# Development of conformational and tissue specific antibody against a GPCR as potential drug candidate in immuno-oncology

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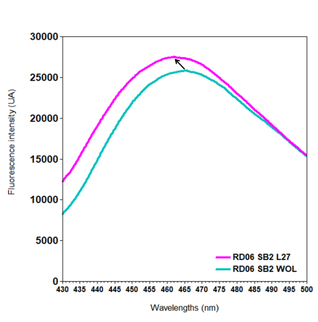
G protein-coupled receptors and more specifically chemokines receptors are widely implicated in several oncological indications. They can play a role in many processes such as tumor growth, angiogenesis, chemotaxis, and metastasis. Thus, they have great potential as drug targets in immuno-oncology.
Our project is to develop a tissue selective and conformational specific drug candidate against a chemokine receptor implicated in hepatocarcinoma. The GPCR of interest for this study is RD06 (anonymous name). RD06 is highly expressed in the liver and is known to be involved in several cancers, notably liver cancer, where it has already been considered a key factor in the progression of HCC.
To achieve this goal, the first step was to obtain the antigen of interest (RD06 stabilized in physio-pathological conditions) and use it for antibody discovery. G.CLIPS Biotech has developed an enabled technology to produce *in vitro* specific conformations and native lipid environments on purified membrane proteins. This innovative technology overcomes one of the problems encountered in the development of antibodies targeting membrane proteins. In this study, a combination of different propriety methods was used: (1) Purification of recombinant RD06 with G.mixes® in bicelles and verification of the conformations of the protein with G.Select®; (2) Reconstitution of RD06 in nanodiscs in a specific environment representing liver and lipids raft, and finally ;(3) Pharmacological profiling of benchmark ligand on the receptor in different environment and conformation; (4) Preliminary yeast display experiment on the antigens obtained above for antibody discovery.
Our data show that our technology can unlock selection of conformation/environment sensitive ligand/drug candidates. This finding pave the way towards de-risked candidates on efficiency/ toxicity balance and open opportunities towards development of innovative and selective therapies for unmet medical needs.

## 1- G. Select assay : structural and conformational stabilization of the membrane protein in different and specific environments with G.mixes

**G. Select assay: This assay uses proprietary mixes (G. mixes) to identify the best conditions for the receptor extraction, purification and structural/conformational stabilization.**

### G.CLIPS proprietary technology



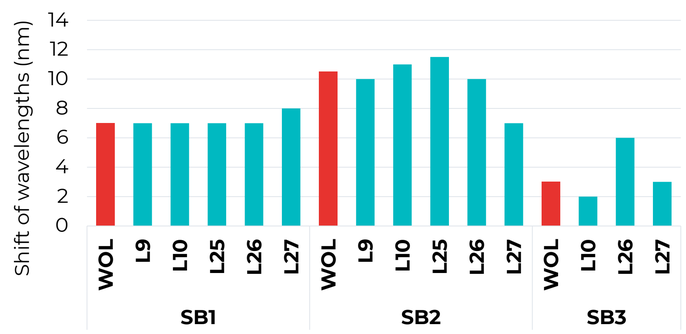

WOL

SB2 L27 mix

| Solubilization buffer | Features |
|---|---|
| **SB1** | Small micelles |
| **SB2** | Small micelles + ions bivalents |
| **SB3** | Large micelles |

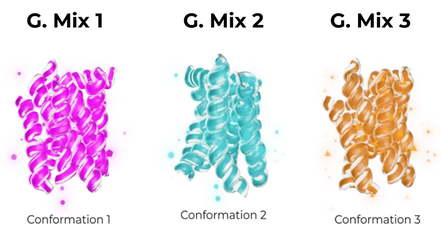
| Lipidic mix | Environnent |
|---|---|
| ***WOL*** | *Without lipids* |
| **L7** | Liver like |
| **L9** | Positively charged |
| **L10** | Macrophage like /DC |
| **L25** | Negatively charged |
| **L26** | Lung like |
| **L27** | Stomach like |

### G. mixes reflect physio-pathological environments.




Basal activity of RD06 in different G.mixes monitored using G.Validation assay (see above). Structural change upon RD06 activation/inactivation is translated by a fluorescence emission spectra change of the fluorophore based on the receptor.

G. Mix 1 | G. Mix 2 | G. Mix 3

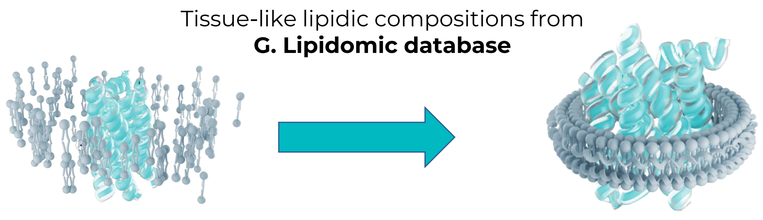



**A-** The importance of ions can be seen in the figure above. Indeed, the variation in maximum wavelength (nm) depending on the buffer in which the protein is present reflects more or less active conformations of RD06.
**B-** Lipids associated with buffers (G. mixes) may have no impact on RD06 (e.g. lipids in the presence of SB1) or may influence receptor conformation.
Maximum wavelength shifts are observed to a greater or lesser extent depending on the involvement of G.mixes.

