

# Detection of Ligand-Dependent Interaction of Nuclear Receptors with their Cofactor Using the Corning<sup>®</sup> Epic<sup>®</sup> System

Ingo Kober<sup>1</sup>, L. Jack Fang<sup>2</sup>, Johannes Gleitz<sup>1</sup>, and Anthony G. Frutos<sup>2</sup>

<sup>1</sup>Merck Serono, Darmstadt, Germany

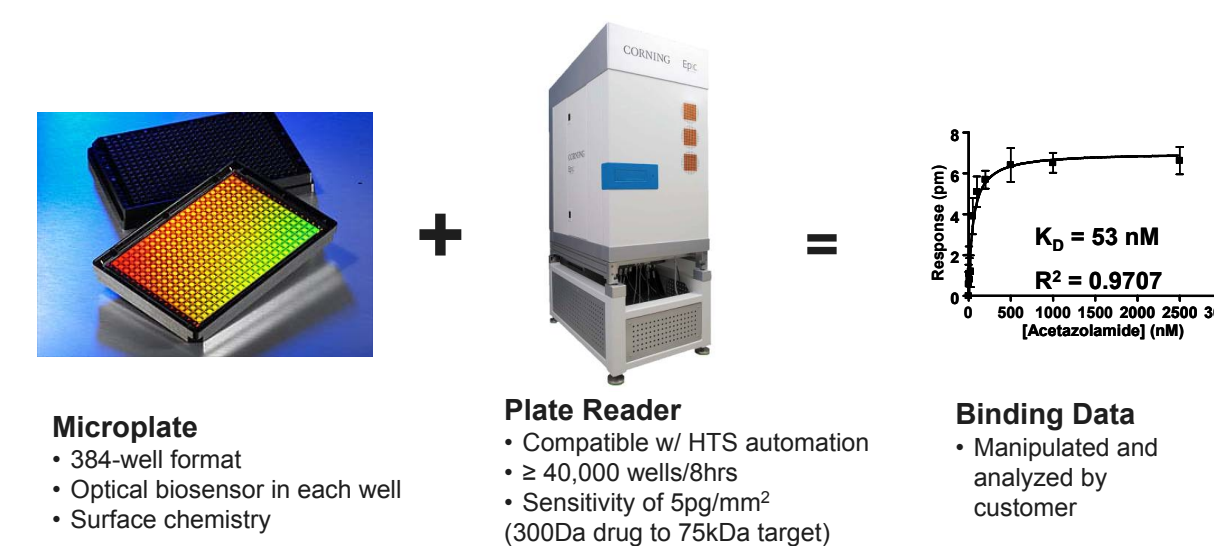
<sup>2</sup>Corning Incorporated, Life Sciences, One Science Drive, Corning, NY 14831

