

CORNING

Epic[®]
system

LOPAC[™] Screen Against Trypsin Using the Corning[®] Epic[®] System

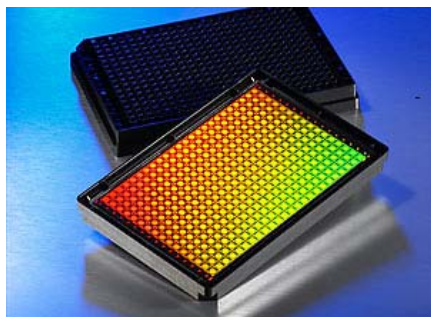
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Abstract

The Corning® Epic® System is a high-throughput, label-free screening platform that allows the detection of direct binding of a compound to an immobilized target without the use of fluorescent or radioactive tags. This enables an easier and less complex way of measuring drug-binding events. It also allows for screening of previously difficult targets, eliminates false responses caused by labels, and reduces the amount of time and effort needed to perform a screen. This work details a screen of 1280 compounds against an immobilized target of trypsin. Of the 1280 tested, 11 “hits” were identified. Two different functional assays — Pierce QuantiCleave™ fluorescent assay and the Corning Epic label-free functional assay — were used to verify the Epic direct bind screening results.

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.



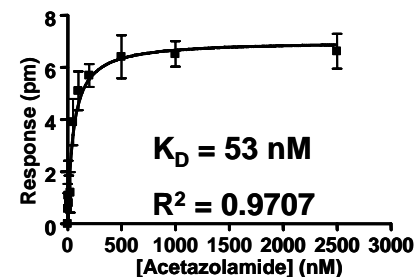
Microplate

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



Microplate Reader

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

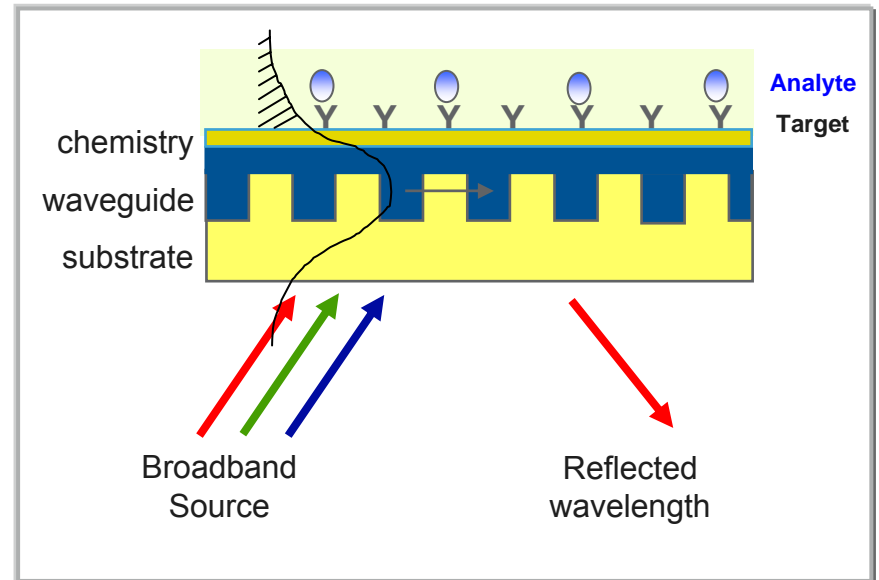
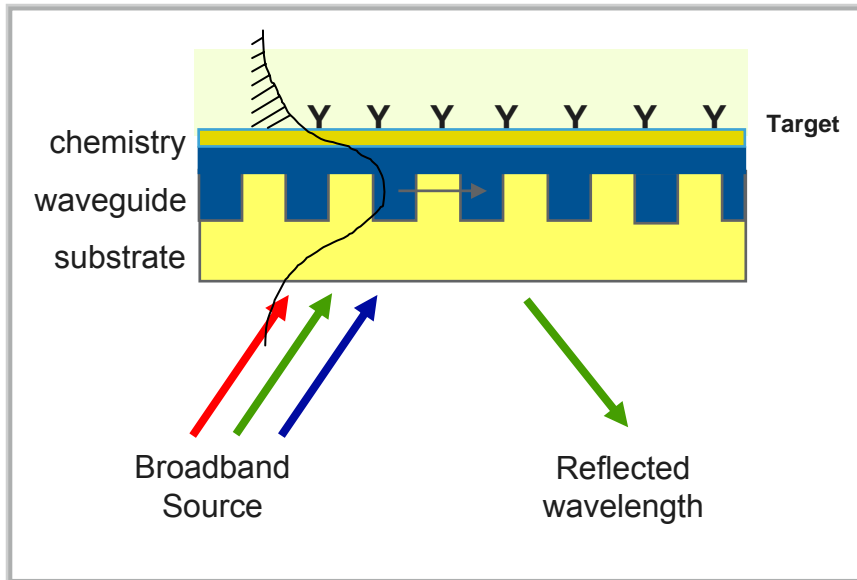


