

# CORNING

The logo for Epic system, featuring the word "Epic" in a large, black, sans-serif font with a registered trademark symbol (®) to its upper right. The word "system" is written in a smaller, black, lowercase sans-serif font directly below "Epic". The letter "i" in "Epic" has a small orange dot above it. A thin vertical orange line is positioned to the left of the logo.

Epic<sup>®</sup>  
system

## **Evaluation of the Corning<sup>®</sup> Epic<sup>®</sup> System for Detection and Validation of Small-Molecule/Protein Interactions**

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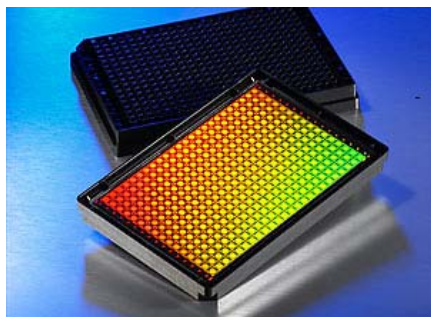
## Abstract

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We evaluated the Corning® Epic® System, a label-free, high-throughput detection technology, for detection and validation of interactions between small-molecules and two different protein targets, a nuclear receptor and a biotinylated protease. The nuclear receptor was directly immobilized via amine coupling and the protease was immobilized via biotin-streptavidin capture in a 384-well Epic microplate. A variety of Novartis AG compounds were tested against both protein targets to determine yes/no binding and to estimate the binding affinity ( $K_D$ ). The results presented here demonstrate that the Epic System is able to not only detect the direct interactions and rank relative binding affinities of small-molecule compounds against protein targets but also differentiate promiscuous aggregates from true binders. With relatively short assay development time--approximately two weeks--the Corning Epic System provides a highly efficient and economic solution for secondary and primary drug screening applications.

# Corning® Epic® System

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.



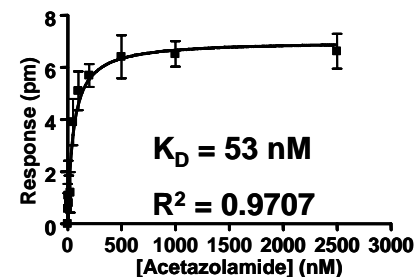
## Microplate

- 384-well format
- Optical biosensor in each well
- Surface chemistry



## Plate Reader

- Compatible w/ HTS automation
- $\geq 40,000$  wells/8hrs
- Sensitivity of  $5\text{pg}/\text{mm}^2$   
(300Da drug to 75kDa target)