## Abstract

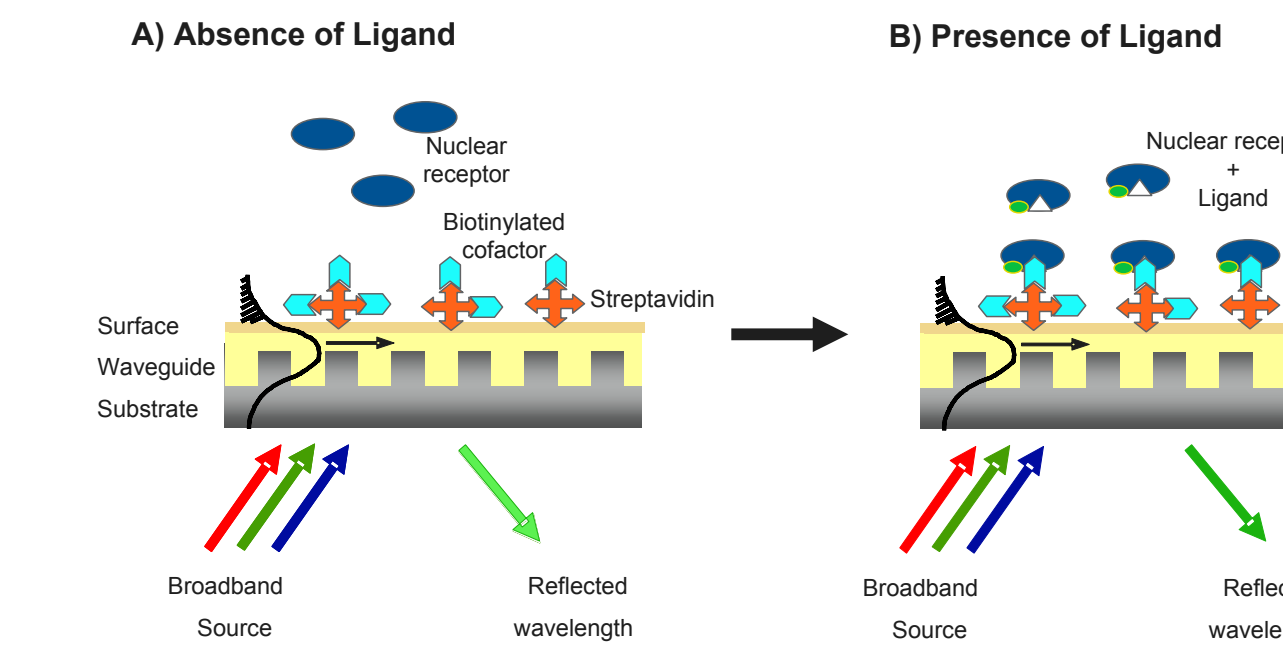
Realization of the complexity of G protein coupled receptor (GPCR) signaling and ligand-directed functional selectivity demands high resolution tools for studying GPCR behavior. Given its well-characterized agonist binding site and the availability of a wealth of structurally related ligands with functionally diverse properties,  $\beta_2$ -adrenergic receptor ( $\beta_2$ AR) has been used as an excellent model system for studying the mechanism of GPCR activation and signaling. Here we use non-invasive resonant waveguide grating (RWG) biosensor to systematically examine the kinetic profiles of human epidermoid carcinoma A431 cells in response to stimulation with diverse arrays of  $\beta_2$ AR ligands. The RWG biosensor offers an integrated signal, termed as dynamic mass redistribution (DMR) signal, which is in close proximity to a global representation of GPCR signaling in native cells. Multi-parameter analysis of agonist-induced DMR signals indicates the presence of multiple ligand-specific states of the  $\beta_2$ AR. The implications of DMR signals for screening biased ligands are discussed.

## Corning<sup>®</sup> Epic<sup>®</sup> System Concept

The Corning Epic System is a high-throughput, label-free detection platform that consists of SBS-standard 384-well microplates with optical sensors inside each well, an HTS-compatible microplate reader and a set of label-independent assay protocols. The Epic System is applicable to both biochemical and cell-based assays, and enables high-throughput screening of "intractable" targets.



## Ligand-dependent Interaction Between Nuclear Receptor and its Cofactor on Epic<sup>®</sup> System



## Materials and Methods

### Materials:

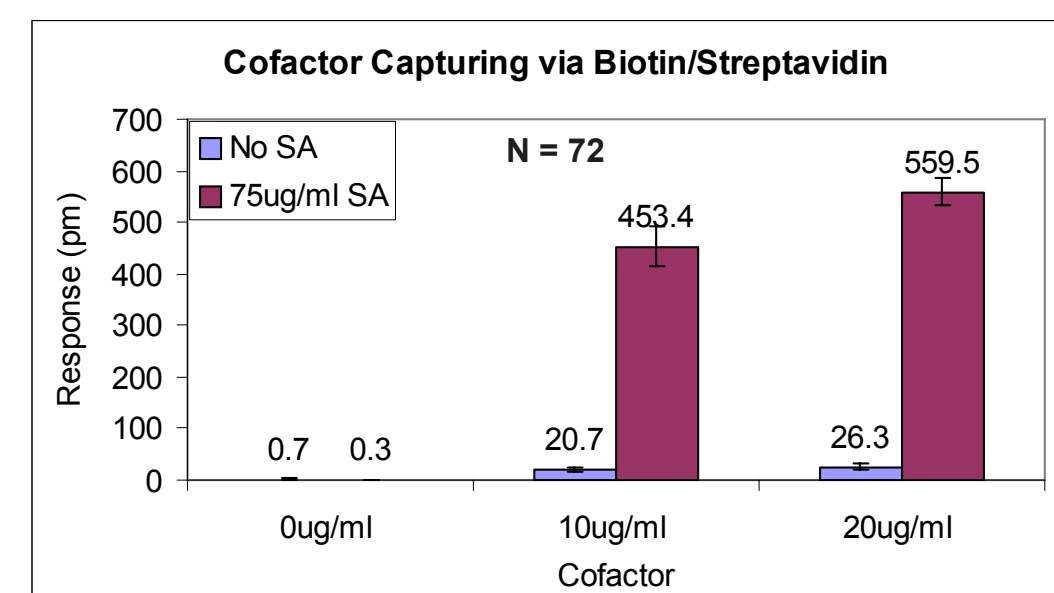
- NR1 (nuclear transport receptor 1): MW=59.6kDa, pI=6.05
- NR2 (nuclear transport receptor 2): MW=55.4kDa, pI=5.89
- Cofactor NR1/2: MW=3,026Da, biotinylated
- Ligand 1: ~200ul of 5mg/ml (~10mM), MW=481Da in 100%DMSO
- Ligand 2: ~1000ul of 10mM, MW=393Da in 100%DMSO

