A single domain intrabody specifically targeting the follicle-stimulating hormone receptor (FSHR) affects receptor signaling and trafficking

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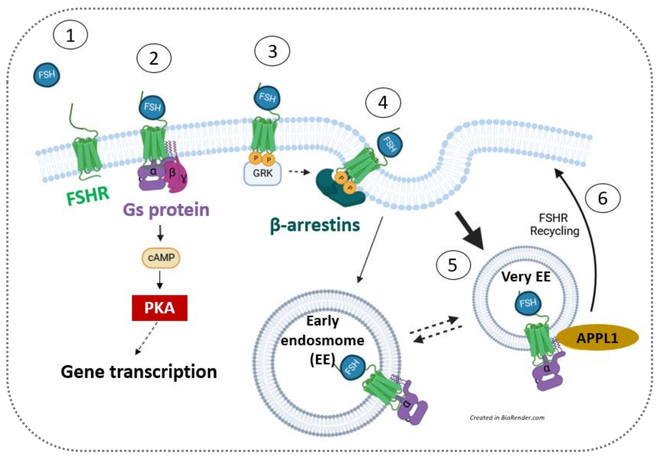
INTRODUCTION

The **follicle-stimulating hormone** receptor (**FSHR**) is a **G protein-coupled receptor (GPCR)** involved in the **growth** and **maturation** of **ovarian follicles** and in **spermatogenesis.** Therefore, it is the main target **of medically-assisted therapies,** and also a putative target for hormonal **contraception**.

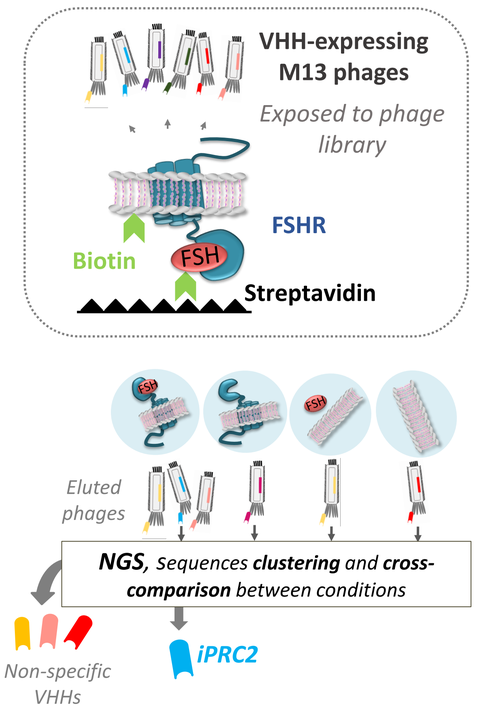
At a cellular level, **FSH-activated FSHR** primarily activates the **Gs protein** pathway. Following GRK-phosphorylation, FSHR then recruits **β-arrestins**, leading to its **internalization** to the early endosome (EE), but **mainly to the very EE**, from witch it is quickly **recycled** back to the plasma membrane.



Intracellular variable fragments from heavy-chain antibody from camelids (**intra-VHH**) provide a **better understanding** on the relationships between **receptor conformation and signaling activity**, but so far, this property has been explored only for five GPCRs (Raynaud et al., 2022).

Intra-VHHs targeting the FSHR with **different functional effects** constitute precious **tools** to correlate **discrete conformations** of the FSHR with the **signaling network engaged** and the physiological outcome.

INTRA-VHH SELECTION



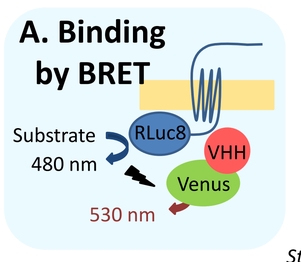
Intra-VHH selection was performed by **phage display.** FSHR-expressing HEK293 cell **membrane fragments** were used, in presence or absence of FSH, with the **intracellular side** oriented to the phage library.