Binding Data

- Manipulated and analyzed by customer

Operating Principle: Biochemical Assays

- Measures changes in index of refraction upon a binding event
- Change in index manifested by a shift in resonant wavelength



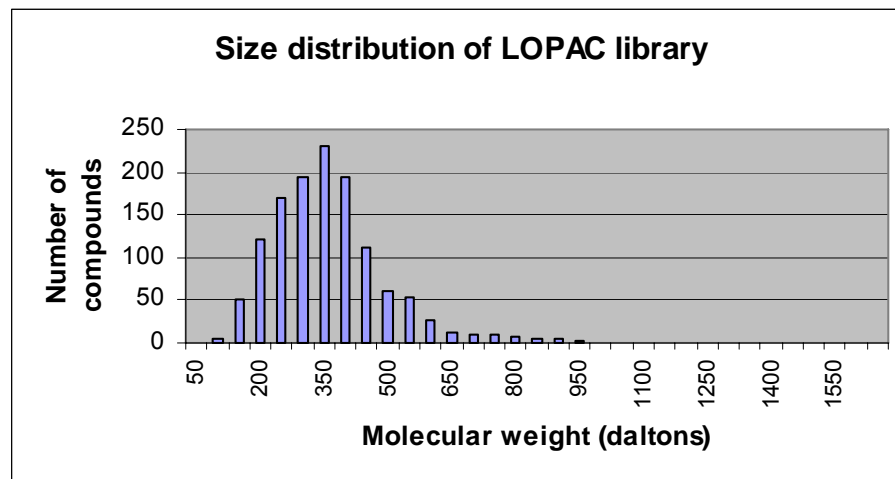
Introduction

- We performed a screen of the LOPAC™ 1280 compound library from Sigma using a direct binding assay on the Epic® System.
- The library was screened against immobilized trypsin.
- The screen consisted of 16 384-well microplates. For each drug compound there were four replicates (n=4).
- A compound concentration of 10uM in 1XPBS3% DMSO was used. Antipain and leupeptin, known trypsin inhibitors, were included in each microplate as positive controls.
- Pierce QuantiCleave™ fluorescent assay and protease functional assay on the Epic System were used to verify the Epic screening results.