### Methods:

- Immobilizing streptavidin on a Epic<sup>®</sup> plate at room temperature for 1 hour
- Capturing the cofactor via biotin/streptavidin
- Reading the baseline on the Epic system
- Binding of nuclear receptors in the presence or absence of a ligand
- Reading the response on the Epic system

## Cofactor Capturing via Biotin/Streptavidin

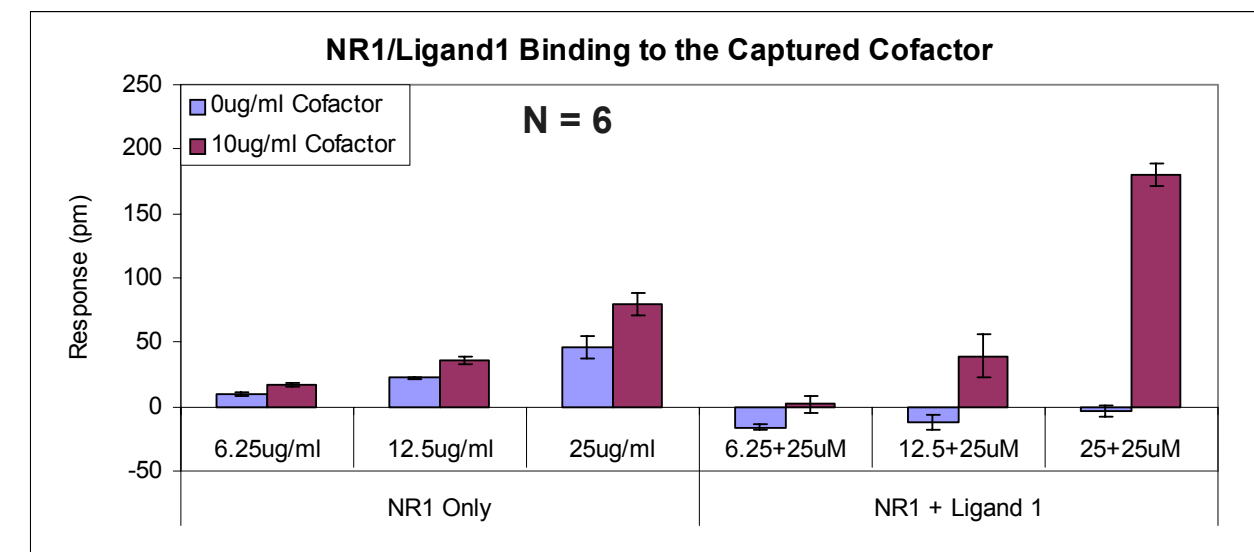
- Streptavidin (SA) was immobilized in the wells of an Epic<sup>®</sup> plate
- Different amount of the cofactor was captured in PBS for 1 hour at RT



Conclusions: Good levels of cofactor were captured via biotin/streptavidin at different concentrations.