## Binding Data

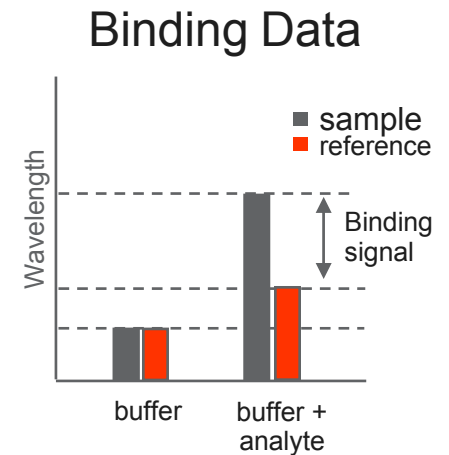
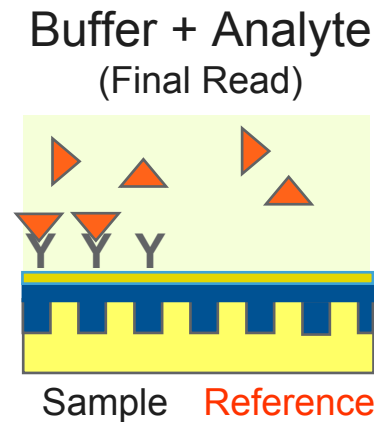
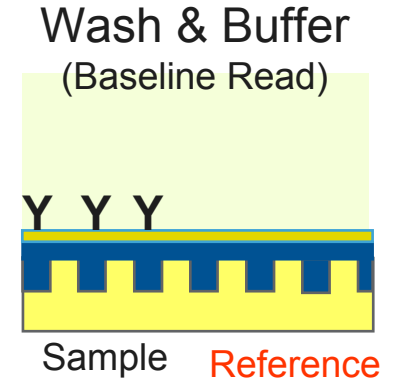
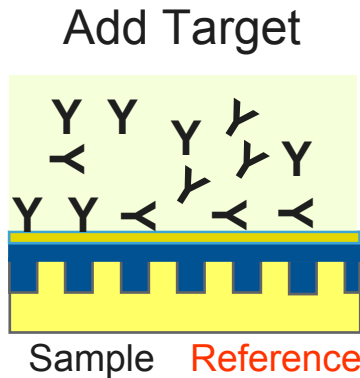
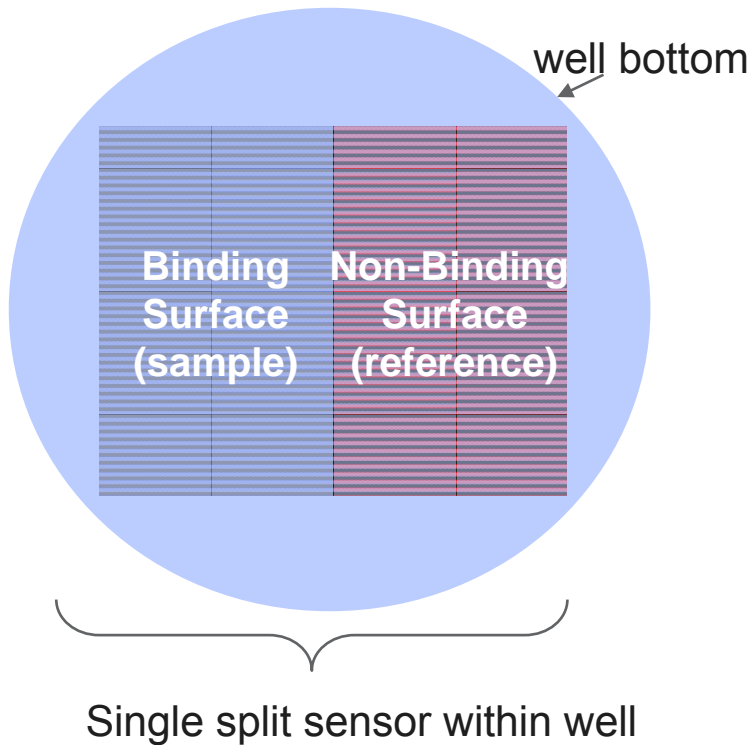
- Manipulated and analyzed by customer