**The selection of the right G.mix is crucial for the receptor purification, stabilization and basal activation state.**

## 2- Reconstitution of the receptor in lipidic Bilayer mimicking Liver lipid composition as characterized in G. Lipidomic database

Tissue-like lipidic compositions from **G. Lipidomic database**

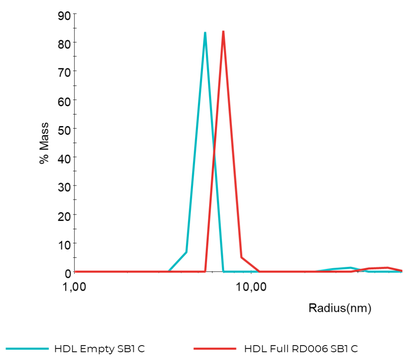


**RD06 was reconstituted in HDL particles of G. Mix SB1 L7/C.**

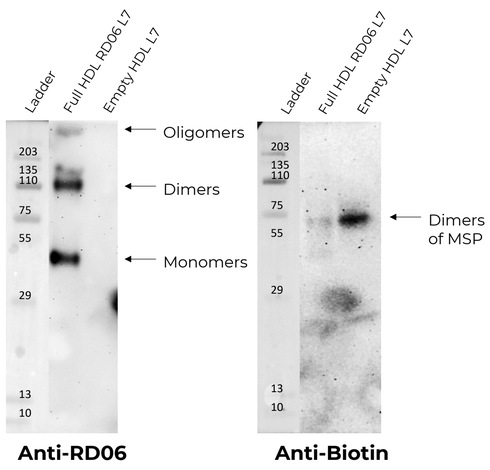
Empty HDL particles were also reconstituted in the same G.mix for comparison and as a negative control.

### A- Nanodiscs homogeneous and with consistent sizes.

**A-** To verify the correct reconstitution of GPCR in the nanodiscs, a Dynamic Light Scattering (DLS) is used to check the homogeneity of the solution and the size of the nanodiscs. For RD06, DLS shows homogeneous solutions. In the same way, full HDL containing the protein are larger than empty HDL.




### B- HDL full contains GPCR RD06.



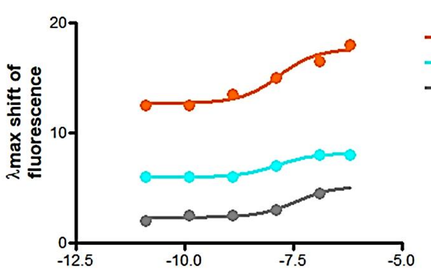

**B-** Western blots were performed using the antibody against RD06 and determined the efficiency of the receptor integration in nanodiscs (HDL) full and not in the empty. We observed monomers, dimers and even oligomers of the receptor in HDL full.
Anti-biotin enables us to recognize biotinylated MSP. MSP dimers are recognized in both full and empty HDL, as they are present in both cases for reconstitution.

**RD06 is reconstituted in nanodiscs mimicking a physio-pathological membrane bilayer and with G. mix will be used as antigen for antibody discovery.**

## 3- Pharmacological profiling of benchmark ligand on the receptor in different environment and conformation

**Dose response via G. Validation assay**
Concentrations of natural ligand : 12,5 pM to 625 nM

| Lipidic mix | Environment |
|---|---|
| C | Liver like |
| H | Macrophage like DC |
| Q | Lipids rafts like |

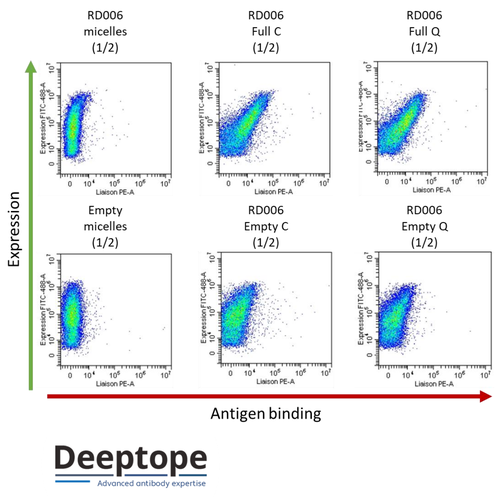



The basal state differs according to the stabilized RD06 conformation. The physiological environment close to the liver stabilizes a more active protein than other environments.

| G.Mix | EC50 (nM) | BMAX (nm) | KD (pM) |
|---|---|---|---|
| RD06 SB1 C | 14,92 | 18 | 6,12 |
| RD06 SB1 H | 13,52 | 8 | 5,41 |
| RD06 SB1 Q | 35,85 | 3,12 | 7,65 |

The natural ligand has better affinity, efficacy and efficiency when RD06 is stabilized by **G.Mix liver like** than in **lipid rafts like**. This is consistent with previous results.
Moreover, the conformation of RD06 in a **macrophage environment** seems to combine both high efficiency and high efficacy. In this condition, the ligand has a great affinity for RD06.