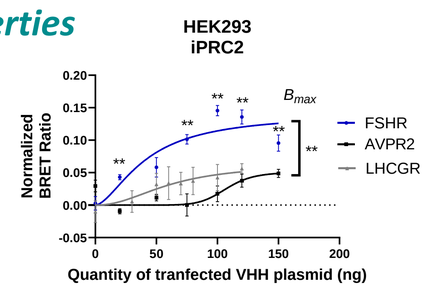
The VHHs carried by the phages recognising the antigens were **sequenced** by NGS, and sequences were **aligned**, **clustered** and **compared** to control conditions in order to identify **specific clones**.
This strategy allowed the selection of **an intra-VHH** of interest : **iPRC2**

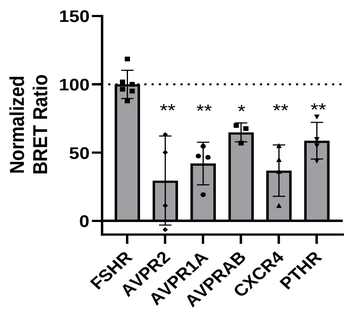
INTRA-VHH CHARACTERIZATION

1 *Binding properties*

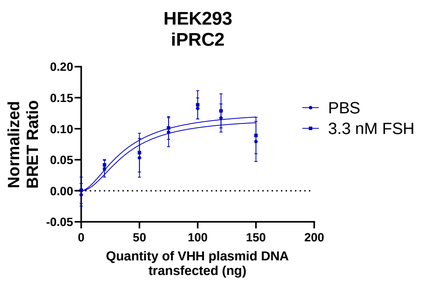
A. Binding by BRET





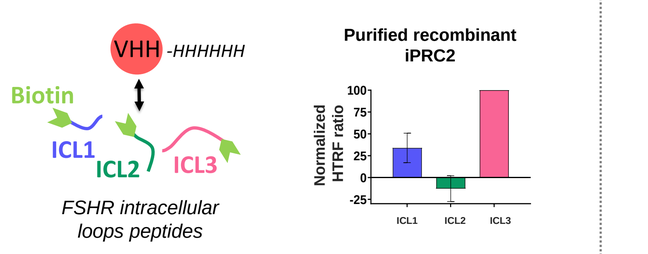


Stimulation with 3,3 nM of FSH, 3,3 nM of LH, 100 nM AVP,



➔ iPRC2 specifically binds the FSHR in presence of FSH or not

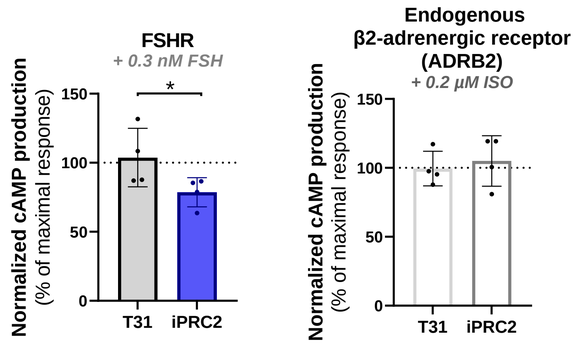
B. Epitope mapping by HTRF



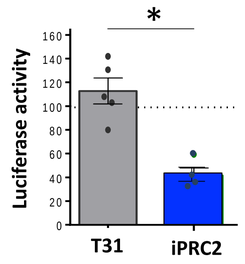
➔ iPRC2 recognizes FSHR ICL1 and ICL3

2 *G protein signaling pathway* *(ICL3 being involved in Gs protein recruitment)*

A. cAMP production



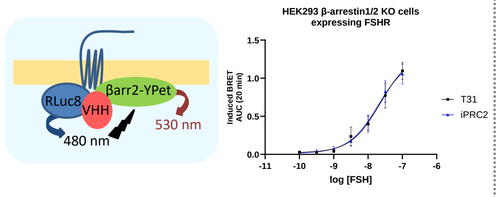
B. CRE-dependent transcription



➔ iPRC2 **decreases G protein-dependent cAMP production** in response to FSH, but have no effect on ADRB2 response to isoproterenol (ISO)
➔ It **reduces CRE-dependent transcription** in response to FSH