## Assay Systems and Objectives

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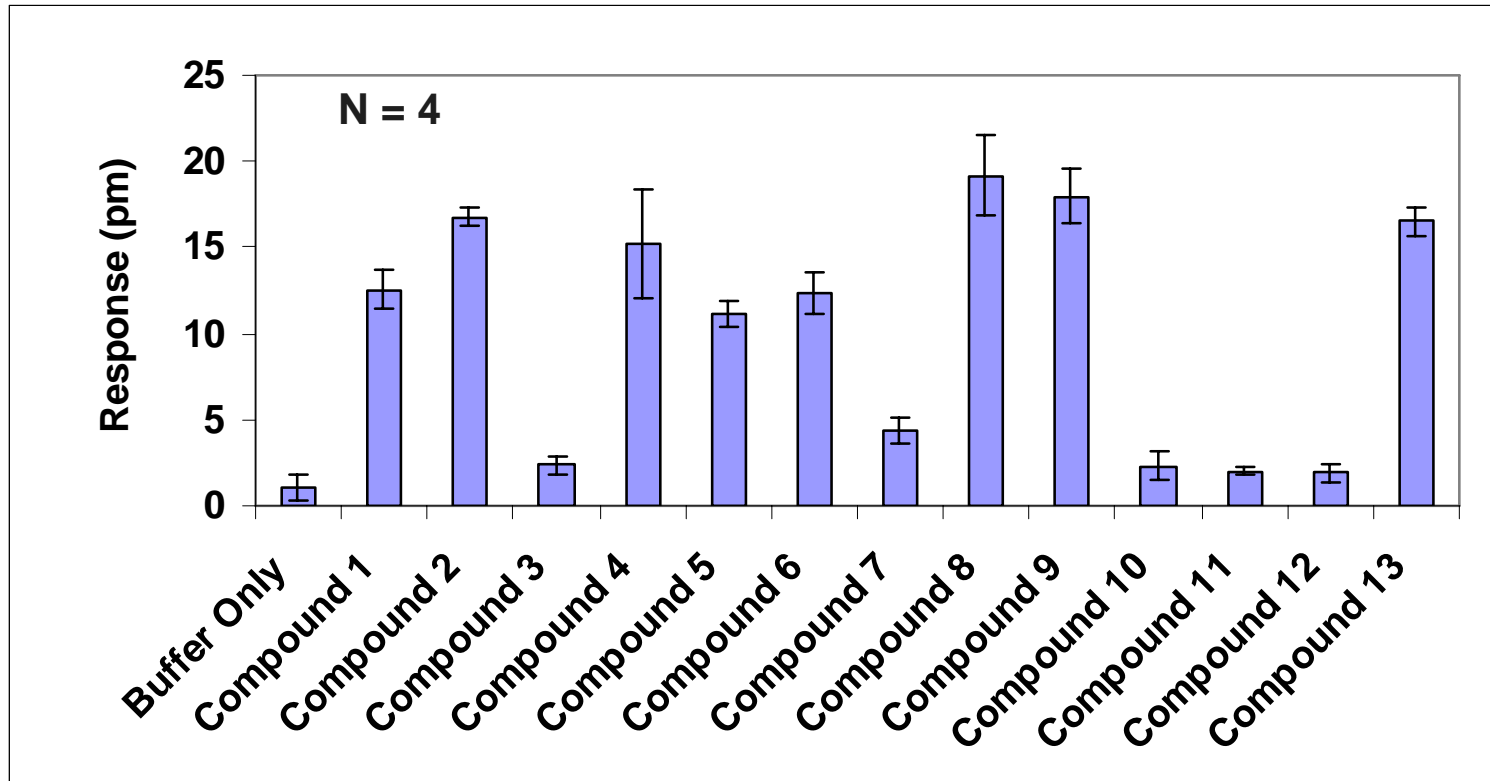
- Two assay systems were tested:
  - The binding of small-molecules to a nuclear receptor (~30kDa)
  - The binding of small-molecules to a biotinylated protease (~23kDa).
- Objectives:
  - Demonstrate the ability of the Epic<sup>®</sup> System for “Yes/No” binding of small-molecule compounds to both targets.
  - Demonstrate the ability of the Epic System for rough  $K_D$  estimation of small-molecule compound binding.
  - Test the ability of the Epic System for identification of promiscuous compounds.

# Dual-Sensor Self-Referencing Technology



# Mini-screen of 13 Compounds Against a Nuclear Receptor

- Nuclear receptor was immobilized at 50  $\mu\text{g/mL}$  in Na-Citrate pH 5/1mM DTT/0.02% CHAPS for 1 hour at room temperature
- Inhibitor (5 $\mu\text{M}$ ) binding assay performed in PBS with 0.1% DMSO/1mM DTT/0.02% CHAPS



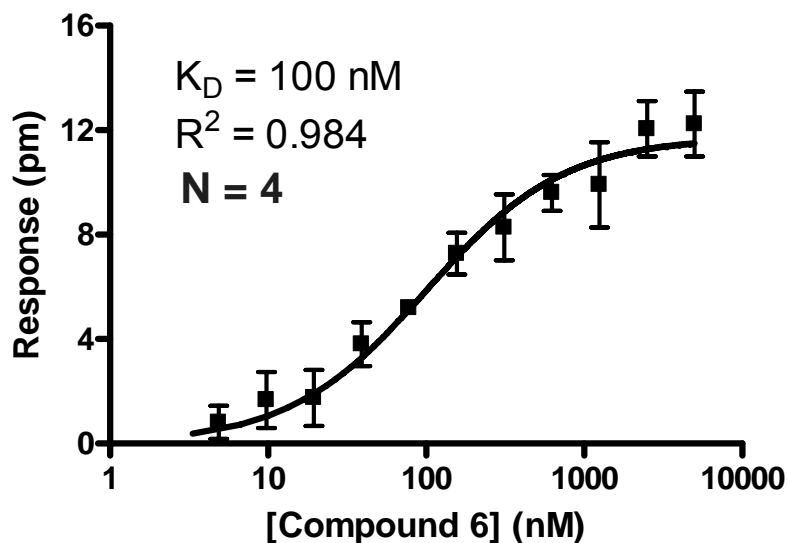
## Conclusions:

Eight compounds show binding to the nuclear receptor, consistent with Novartis internal data.

