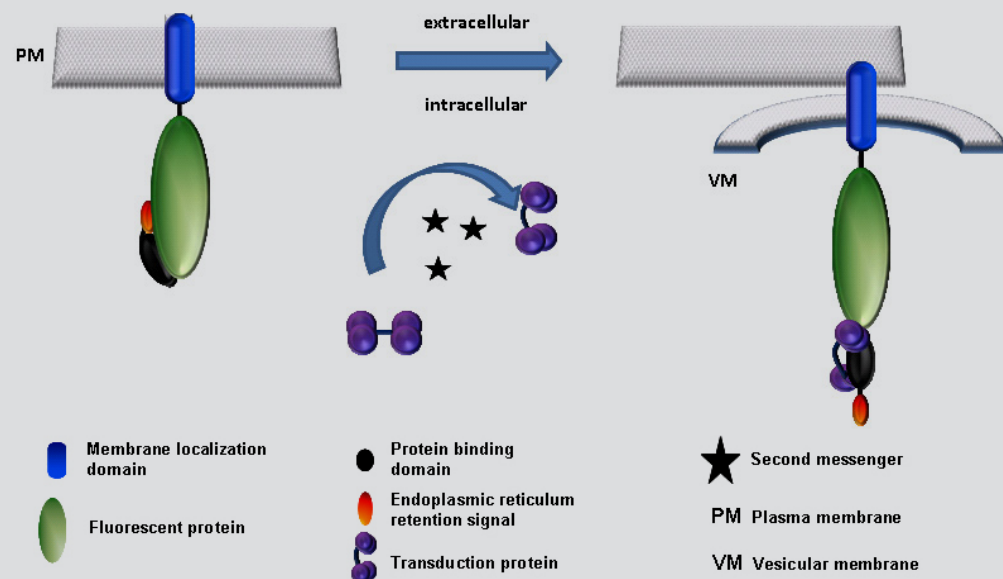
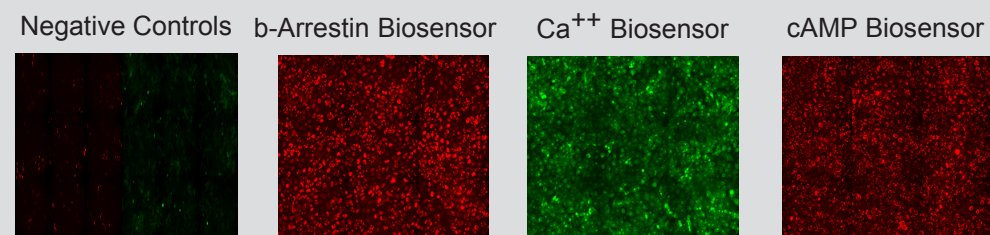
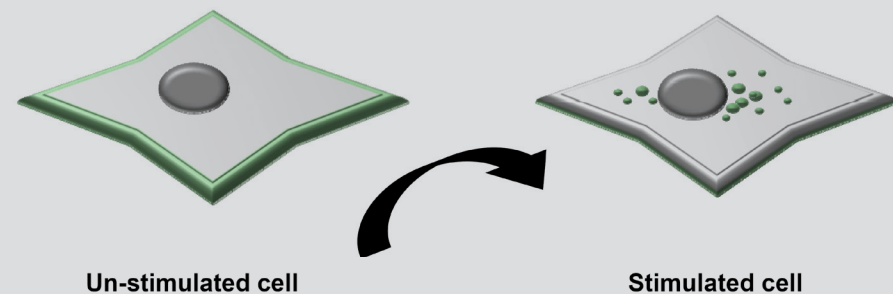


## Schematic representation about the biosensors operating



Biosensors comprise a membrane localization peptide, a second messenger transduction protein binding peptide, a reticulum retention signal and a fluorescent peptide. When a receptor is activated, second messengers involved in the GPCR pathway vary their concentration. This induces a change in the biosensor localization.

Fluorescence distribution within the cell



**Innoprot**  
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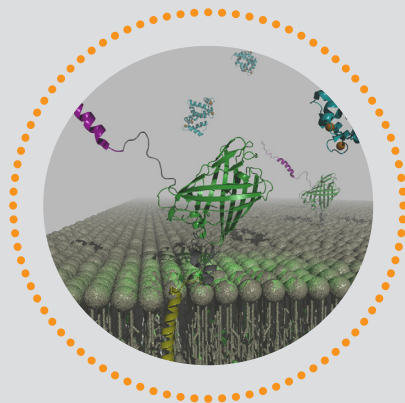
NOMAD™  
BIOSENSORS  
TECHNOLOGY

THE NEW  
GENERATION OF  
BIOSENSORS  
FOR HCS

**Innoprot**  
Speeding up drug discovery

# NEW GENERATION OF BIOSENSORS FOR HCS

Innoprot has developed a novel family of biosensors for measuring GPCR activity in living cells using the same fluorescent backbone. Each biosensor of the family co-expressed in a cell line with a GPCR, provides an innovative and sensitive research tool for studying the molecular mechanism and kinetics of GPCR activation. Nomad biosensors enable the measurement of second messenger concentration changes involved in GPCR activation in combination with b-arrestin recruitment. An activation of the GPCR leads to a change in the structural folding of Nomad biosensor that promotes its cellular relocation. The molecular structure of Nomad biosensors comprises: membrane localization peptide, second messenger transduction protein binding peptide, reticulum retention signal and a fluorescent peptide. The second messenger transduction protein binding peptide could be replaced depending on the second messenger involved in the GPCR activation pathway, resulting 4 different versions of Nomad biosensors: cAMP, Ca<sup>++</sup>, DAG & b-arrestin