LOPAC™ Distribution Data

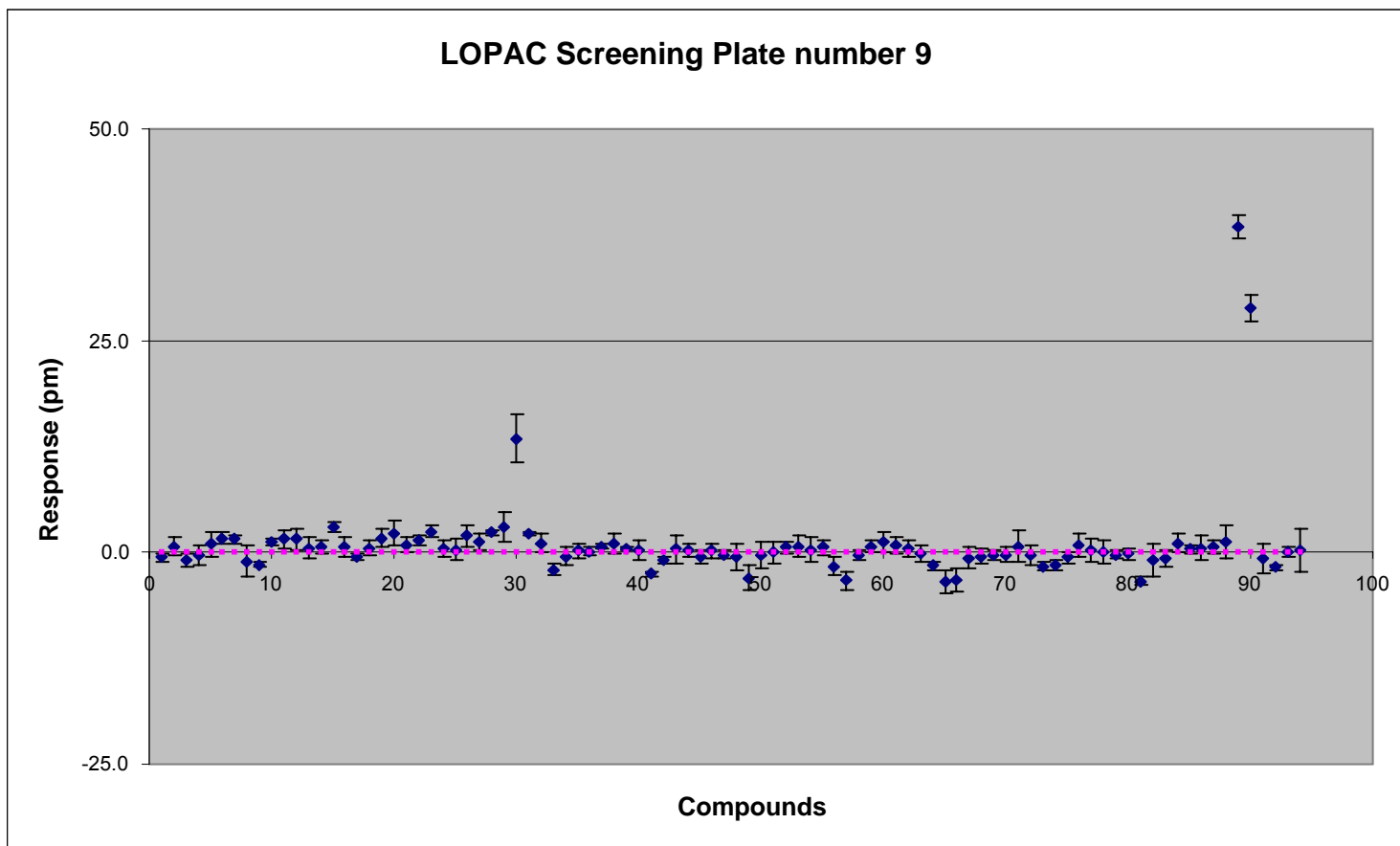
- LOPAC™ (Library of Pharmacologically Active Compounds) is a collection of 1280 molecules that span a broad range of cell signaling and neuroscience areas. The library is most commonly used to validate new drug discovery assays and characterize orphan receptors.

- Breakdown by function:
 - Neurotransmission 58%
 - Cell signaling/other 9%
 - Phosphorylation 8%
 - Cell stress 4%
 - Lipids 4%
 - Ion channel 6%
 - G protein 3%
 - Gene regulation 3%
 - Hormones 3%
 - Apoptosis cycle 2%