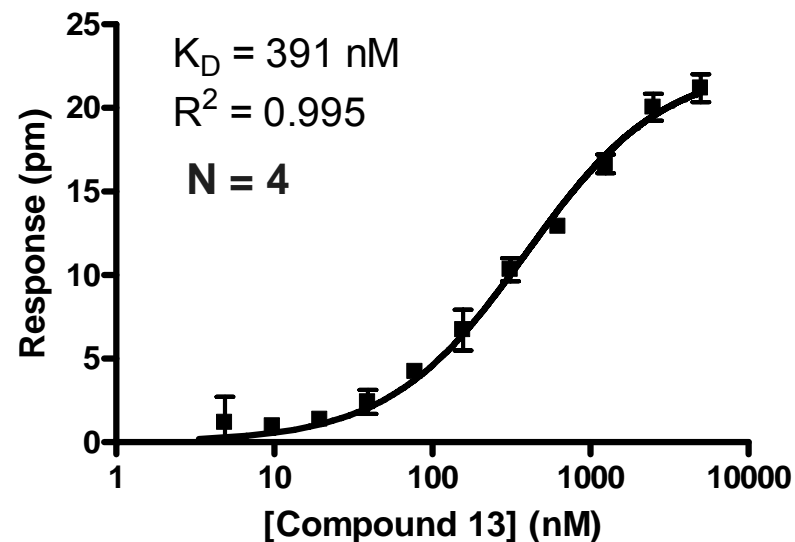
# $K_D$ Estimation of Compound 6 and 13 Binding to a Nuclear Receptor

- Nuclear receptor was immobilized at 50  $\mu\text{g/mL}$  in Na-Citrate pH 5/1mM DTT/0.02% CHAPS for 1 hour at room temperature
- Inhibitor titration performed in PBS with 0.1% DMSO/1mM DTT/0.02% CHAPS

TR-FRET:  $EC_{50} = 50 \text{ nM}$



TR-FRET:  $EC_{50} = 700 \text{ nM}$



## Conclusions:

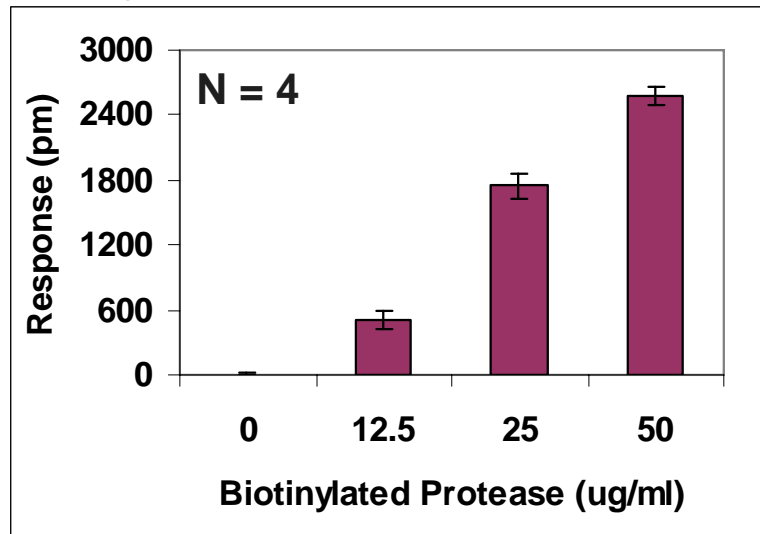
Dose-dependent binding was observed for both Compound 6 (~160 Da) and 13 (~330 Da).

$K_D$  estimations of both compounds were consistent with Novartis internal data.

