

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". A thin vertical orange line is positioned to the left of the logo.

Epic[®]
system

Analysis of Protein/Small Molecule Interactions Using the Corning[®] Epic[®] System

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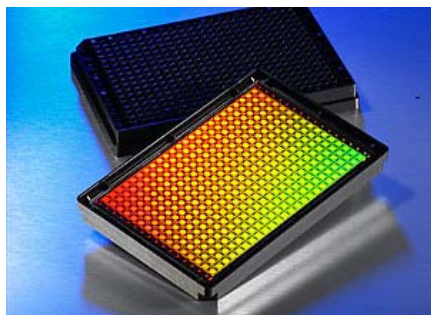
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Abstract

The discovery of antiviral drugs has been primarily focused on inhibitors of viral enzymes and there are growing needs in finding drugs against novel targets to overcome viral resistance against current drugs. Conventional HTS assays are often based on the enzymatic activity of the targets and are not suitable for the discovery of small molecules that bind to unknown sites on a protein target. The Corning® Epic® System is a high-throughput, label-free, direct bind screening system that overcomes many of the limitations inherent with currently available assay technologies. We used the Epic System to study the direct interaction between published small molecule binders and three different protein targets: an HIV protease, an HIV reverse transcriptase, and an HCV protease. The targets were immobilized in the wells of a 384-well Epic microplate via amine coupling and a yes/no binding screen of the 10 literature compounds was performed. The Epic System correctly identified as ‘hits’ those compounds which are known binders of each target. In a titration series, responses were shown to be dose dependent and saturable. This data shows the utility of the Epic System for analysis and discovery of small molecule/protein interactions.

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.



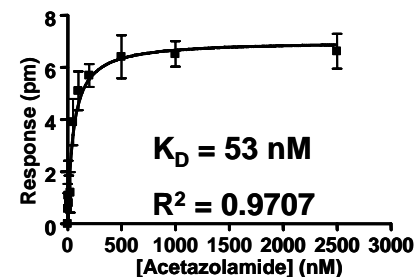
Microplate

- 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

Introduction

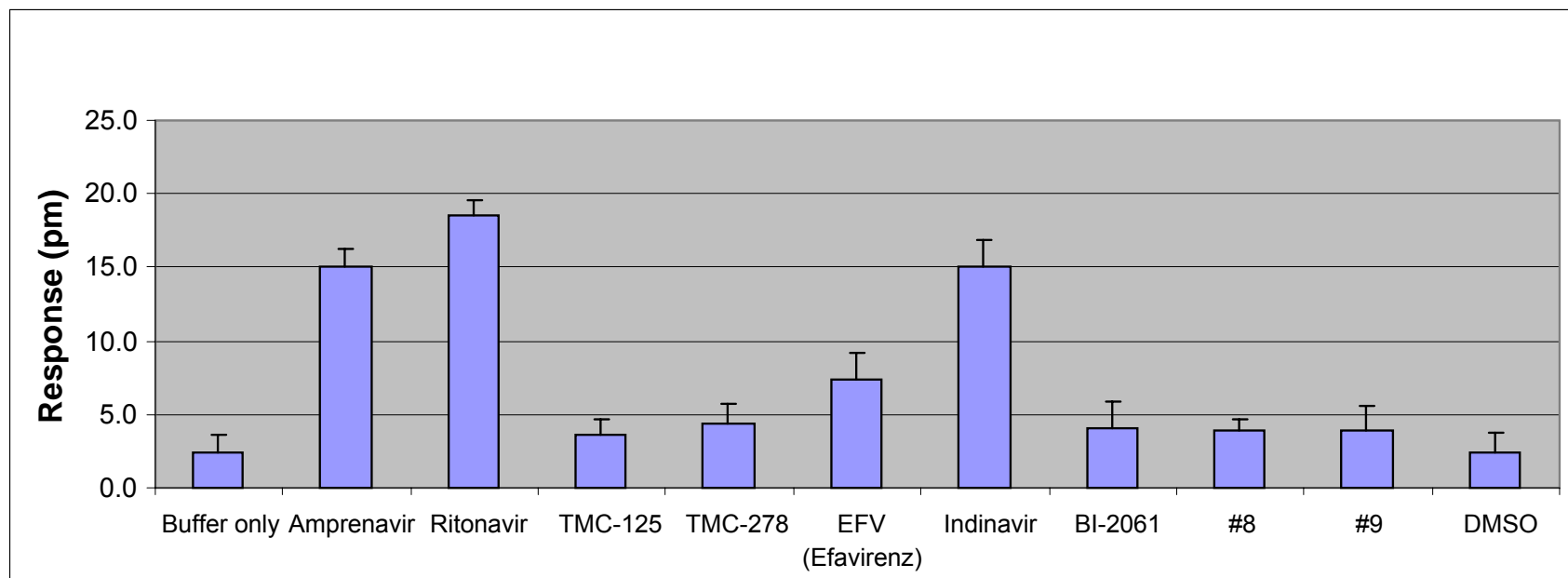
- Three systems provided by Gilead were assayed on Corning Epic System.
 - An HIV protease (~22kDa);
 - A hepatitis C virus (HCV) protease (~21kDa);
 - An HIV reverse transcriptase (~118kDa).
- The primary objective of the study was to demonstrate the ability to distinguish compounds that bind from those that do not.
 - This was accomplished by a screen of 10 compounds at a fixed concentration of 10uM.
- Estimates of affinity (K_D) were made for compounds that were identified as hits in the screen.
 - This was accomplished by a titration series of 8 -10 concentrations per compound