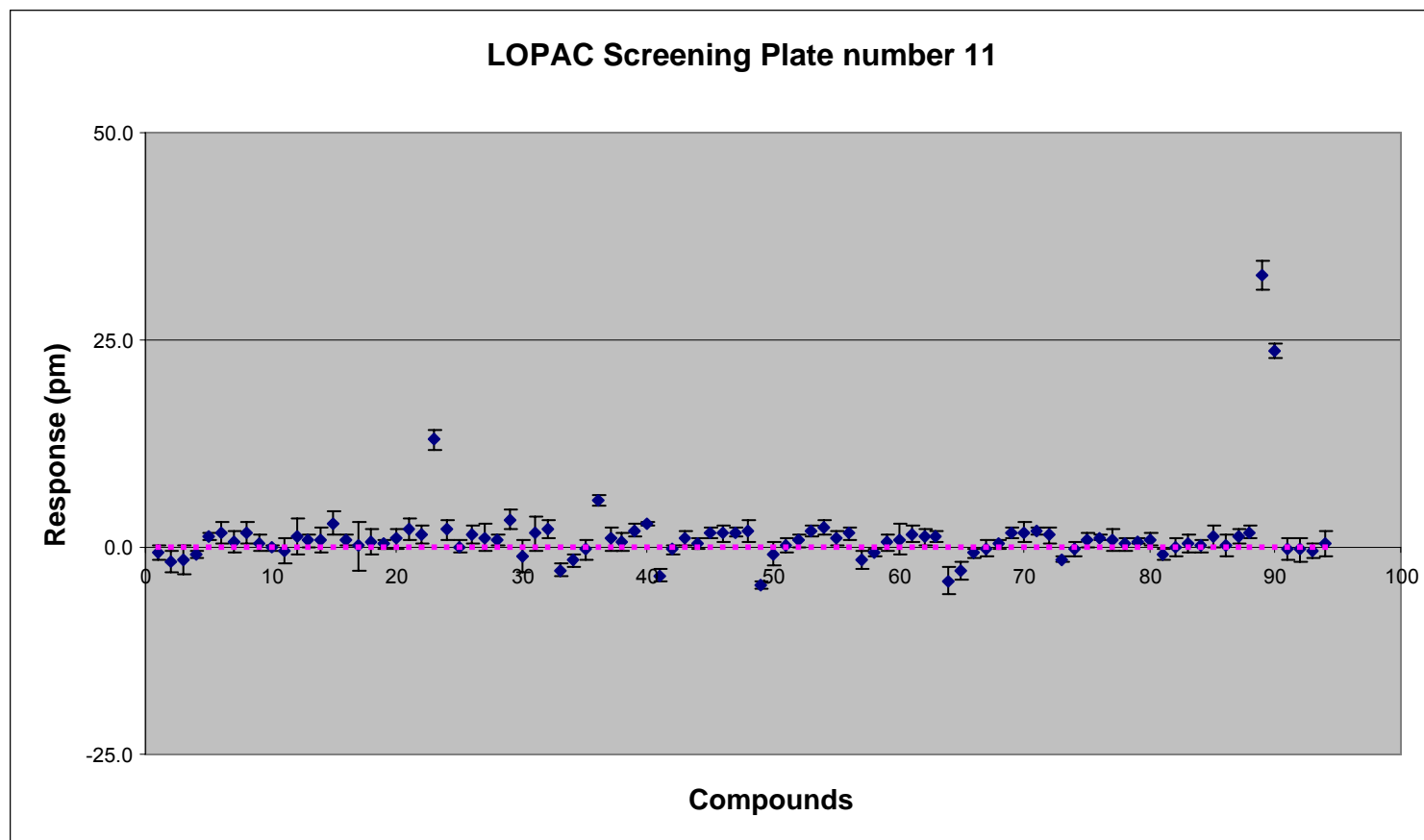
LOPAC™ Stats	Daltons
MW Min	42.39
MW Max	1505.1
Mean MW	337.92
Median MW	317.98
MW Range	1462.71

Individual Plate Results



[Compound] = 10uM
Binding buffer: PBS/3%DMSO.

Individual Plate Results



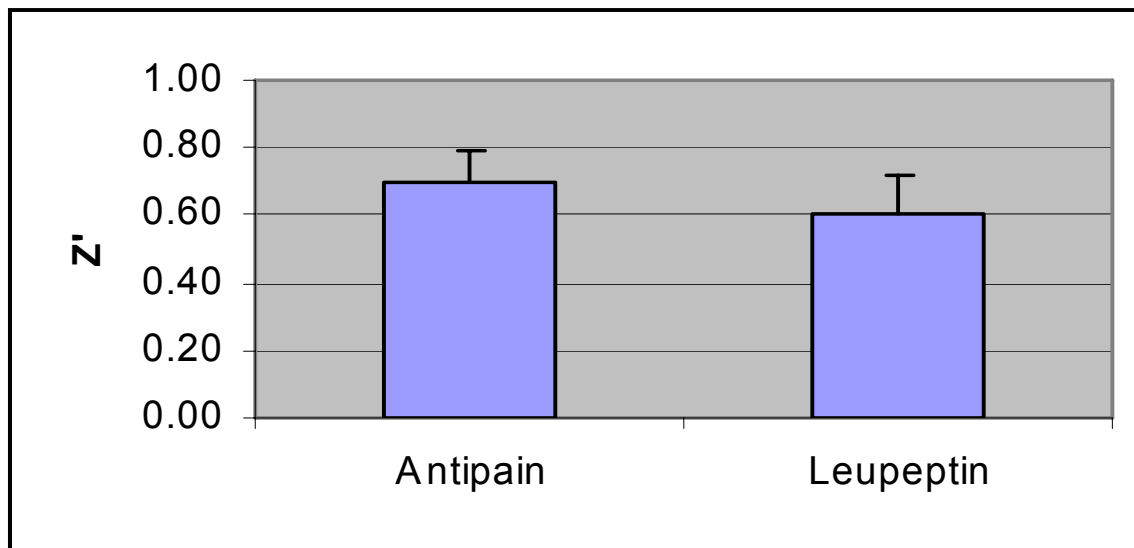
[Compound] = 10uM
Binding buffer: PBS/3%DMSO.