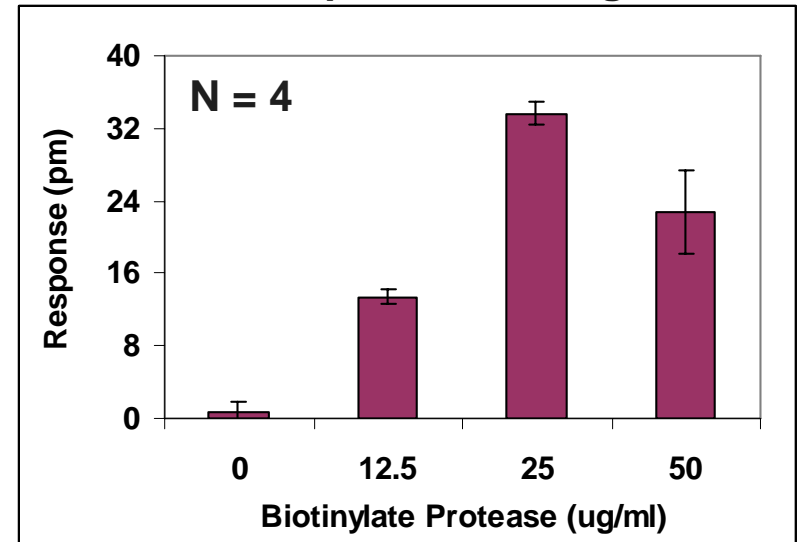
# Capture and Inhibitor Binding of a Biotinylated Protease

- Streptavidin was immobilized at 50  $\mu\text{g/mL}$  in 20 mM Na-Acetate pH 5.5 for 1 hour at room temperature
- Biotinylated protease was captured at different concentrations in PBS for 60 min at room temperature
- Inhibitor (5 $\mu\text{M}$ ) binding assay performed in PBS with 0.1% DMSO/0.02% CHAPS

## Biotinylated Protease Capture



## Reference Compound Binding



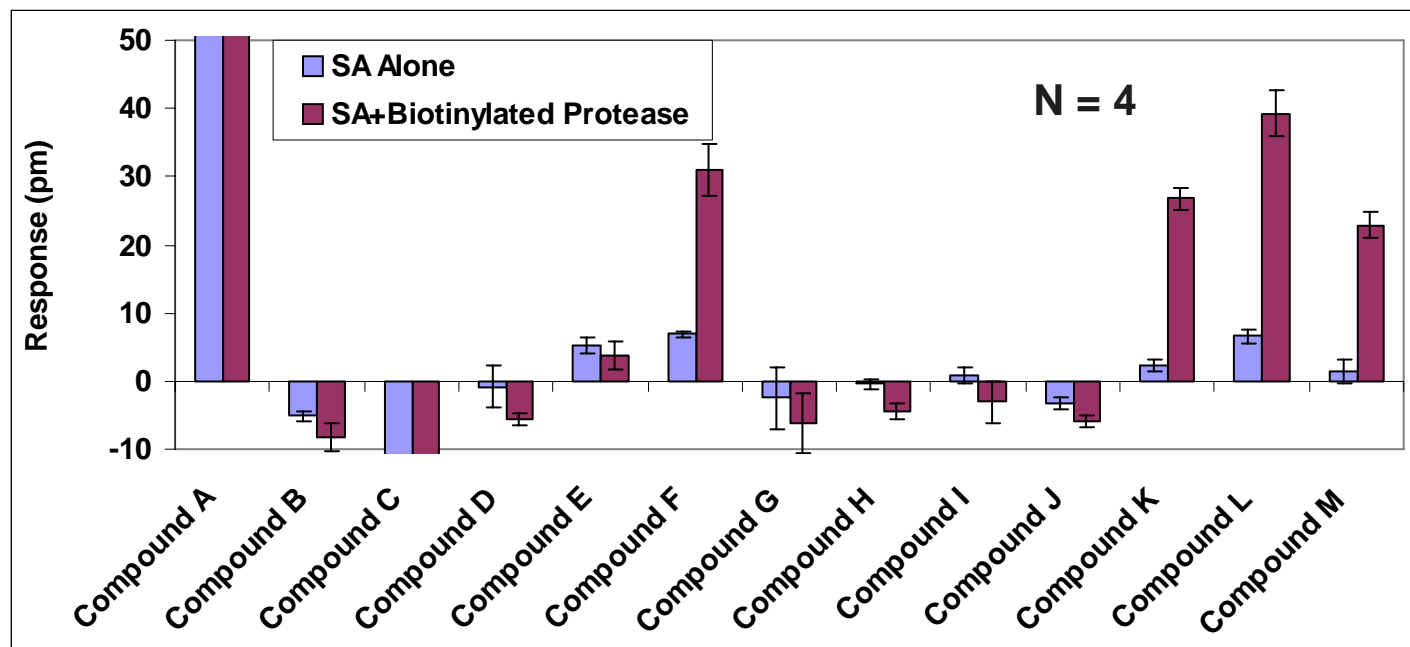
### Conclusions:

Dose-dependent capturing of the biotinylated protease to immobilized streptavidin observed.