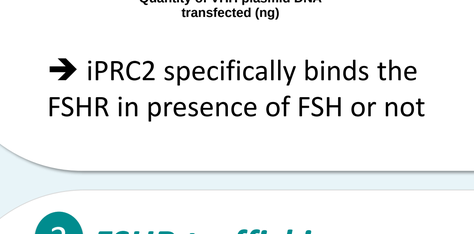
3 *FSHR trafficking*

A. β-arrestins



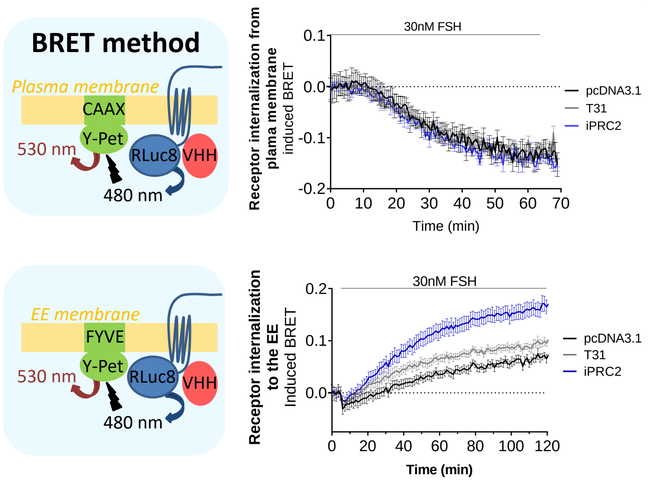
➔ iPRC2 has **no effect on β-arrestin 2** recruitment

C. FSHR Recycling



➔ iPRC2 **decreases FSHR recycling**

B. FSHR intracellular trafficking



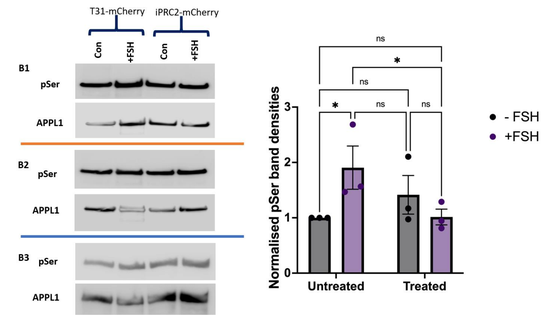
➔ iPRC2 does **not impact FSHR internalisation** but **increases** its **trafficking to the early endosomes** (EE)

=> **Location biais**

4 *Role of APPL1*

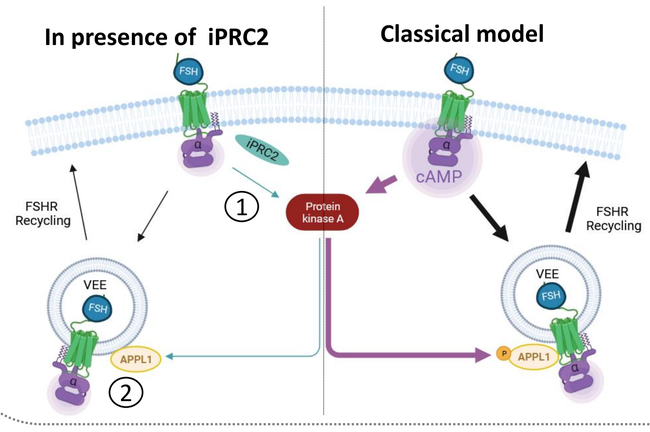
The protein **APPL1** interacts with FSHR on **ICL1, and** is involved in **FSHR recycling** when phosphorylated by the **PKA**.

What is the phosphorylation status of APPL1 ?



➔iPRC2 induces a **decrease** of **APPL1 phosphorylation**

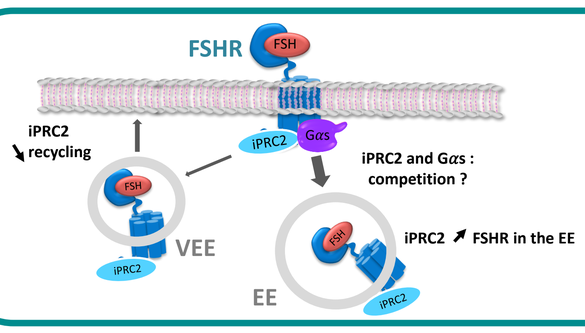
Mechanistic hypotheses regarding the recycling defect of FSHR



① iPRC2 impairs **APPL1 phosphorylation as a result of the reduced cAMP**

② iPRC2 could also directly interfere with **APPL1 binding** to the FSHR

CONCLUSIONS AND PERSPECTIVES



In summary, **iPRC2**

- recognizes **FSHR ICL1** and **ICL3**
- **reduces cAMP** production (competition ?)
- increases its trafficking to the EE
- and induces a **default in FSHR recycling**
- potentially because it impairs **APPL1 phosphorylation**

Several questions still need to be addressed :

- Do FSHR and iPRC2 **colocalize** in the EE ?
- Is **FSHR trafficking** to the **VEE decreased ?**
- Is the **cAMP production** reduced by a **competition of iPRC2** with the **Gs protein ?**
- increases its trafficking to the EE
- Is FSHR interaction with **APPL1** impaired by iPRC2 ?

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