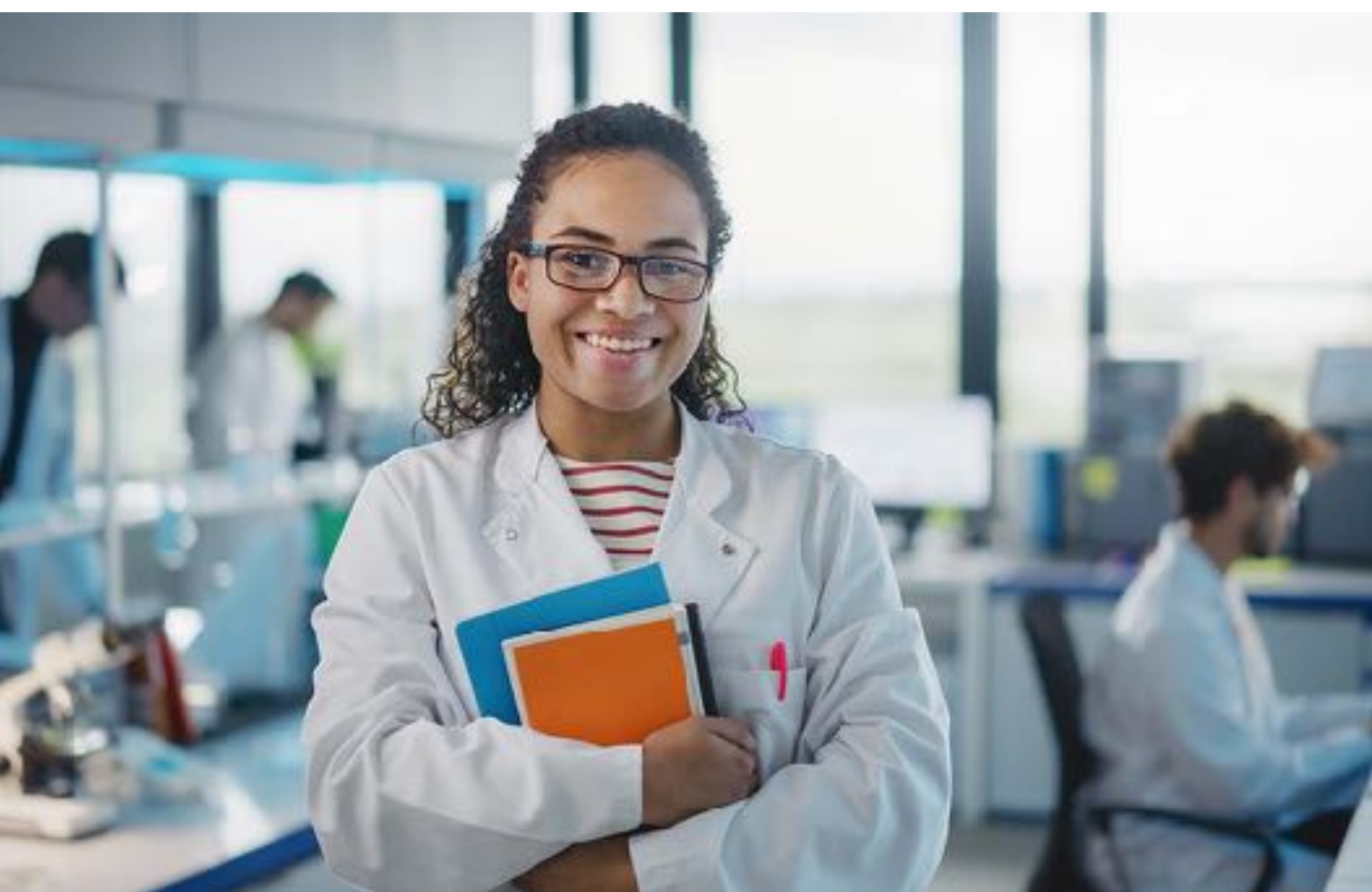
Buffer: Available in Tris 50mM, pH 7.5. Other buffers or customized formulation can be provided upon request.

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



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