Summary of “Hits”

Cat #	mol weight	Name
A 4562	294.15	5-(N,N-Dimethyl)amiloride hydrochloride
A 3085	299.77	5-(N-Ethyl-N-isopropyl)amiloride
A 5585	299.77	5-(N-Methyl-N-isobutyl)amiloride
6880	208.09	4-Aminobenzamidine dihydrochloride
A 9561	311.78	5-(N,N-hexamethylene)amiloride
A 7410	266.09	Amiloride hydrochloride
B 6506	156.62	Benzamidine hydrochloride
I 8898	875.12	Ivermectin
M-166	280.67	MDL 26,630 trihydrochloride
P 0547	592.69	Pentamidine isethionate
S 2501	254.63	Spermidine trihydrochloride

11 showed a response greater than 3σ of the mean response of negative controls.

Robustness of Epic[®] Direct Bind Assays: Z' Results

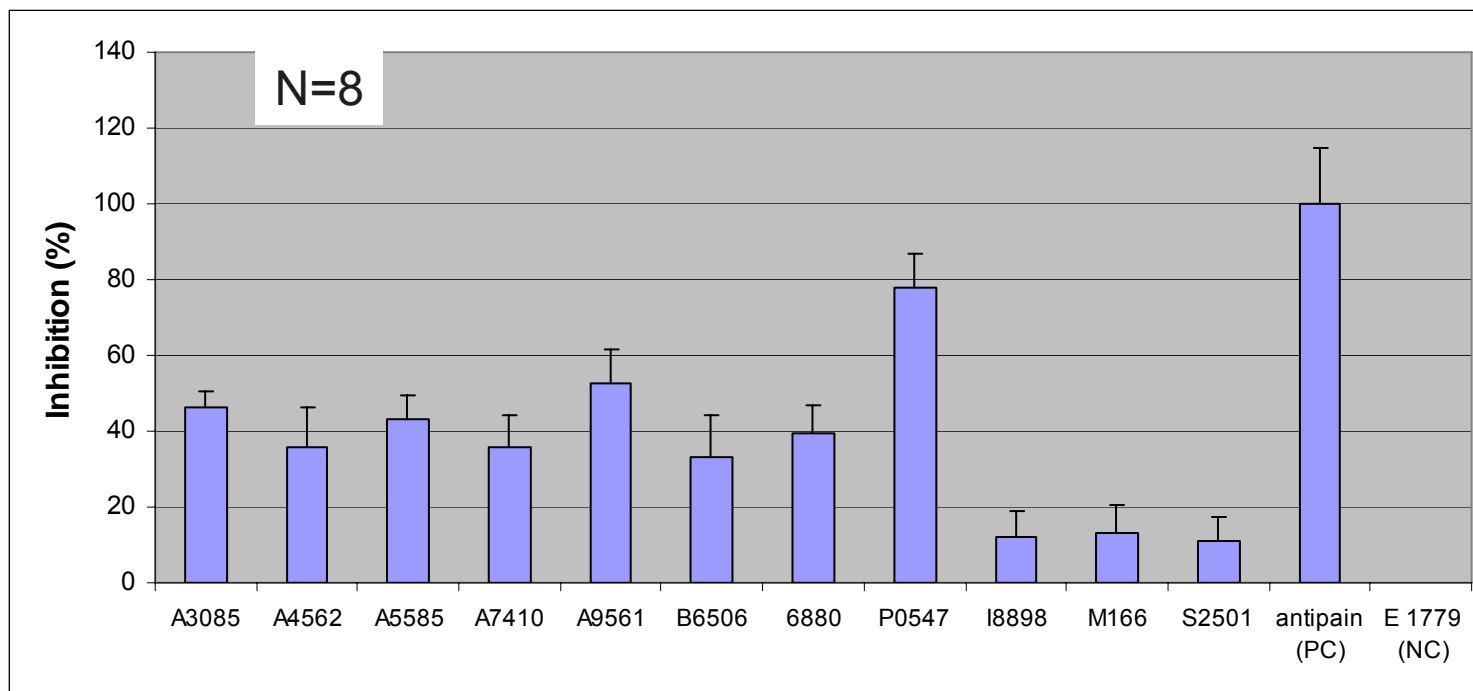


Z' was calculated for 16 plates. The above result is the average Z' of 16 plates.