### Preliminary yeast surface display experiences confirm G.Validation assay results



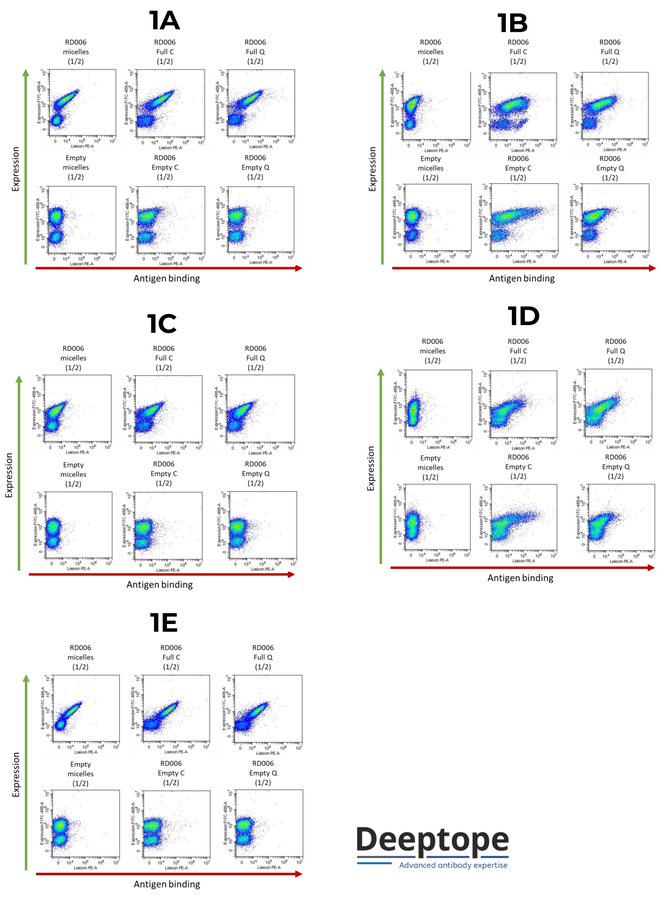

The natural ligand **recognizes RD006** in micelles compared with empty micelles. The ligand also **strongly recognizes the protein** in nanodiscs with two different types of surrounding lipids, in contrast to empty nanodiscs.

The positive control is therefore validated.

**Stabilization of different and specific conformations of RD06 depending on the G.mixes used allow for a change of affinity even on the natural ligand.**

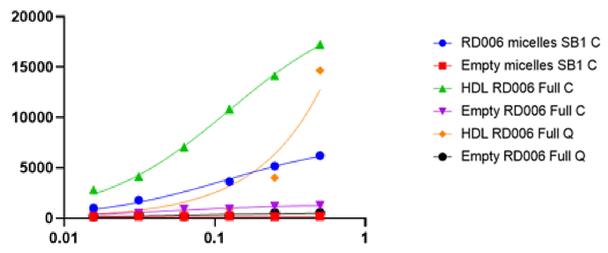
## 4- Preliminary yeast display experiment on the antigen stabilized in different formats and environments for antibody discovery

### G.CLIPS stabilizes specific conformations of RD006 via micelles or nanodiscs, permitting the study of different behaviors of the antibodies tested.




Example of one of the antibodies tested:

**1A**




For some antibodies tested (1A, 1C and 1E), both formats of the RD006 antigen (micelles and nanodiscs) are **strongly binding**. We therefore have **clearly specific binding of antibodies** on micelles and HDL with no binding on the control empty ones.

**Our unique technology enable the selection of conformational and environment sensitive antibodies.**

G.mixes enable *in vitro* stabilization of target membrane proteins such as RD06 in specific conformations to a given environment, reflecting a particular pathophysiological state. These mixes were designed to stabilize the RD06 protein in a more or less active at basal, ligand-free state. The nanodiscs enable the GPCR to be reconstituted in a membrane bilayer even closer to its native conformation. We were able to show that environment play a role in pharmacological behavior of the target and candidates. Both G.Validation assay and yeast display experiment confirmed the power of our technology in selecting pathology specific (conformational and environment) drug candidates. Theses finding pave the way towards new innovative drugs and mechanism of function in immuno-oncology to answer patient's unmet medical needs.

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