## Ligand-Dependent Nuclear Receptor 1 (NR1) Binding

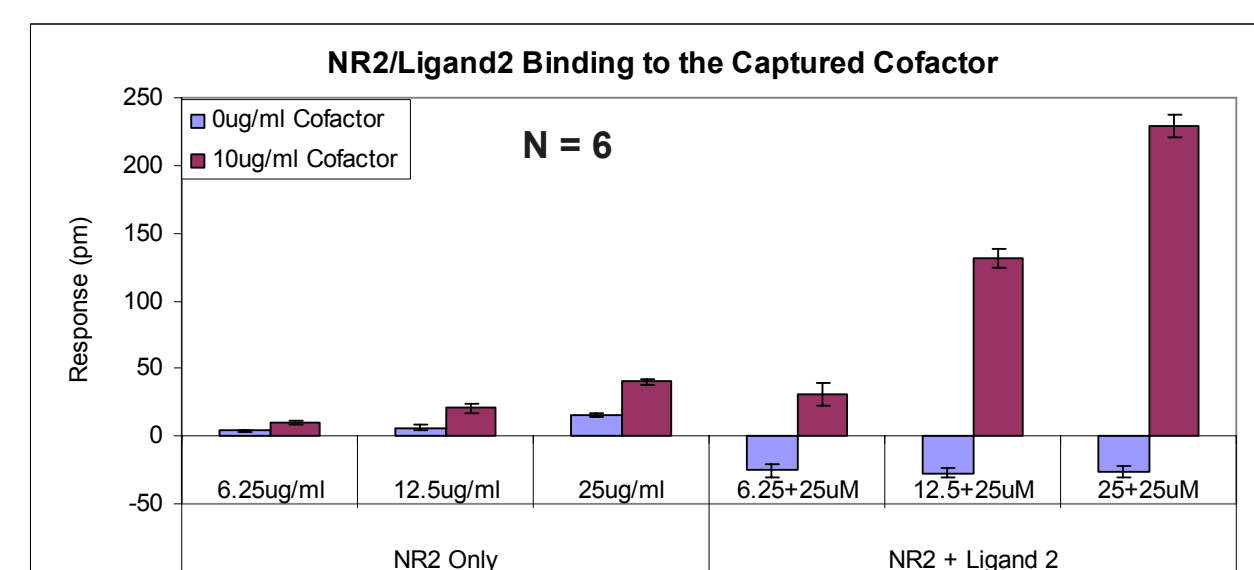
- Cofactor was captured via biotin/streptavidin
- Different amount of NR1 was added in the presence or absence of 25uM Ligand 1



Conclusions: Dose-dependent binding of NR1 observed. Cofactor-dependent binding of NR1 observed in the presence of Ligand 1. Some non-specific binding of NR1 observed only in the absence of Ligand 1.

## Ligand-Dependent Nuclear Receptor 2 (NR2) Binding

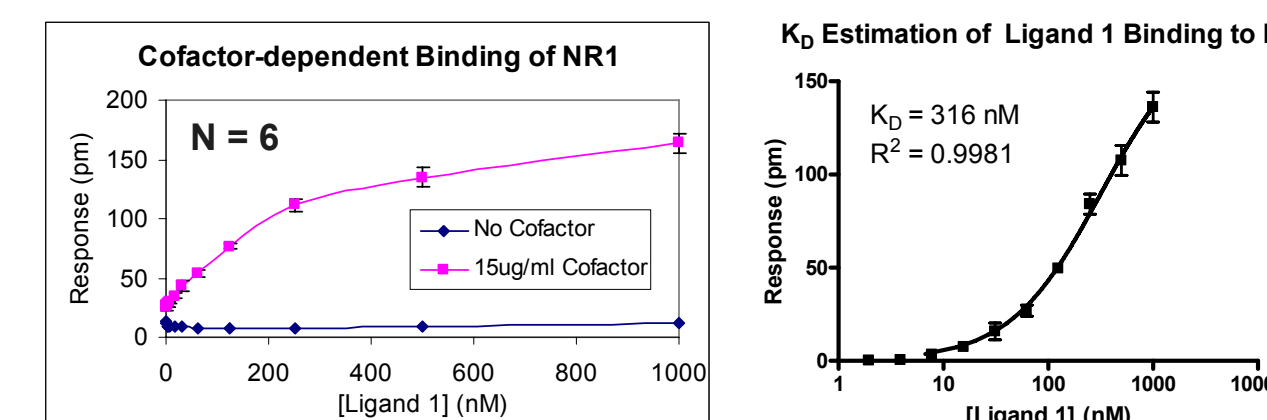
- Cofactor was captured via biotin/streptavidin
- Different amount of NR2 was added in the presence or absence of 25uM Ligand 2



Conclusions: Dose- and cofactor-dependent binding of NR2 observed.

