Fidabio
Mobilize your complex biology

Ligand-binding characterization of Nanodisc-embedded GPCR (β2AR).

Key Fidabio benefits

- Detailed in-solution characterization of receptor-ligand interaction
  - *Binding affinity (Kd)*
  - *Nanodisc sizing and quality check*
- Native conditions and ultra-low sample volume
- Built in quality control - oligomerization and aggregation check

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Introduction

G protein-coupled receptors (GPCRs) are the largest family of membrane proteins and they are involved in many vital physiological cell mechanisms, amongst others, hormones signaling, cell cycle regulation, neurotransmitters signaling and smell receptors. GPCRs-targeting drugs represent 27% of the global commercial drugs. Often, the complexity of GPCR presents challenges to current technologies. It does require new technologies to advance discoveries and treatments for GPCR related disorders. This work demonstrates that Flow-Induced Dispersion Analysis (FIDA) can be used to fully characterize the binding between Nanodisc-embedded β2-adrenergic receptor and Nanobody (Nb80), probe the active or inactive state and potential oligomerization.

Fidabio is applying a new capillary-based technology for measuring binding affinity through absolute and accurate size determinations of analytes in a pressure-driven flow system. In this study the change in apparent size of Nb80 forms the basis for accurate assessments of the functionality of GPCRs.

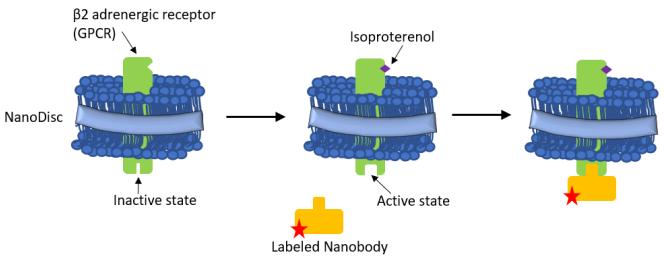

Figure 1. Schematic representation of the system analyzed in this work, composed of β2-adrenergic receptor (in green) embedded in a NanoDisc and Nanobody Nb80 binding into the G-protein binding pocket.

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Materials & Methods

Fida 1 instrument with 480 nm LED fluorescence detection for binding experiments (Fidabio ApS). Fidabio standard capillary (i.d.: 75 µm, LT: 100 cm, Leff: 84 cm). HEPES buffer 10mM pH 7.4, 0.5 mg/ml BSA was used as the working buffer. Nanobody Nb80 was used as the indicator. Nb80 was labeled with an Alexa Fluor® 488 Protein Labeling Kit from ThermoFisher Scientific. NanoDisc-embedded β2-adrenergic receptor (β2AR) was used as the analyte (0-1 µM). Sample analysis was performed by filling the capillary with the analyte, followed by an injection of 39 nL of preincubated indicator+analyte, which was mobilized towards the detector at 400 mbar.

Results

Nb80 binds to the active GPCR.

Fidabio provides an absolute measurement of hydrodynamic radius (Rh). It was used to measure the size change of Alexa488-labeled Nb80 upon increasing concentration of Nanodisc-embedded β2AR (ND-β2AR). The change in apparent Rh of Nb80 was plotted as a function of increasing ND-β2AR concentration (0-1 µM) in presence of isoproterenol 10µM, an agonist that binds to the extracellular side and turns the GPCR into the active state (Figure 1, green line). The Rh of Nb80 without ND-β2AR was 1,76 nm, and upon addition of ND-β2AR a size increase up to 5.25 nm was detected, clearly indicating a binding.

The Rh of 5.25 nm fits very well the average size of these NanoDiscs which are around 10nm in diameter.
To demonstrate the specificity of the binding, we ran two additional controls. The first one was in the presence of an antagonist, alprenolol (APNL) 10µM, which turned the receptor into the inactive state. The second control was run without agonist or antagonist, so the receptor was mostly inactive.
With alprenolol and without any drug, the Rh of Nb80 in the presence of high GPCR concentration was 3.6 nm (orange dot) and 3.4 nm (blu dot) respectively. This demonstrates significantly reduced binding ability of the inactive GPCR.

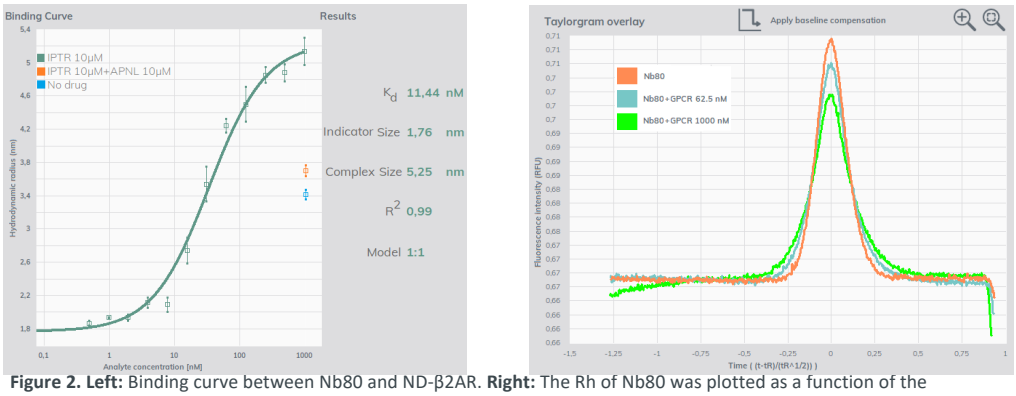

Figure 2. Left: Binding curve between Nb80 and ND-β2AR. **Right:** The Rh of Nb80 was plotted as a function of the concentration of ND-β2AR. Raw data showing the indicator peak getting wider as the concentration of ND-β2AR increased, due to increase in apparent size upon binding.

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Conclusion

The data presented show how functional characterization of proteins embedded in Nanodiscs can be performed in-solution and non-invasively using FIDA-technology. Besides receptor binding, FIDA technology also provides the possibility of assessing dimerization and oligomerization of GPCRs, and it can work with any scaffold up to 500 nm radius, e.g. proteoliposomes.

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