QuantiCleave™ Fluorescent Protease Assay

- The assay uses fluorescein-labeled casein as a substrate for assessing protease activity in a sample by fluorescence resonance energy transfer (FRET) with a standard fluorometer.
- It measures the decrease in fluorescence quenching (=increased total fluorescence) that occurs as FITC-Casein substrate is digested into smaller fluorescein-labeled fragments.
- The presence of inhibitor will block the expected decrease in fluorescence quenching.
- The assay uses standard fluorescein excitation/emission filters (485nm/538nm).

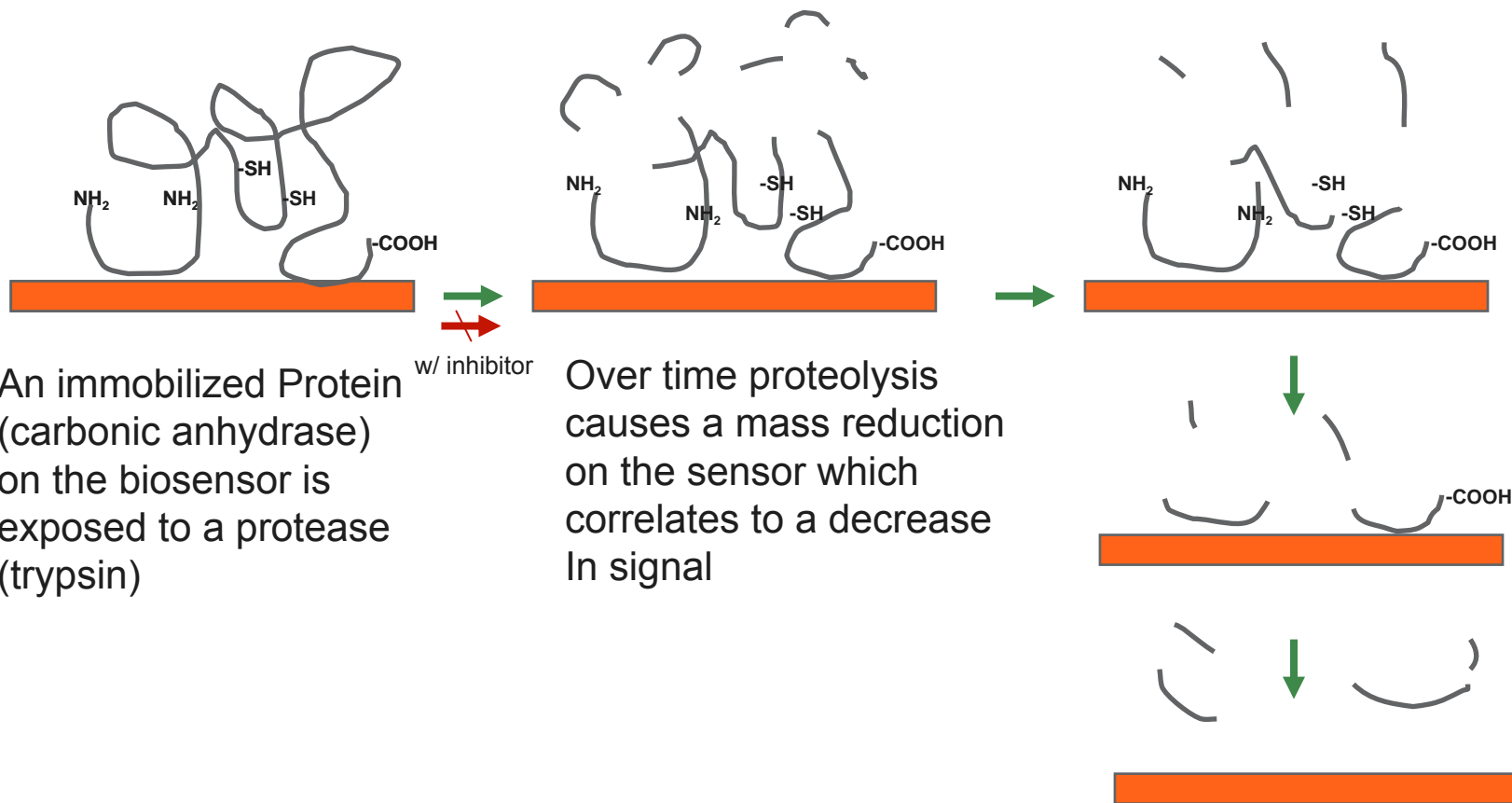
Validation of “Hits” Identified by Epic[®] Using a Fluorescent Functional Assay