## K<sub>D</sub> Estimation of Ligand 1 Binding to NR1

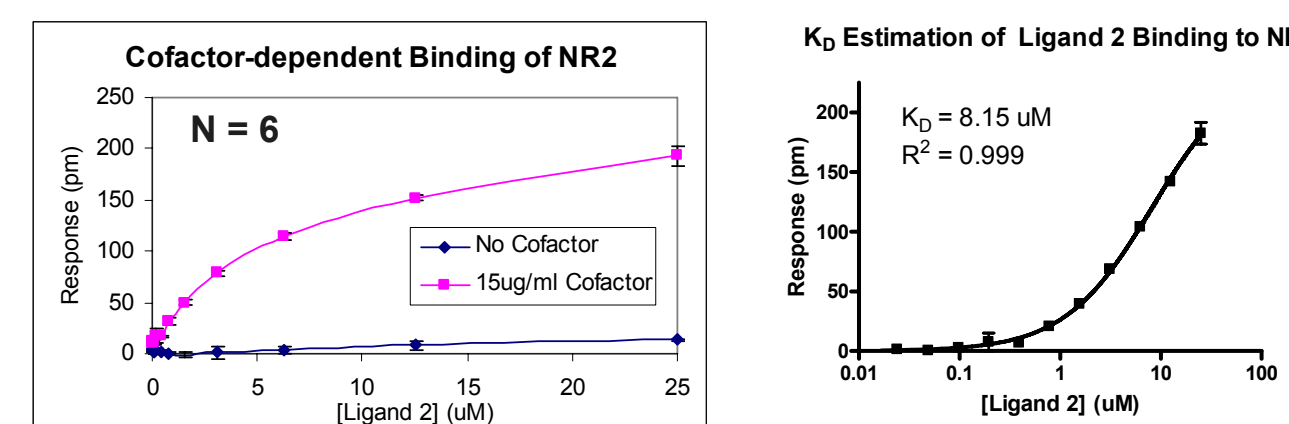
- 15ug/ml cofactor was captured via biotin/streptavidin
- Different amounts of Ligand 1 were mixed with 25ug/ml NR1
- The mixture was added to wells with captured cofactor and the response was measured on Epic<sup>®</sup> System



Conclusions: Cofactor- and Ligand 1-dependent binding of NR1 observed. The estimated K<sub>D</sub> of Ligand1 binding to NR1 is 316nM.

## K<sub>D</sub> Estimation of Ligand 2 Binding to NR2

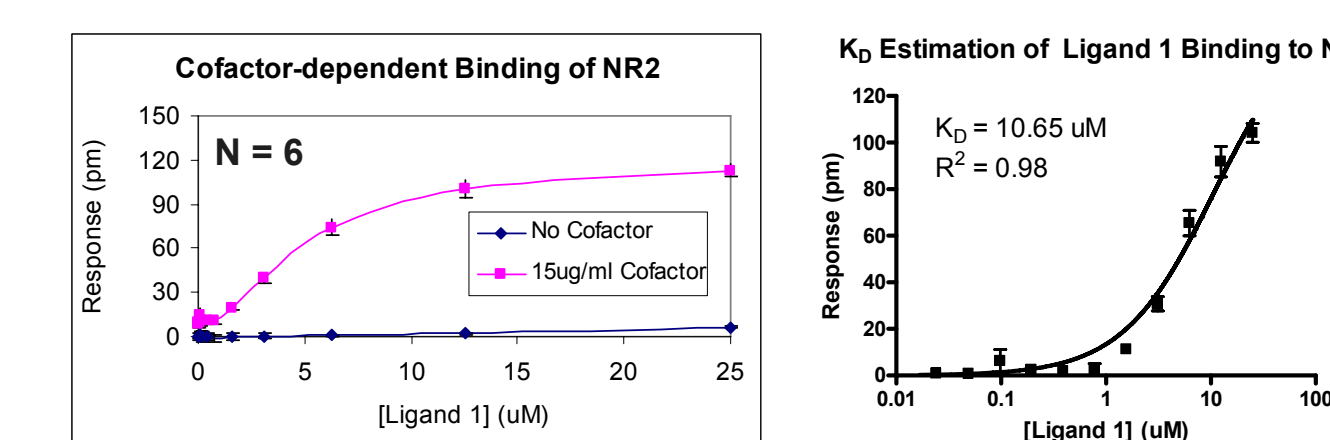
- 15ug/ml cofactor was captured via biotin/streptavidin
- Different amounts of Ligand 2 were mixed with 25ug/ml NR2
- The mixture was added to wells with captured cofactor and the response was measured on Epic<sup>®</sup> System