Materials

	Category	MW (kD)	pI
Protein A	HIV protease	22	8.84
Protein B	HIV reverse transcriptase	118	8.9
Protein C	HCV protease	21	9.6

Compound	Amprenavir	Ritonavir	TMC-125	TMC-278	EFV (Efavirenz)	Indinavir	BI-2061	#8	#9	DMSO
MW	505.6	721	435.3	366.4	315.7	613.8	774.9	801.9	775.9	78.13
Note		Negative Control for B	Negative Control for A	Negative Control for C	Positive Control for B	Positive Control for A	Positive Control for C			

Compound Screening for HIV Protease



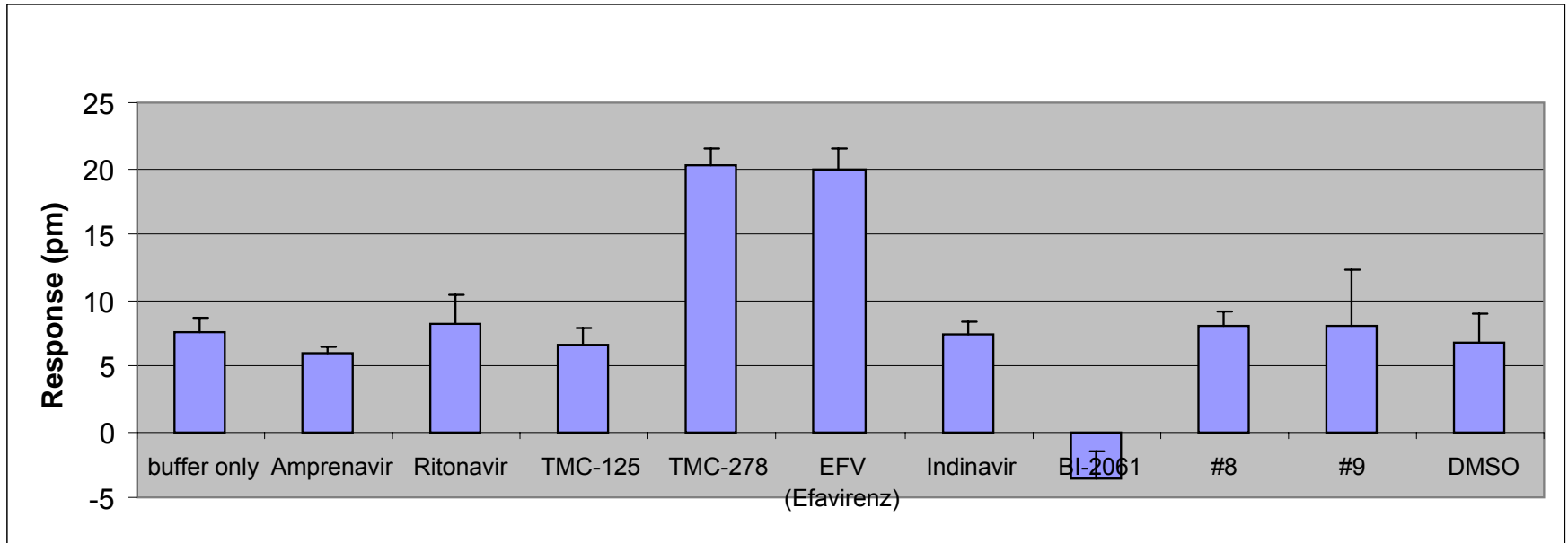
- 50ug/ml of HIV Protease was immobilized in pH 8 phosphate buffer.
- [Compound] was 10uM in 0.1% DMSO/PBS.
- Amprenavir, Ritonavir, Indinavir (positive control) show binding.
- TMC-125 (negative control) shows no binding.