Highest compound binding signal observed when the biotinylated protease was captured at 25  $\mu\text{g/ml}$ .

# Mini-screen of 13 Compounds Against a Captured Biotinylated Protease

- Streptavidin (SA) was immobilized at 50  $\mu\text{g/mL}$  in 20 mM Na-Acetate pH 5.5 for 1 hour at room temperature
- Biotinylated protease was captured at different concentrations in PBS for 60 min at room temperature
- Inhibitor (5 $\mu\text{M}$ ) binding assay performed in PBS with 0.1% DMSO/0.02% CHAPS



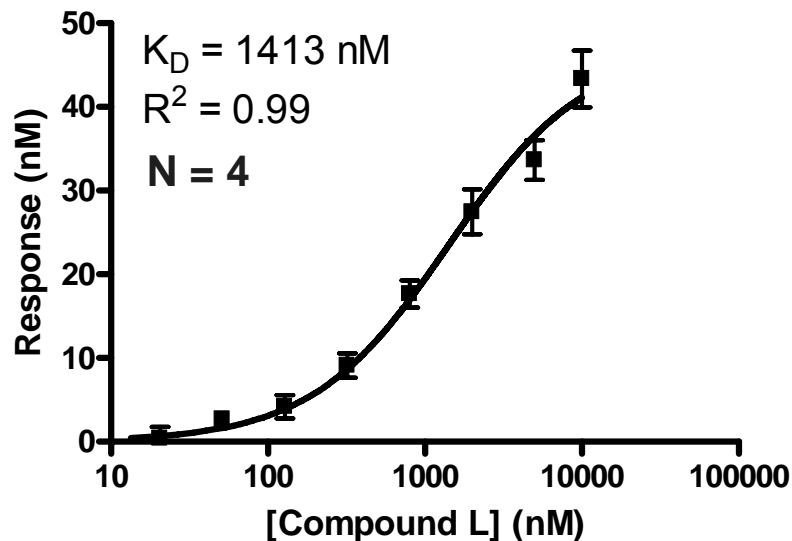
## Conclusions:

- Four compounds show binding to the protease, consistent with Novartis internal data.
- Two problematic compounds were observed: abnormal binding signals were observed for Compound A (161pm and 294pm) and Compound C (-82pm and -96pm).

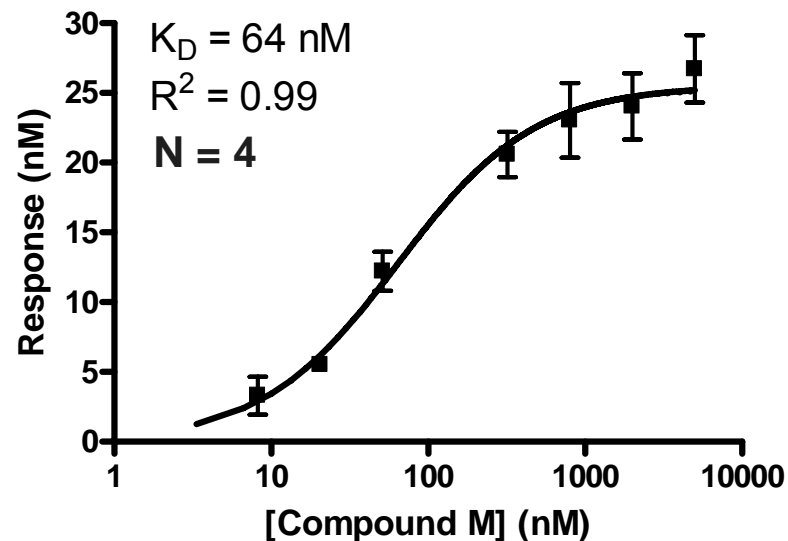
# $K_D$ Estimation of Compound L and M Binding to a Biotinylated Protease

- Streptavidin (SA) was immobilized at 50  $\mu\text{g}/\text{mL}$  in 20 mM Na-Acetate pH 5.5 for 1 hour at room temperature
- Biotinylated protease was captured at different concentrations in PBS for 60 min at room temperature
- Inhibitor titration performed in PBS with 0.1% DMSO/0.02% CHAPS