- 8 out of 11 “hits” identified through Epic direct binding assay show >30% inhibition using fluorescent functional assay.
- Antipain, a known trypsin inhibitor, was used as a positive control; E1779, was used as a negative control.

Validation of Direct Bind Hits Using a Functional Assay on the Epic[®] System

Protease Digestion on Epic System Biosensor

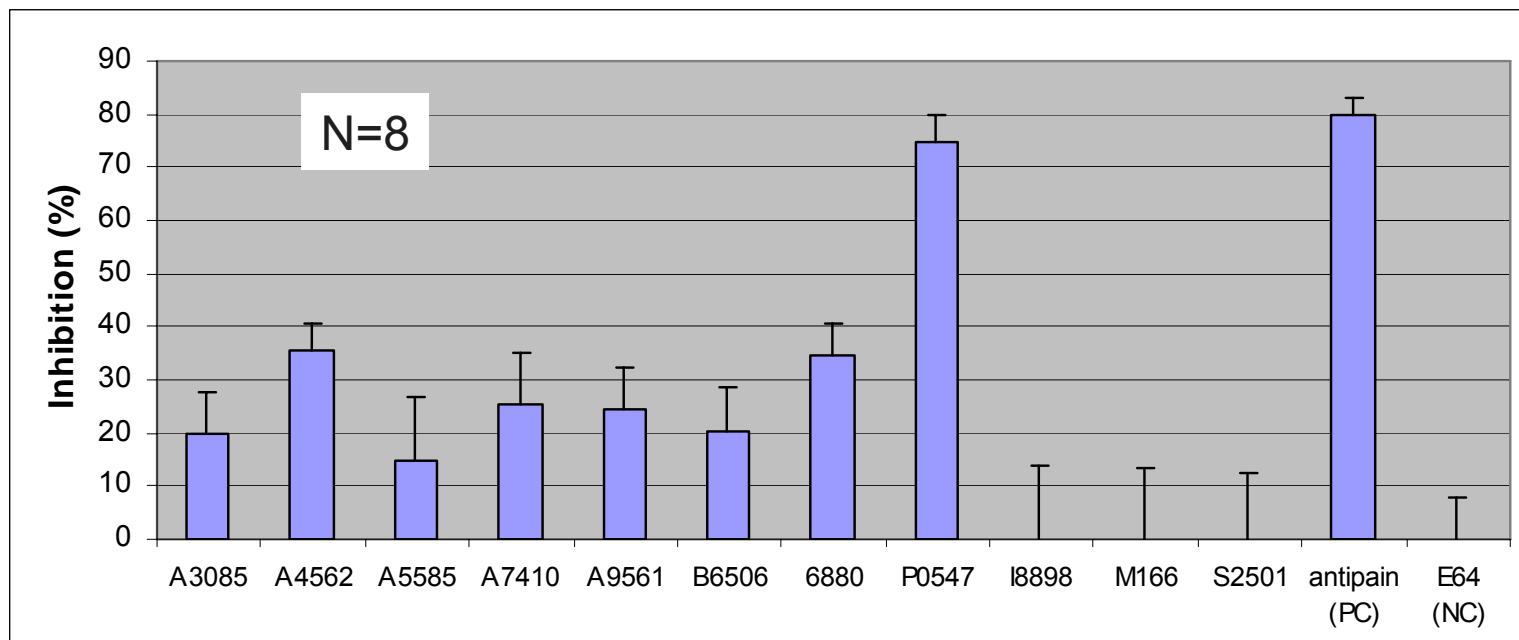


An immobilized Protein (carbonic anhydrase) on the biosensor is exposed to a protease (trypsin)