Statistics of Compound Binding to HIV Protease

	Avg (pm)	SD	% CV
Buffer only	2.37	1.31	55.45
Amprenavir	15.10	1.11	7.36
Ritonavir	18.55	1.00	5.37
TMC-125	3.68	0.98	26.61
TMC-278	4.38	1.27	28.96
EFV (Efavirenz)	7.35	1.80	24.50
Indinavir	15.05	1.76	11.70
BI-2061	4.10	1.84	44.77
#8	3.96	0.66	16.62
#9	3.95	1.57	39.83
DMSO	2.41	1.28	53.12

- Good reproducibility observed for compounds that bind as shown by CVs of 5-12%.

Compound Screening for HIV Reverse Transcriptase



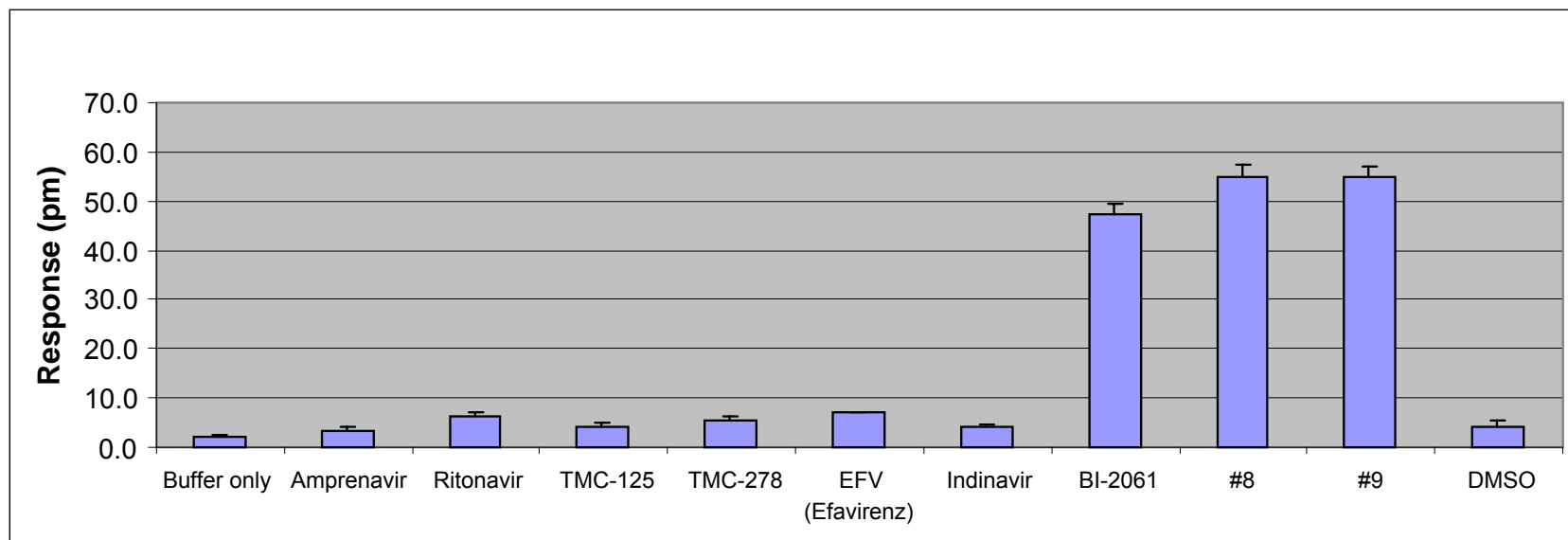
- 100ug/ml of HIV reverse transcriptase was immobilized in pH 8 phosphate buffer.
- [Compound] was 10uM in 0.2% DMSO/PBS.
- TMC-278, EFV (Efavirenz) (positive control) show binding.
- Ritonavir (negative control) shows no binding.