## Advantages of Nomad™ Biosensors

- Different second messengers application
- Assays in living cells to study GPCR kinetics
- High sensitivity in robust assays
- Economic assay for cell processing and data analysis without any additional reagent
- b-arrestin recruitment determination combined with second messenger activation pathway
- Stable expression of the biosensor
- Non-tagged GPCRs

## Applications

- High Content Screening for GPCR activity in living cells (b-arrestin & second messenger)
- Live cell imaging to follow cellular effect kinetics of GPCR activation
- Ideal for high throughput screens based in fluorescence

## Proof of Concept

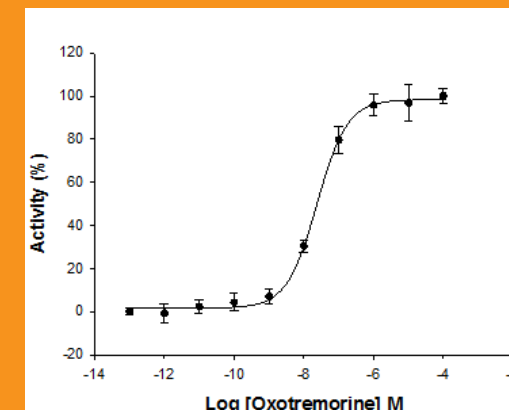
Nomad Biosensors have been validated by co-expressing them with several GPCRs in U2OS cell line. Upon receptor activation using their respective agonists, the activity was easily quantified by both fluorescence intensity and image analysis of cytoplasmic granularity changes following their corresponding second messenger increase. Nomad biosensors also provided a sensitive method for high throughput screening of drug libraries to identify compounds that modulate GPCR or any receptor that induces changes in second messengers levels.

## EXAMPLES

1

### Ca<sup>++</sup> Nomad biosensor:

Measurement of calcium in living cells within a broad dynamic range of physiological concentrations of this second messenger.

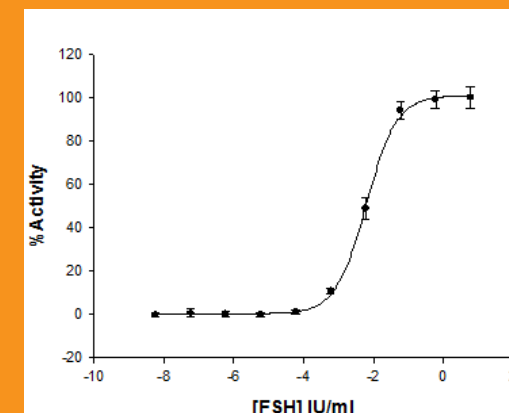


**Concentration response curve for Oxotremorine in M5 cell line cotransfected with Ca<sup>++</sup> Nomad Biosensor.** Cells were treated with 10 log dilution series (n=6). The EC<sub>50</sub> was 2.36 x 10<sup>-8</sup> M after 24 h treatment with the agonist. The assay was validated for High Throughput Screening with an average of Z'=0.84+/- 0.01

2

### cAMP Nomad biosensor:

Measurement of cAMP in living cells within a broad dynamic range of physiological concentrations of this second messenger.

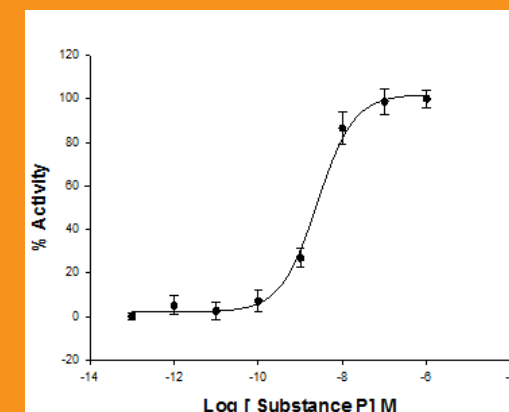


**Concentration response curve for FSH in FSHR cell line co-transfected with cAMP Nomad biosensor.** Cells were treated with 10 log dilution series (n=7). The EC<sub>50</sub> for isoproterenol was 6.15x10<sup>-3</sup> IU/ml after a 24h treatment with the agonist. The assay was validated for High Throughput Screening with an average of Z'=0.82+/- 0.01.

3

### Arrestin Nomad biosensor:

Accurate quantitative measurement of GPCR activation through b-arrestin recruitment in living cells.



**Concentration response curve for Substance P in a TACR1 cell line co-transfected with b-arrestin Nomad biosensor.** Cells were treated with 8 log dilution series (n= 6). The EC<sub>50</sub> for Substance P was 2.73x10<sup>-9</sup> M after a 24h treatment with the agonist. The assay was validated for High Throughput Screening with an average of Z'= 0.84+/- 0.01