Enzymatic assay:  $\text{IC}_{50} = 800 \text{ nM}$



Enzymatic assay:  $\text{IC}_{50} = 30 \text{ nM}$



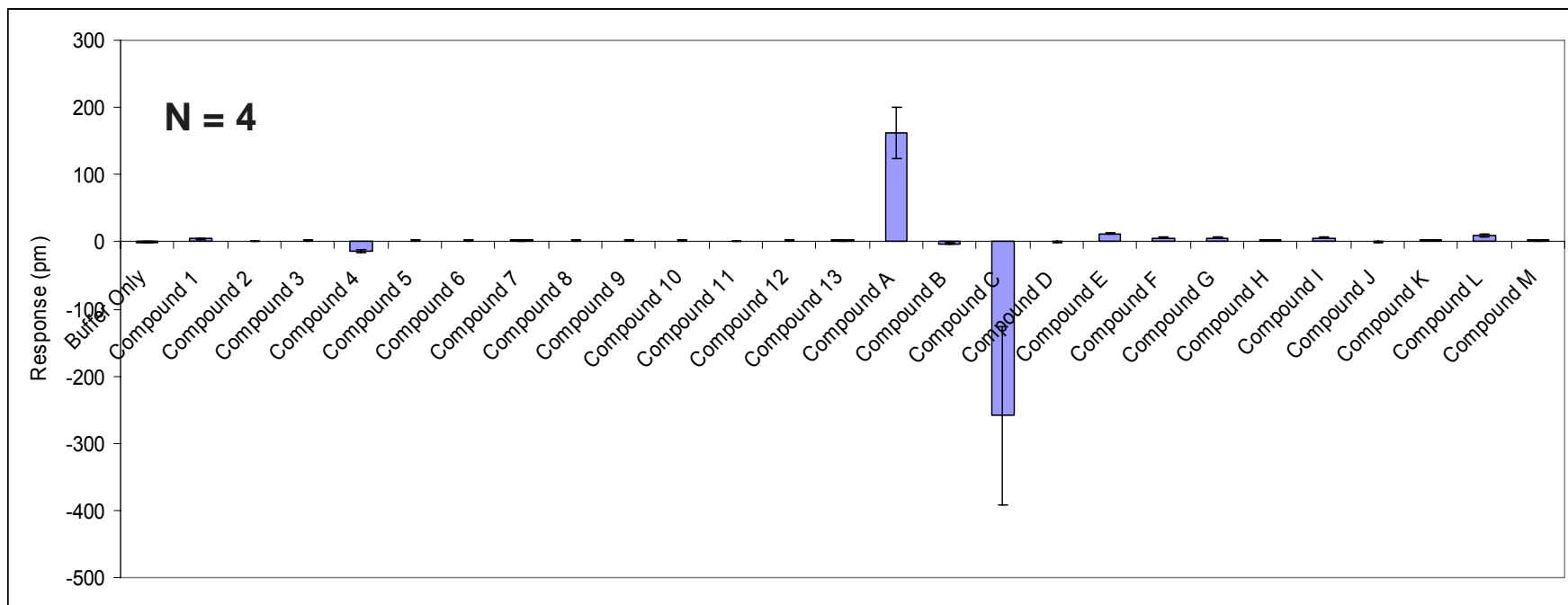
## Conclusions:

Dose-dependent binding was observed for both Compound L (~730 Da) and M (~670 Da).

$K_D$  estimations of both compounds were consistent with Novartis internal data.