Statistics of Compound Binding to HIV Reverse Transcriptase

	Avg (pm)	SD	% CV
Buffer only	7.52	1.15	15.30
Amprenavir	5.96	0.43	7.17
Ritonavir	8.23	2.24	27.27
TMC-125	6.60	1.31	19.83
TMC-278	20.16	1.42	7.06
EFV (Efavirenz)	19.89	1.65	8.29
Indinavir	7.33	1.01	13.71
BI-2061	-3.55	2.00	-56.40
#8	8.08	1.11	13.76
#9	8.04	4.25	52.84
DMSO	6.77	2.18	32.29

- Good reproducibility observed for compounds that bind as shown by CVs of 7-8%.

Compound Screening for HCV Protease



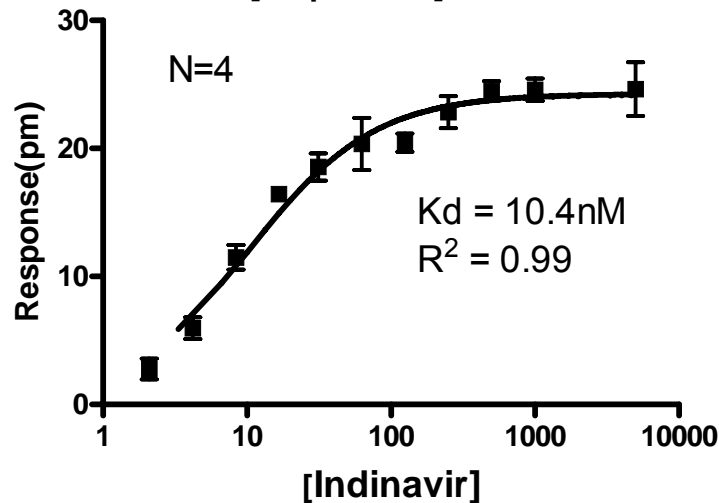
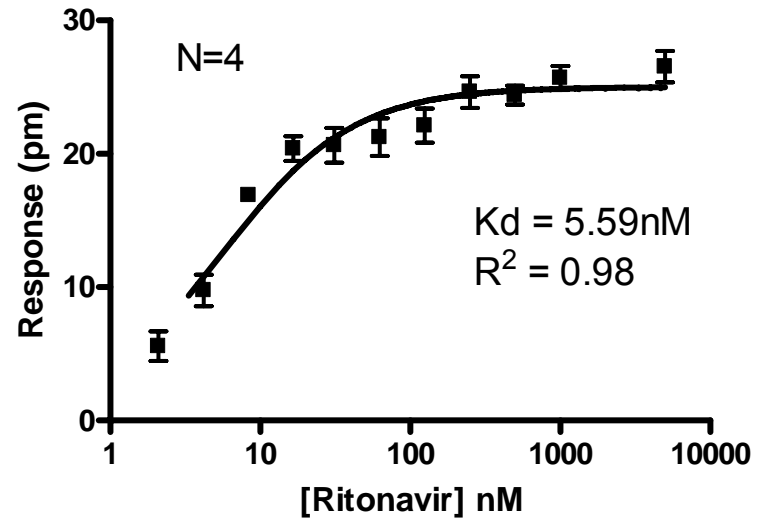
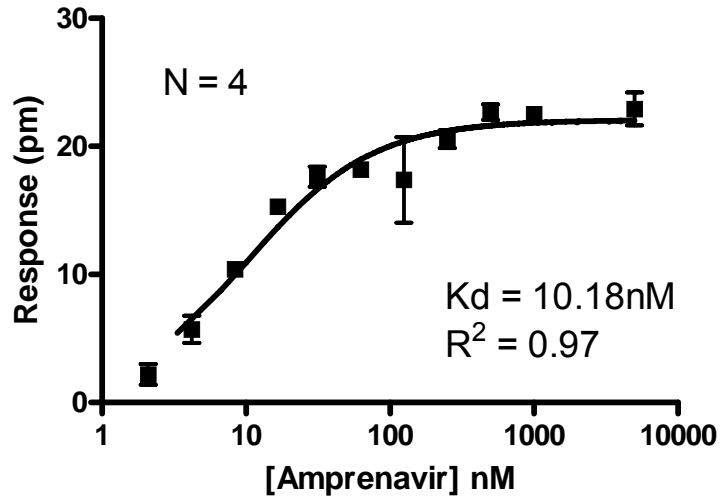
- 50ug/ml of HCV protease was immobilized in pH 8 phosphate buffer.
- [Compound] was 10uM in 0.1% DMSO/PBS.
- BI-2061 (positive control), #8 and #9 show binding.
- TMC-278 (negative control) shows no binding.