w/ inhibitor

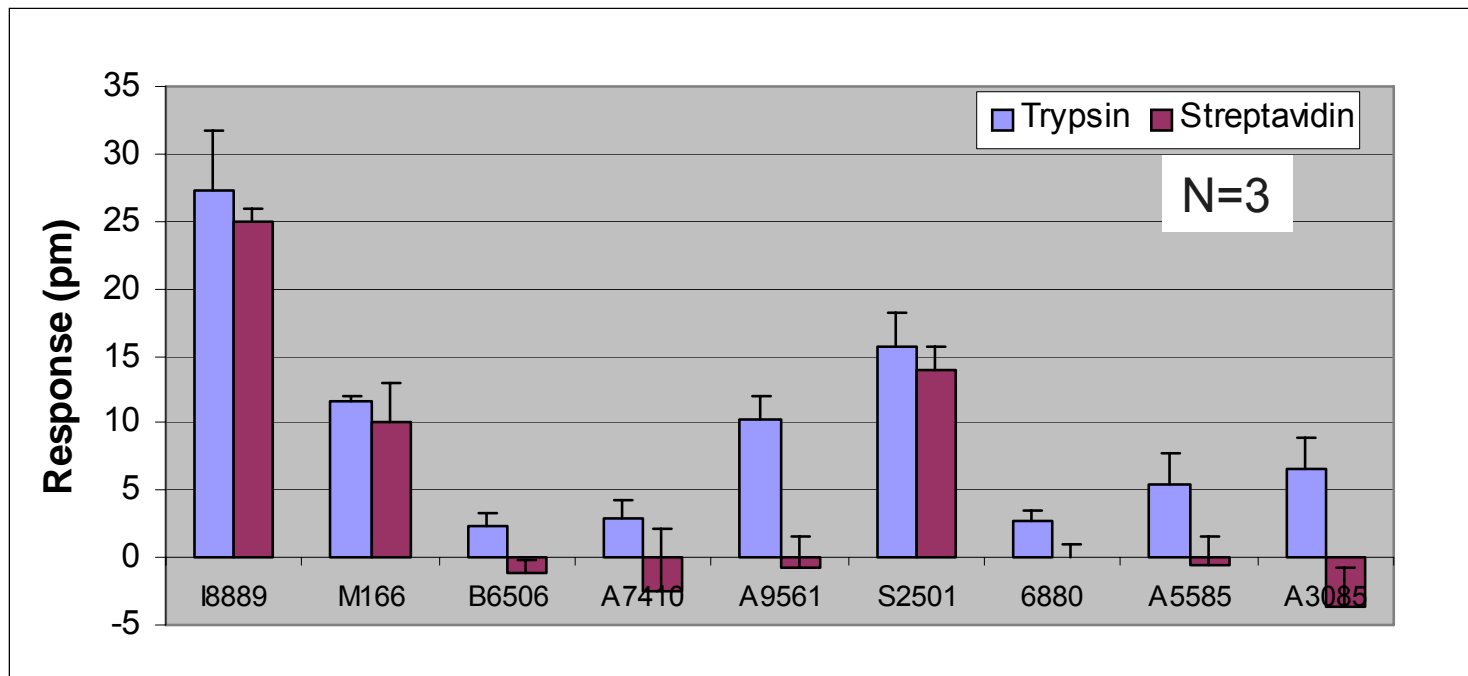
Over time proteolysis causes a mass reduction on the sensor which correlates to a decrease in signal

Validation of “Hits” Identified by Direct Binding Assay Using a Protease Functional Assay on the Epic[®] System



- 8 out of 11 “hits” identified through Epic direct binding assay show >15% inhibition using Protease Functional Assay on Epic System.
- Antipain, a known trypsin inhibitor, was used as a positive control; E64, a known papain inhibitor, was used as a negative control.

Comparison of LOPAC Compound Binding on Trypsin and Streptavidin



Three compounds bind to both trypsin and streptavidin: I8889, M166 and S2501. This suggests that the binding response of these three compounds to trypsin is non-specific. Moreover, these are the three compounds that did not show inhibitory activity in the two functional assays.

Conclusions

- Demonstrated ability to perform small drug screen on the Corning® Epic® System using pharmacologically relevant compounds.
- Of the 1280 compounds tested, 11 showed a response greater than 3σ of the mean response of negative controls.
- Positive controls had Z' of 0.60 (Leupeptin) and 0.70 (antipain) across all microplates in the screen.
- The inhibitors identified on Epic System were validated with Pierce QuantiCleave™ Fluorescent Protease Assay and protease functional assay on the Epic System. The results indicate that 73% of hits identified by direct binding assay show functional inhibition. Moreover, the results from two functional assays were in good agreement.