## Mini-screen of 26 Compounds Against Streptavidin

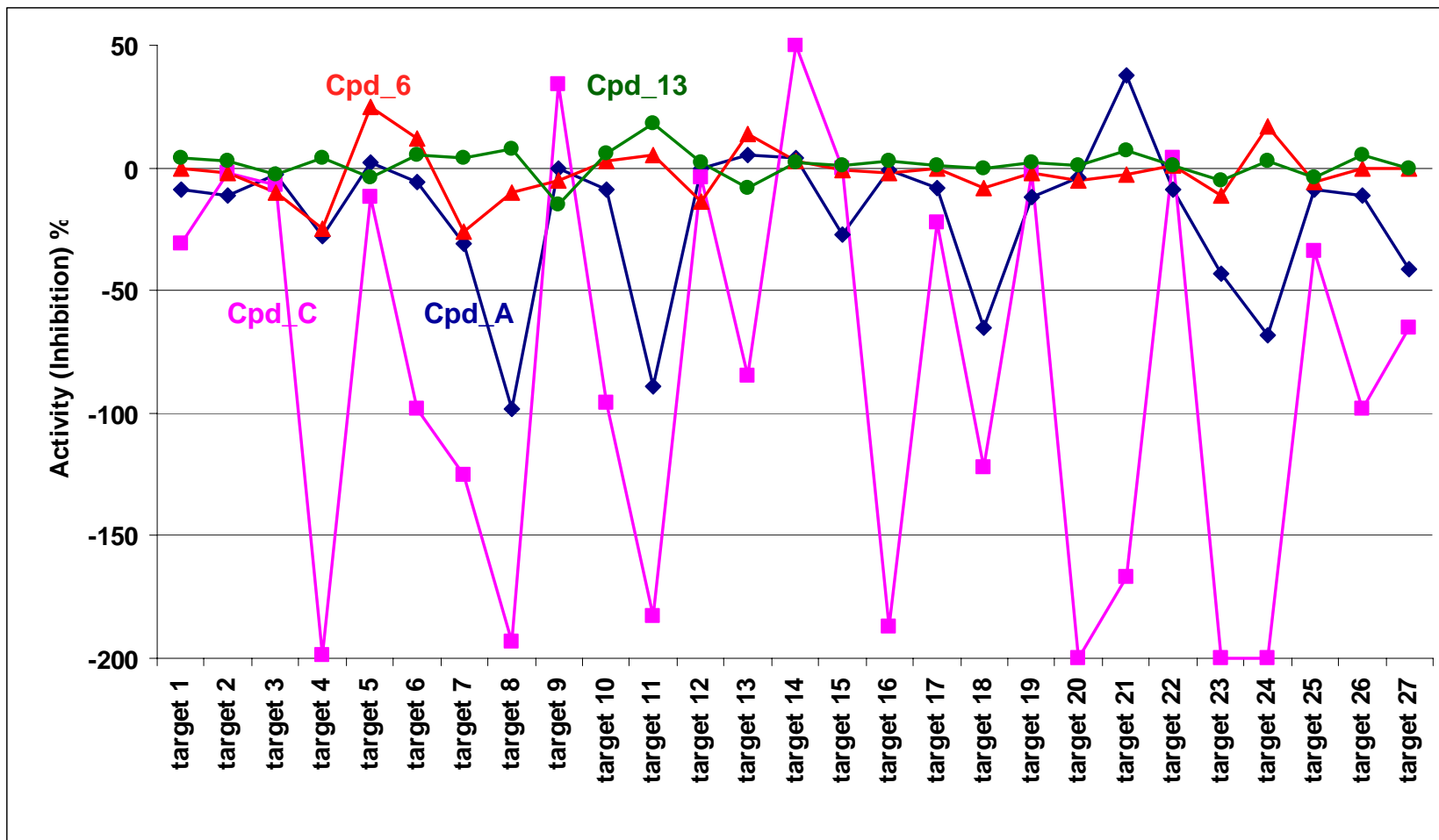
- Streptavidin (SA) was immobilized at 50  $\mu\text{g/mL}$  in 20 mM Na-Acetate pH 5.5 for 1 hour at room temperature
- Inhibitor (5 $\mu\text{M}$ ) binding assay performed in PBS with 0.1% DMSO/1 mM DTT/0.02% CHAPS



### Conclusions:

Two promiscuous compounds (A & C) observed: abnormal binding signals were observed for Compound A and Compound C, consistent with previous results.

# HTS Results of Four Compounds Against 27 Different Targets



## Conclusions:

Two promiscuous compounds (A & C) showed false positive results in a number of unrelated targets.

## Conclusions

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- We correctly identified compounds that bind to each target.
- The rank order affinity ( $K_D$ ) of compounds measured on the Epic<sup>®</sup> System is in agreement with the rank order affinity determined using traditional assays.
- A unique feature of the Epic System is its ability to identify promiscuous compounds and reduce false positives.
- We correctly identified those compounds known to be aggregators and/or promiscuous inhibitors.
- Biotin/streptavidin capture methods for protein immobilization were demonstrated with accurate and reproducible results.