Statistics of Compound Binding to HCV Protease

	Avg (pm)	SD	% CV
Buffer only	1.98	0.43	21.77
Amprenavir	3.28	1.09	33.27
Ritonavir	6.41	0.82	12.86
TMC-125	4.24	0.65	15.38
TMC-278	5.59	0.59	10.48
EFV (Efavirenz)	6.98	0.25	3.61
Indinavir	4.23	0.40	9.44
BI-2061	47.19	2.18	4.63
#8	54.70	2.58	4.72
#9	54.70	2.48	4.52
DMSO	4.15	1.48	35.58

- Good reproducibility observed for compounds that bind as shown by the CVs < 5%.

K_D Estimation of Compounds That Bind to HIV Protease



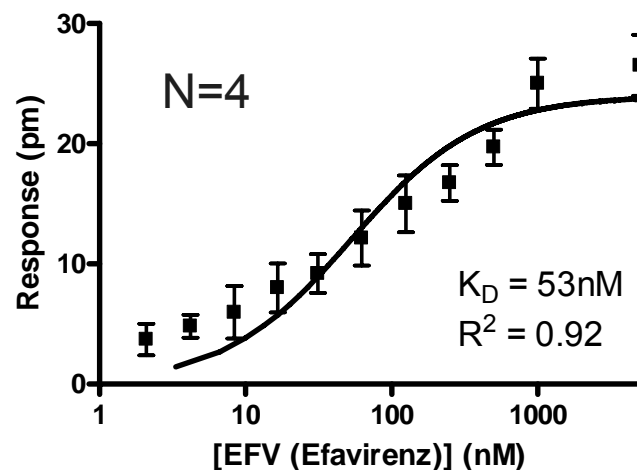
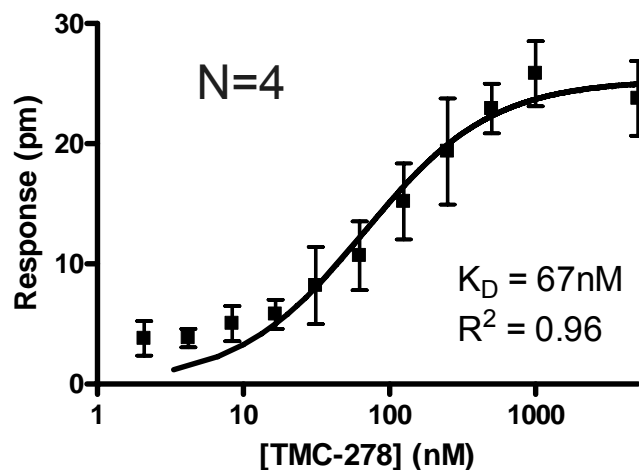
Protocol:

Assay buffer: PBS/0.1%DMSO/0.02% Chaps

Mixing time: 10 minutes

Total volume: 30uL

K_D Estimation of Compounds That Bind to HIV Reverse Transcriptase



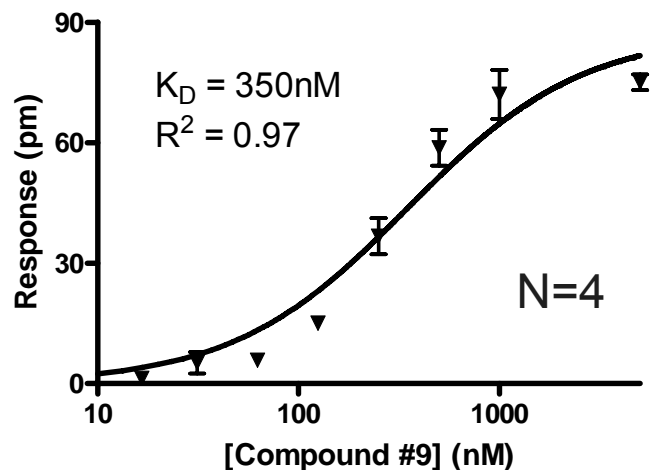
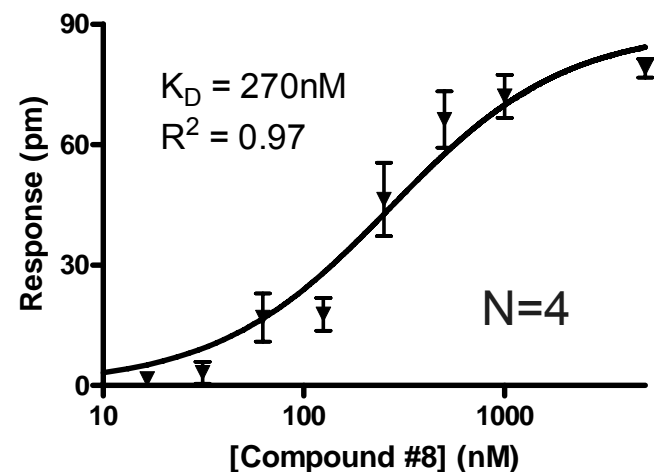
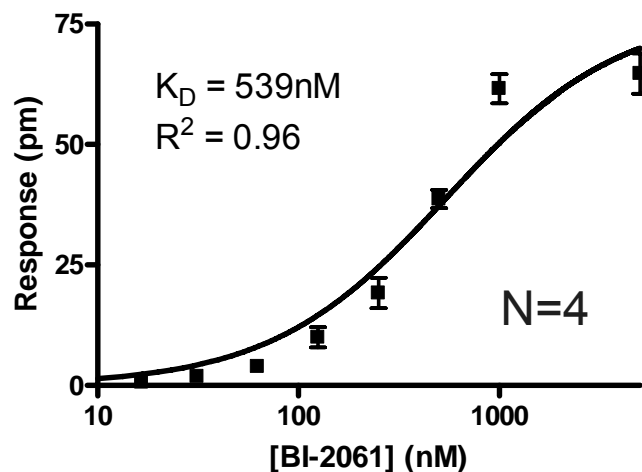
Protocol:

Assay buffer: PBS/0.1%DMSO/0.02% Chaps

Mixing time: 10 minutes

Total volume: 30uL

K_D Estimation of Compounds That Bind to HCV Protease



Protocol:

Assay buffer: PBS/0.1%DMSO/0.02% Chaps

Mixing time: 10 minutes

Total volume: 30uL

Conclusions

- Three proteins were successfully immobilized using amine coupling; immobilization conditions were optimized for each protein.
- A screen of 10 compounds showed clear “hits” for all three proteins. The “hits” are consistent with the literature.
 - HIV Protease: Amprenavir, Ritonavir, Indinavir
 - HIV Reverse Transcriptase: TMC-278 and EFV (Efavirenz)
 - HCV Protease: BI-2061, #8, #9
- Negative control compounds showed no binding; positive control compounds showed binding signals of 20-60pm.
- Compound binding was reproducible with CVs < ~10%.
- Responses were dose dependent and saturable; estimated K_D values were all in the nM range.

	HIV Protease			HIV Reverse Transcriptase		HCV Protease		
Compound	Amprenavir	Ritonavir	Indinavir	TMC-278	EFV (Efavirenz)	BI-2061	#8	#9
K_D (nM)	10.18	5.59	10.4	67.1	52.6	539.1	270.6	348.6
R^2	0.97	0.98	0.99	0.96	0.92	0.96	0.97	0.97