Conclusions: Cofactor- and Ligand 2-dependent binding of NR2 observed. The estimated K<sub>D</sub> of Ligand 2 binding to NR2 is 8uM.

## K<sub>D</sub> Estimation of Ligand 1 Binding to NR2

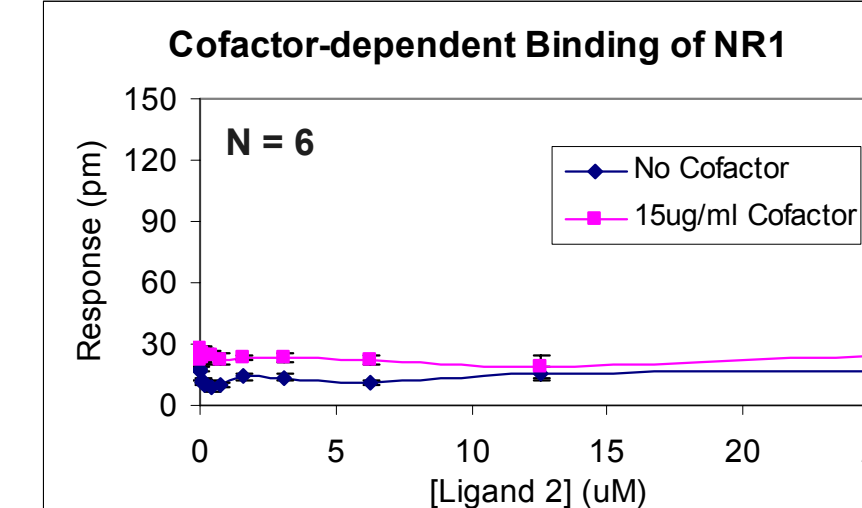
- 15ug/ml cofactor was captured via biotin/streptavidin
- Different amounts of Ligand 1 were mixed with 25ug/ml NR2
- The mixture was added to wells with captured cofactor and the response was measured on Epic<sup>®</sup> System



Conclusions: Cofactor- and Ligand1-dependent binding of NR2 observed. The estimated K<sub>D</sub> of Ligand1 binding to NR2 is 11uM.

## K<sub>D</sub> Estimation of Ligand 2 Binding to NR1

- 15ug/ml cofactor was captured via biotin/streptavidin
- Different amounts of Ligand 2 were mixed with 25ug/ml NR1
- The mixture was added to wells with captured cofactor and the response was measured on Epic<sup>®</sup> System



Conclusions: No apparent binding of Ligand 2 to NR1 observed

## Comparison of K<sub>D</sub> Estimations

	Ligand 1 to NR1	Ligand 1 to NR2	Ligand 2 to NR2	Ligand 2 to NR1
Epic <sup>®</sup> K <sub>D</sub>	316nM	10.7μM	8.15μM	No Binding
HTRFK <sub>D</sub> <sup>+</sup>	~100nM	~10μM	3-9μM	No Binding

\* Merck Serono internal homogenous time-resolved fluorescence (HTRF) assay results or the published data

## Conclusions

- The cofactor was successfully immobilized via biotin/streptavidin capture.
- Specific, ligand-dependent binding of NR1 and NR2 to the captured cofactor was observed
- Estimates of affinity between the receptors and the ligands were made
  - K<sub>D</sub> of Ligand 1 binding to NR1 is ~316 nM
  - K<sub>D</sub> of Ligand 1 binding to NR2 is ~11 μM
  - K<sub>D</sub> of Ligand 2 binding to NR2 is ~8 μM
  - No binding of Ligand 2 binding to NR1 observed
- K<sub>D</sub> estimations on Epic<sup>®</sup> System were consistent with functional assay results