AMGEN

Meso Scale Discovery:

The New Kid on the Block for HTS Protein Kinase Assays

A Beta-Site Evaluation

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Introduction

MSD Contacted Amgen Sept '02
Signed Early Access Program Feb '02

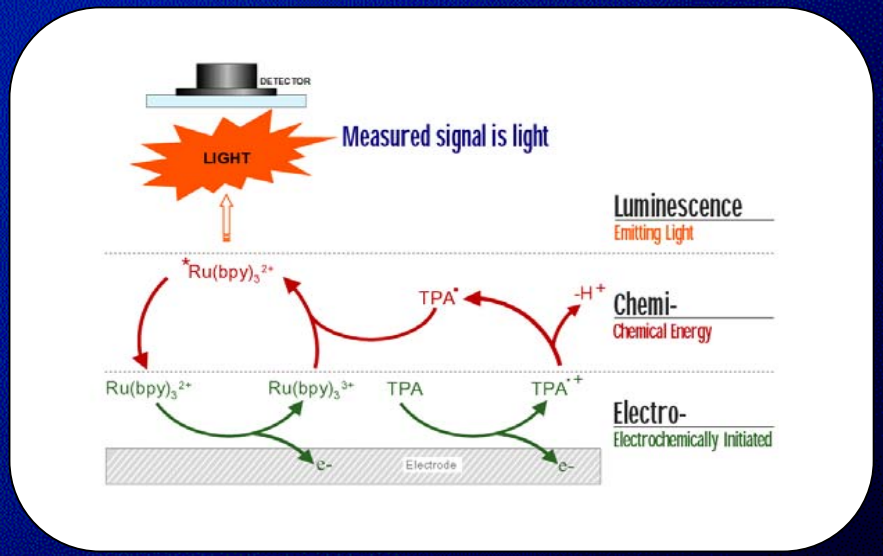
Objectives

- Does it work?
  Technology, Hardware, Biology
- Will it have utility at Amgen?
  Integration it into our Existing Process?
  Future?

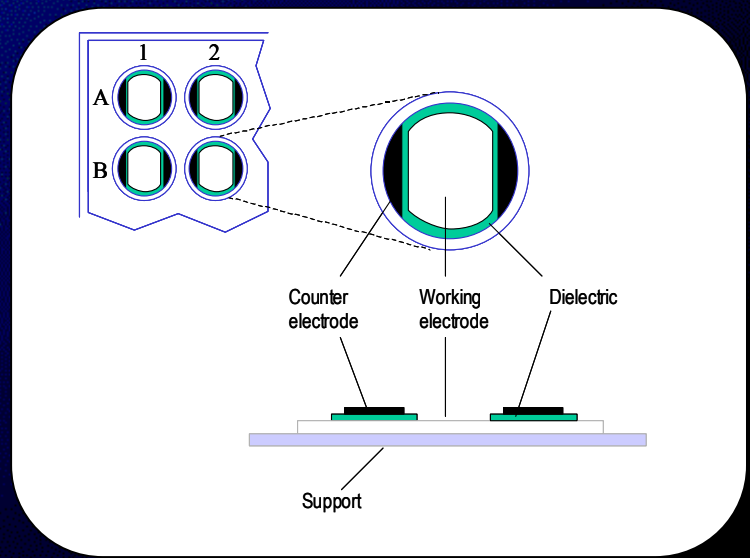
Structure

- Installation and Training
- Conduct 75,000 Multi-Array determination (Ser/Thr Kinase Screens)
- Conduct 35,000 Multi-Spot determinations (Cytokine Secretagogue)
- Comparison of Multi-Array Technology platform against TR-FRET
- Provide Feedback

Meso Scale Sector HTS



- *Plate Based*
- *Electrochemiluminescent*
- *Homogeneous*
- *Imaging Supports High Density Plates*



Hardware

Features

- CCD Imaging (1 min/plate)
- Small Footprint
- 75 plate capacity Stacker
- Stylish Design

- More than 3500 plates cycled
- 1500 plates cycled in three days
  1 instrument failure (loose wire)
- No Stacker Failures
- No Communication Failures

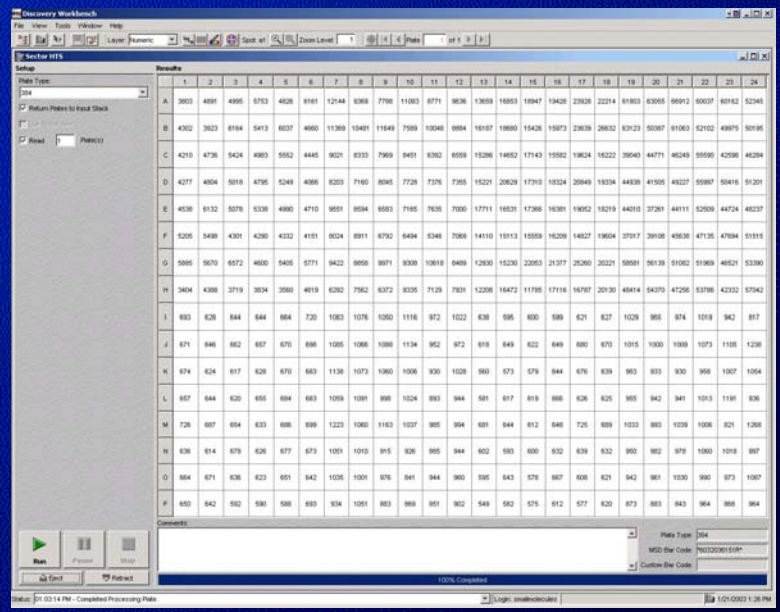
Wish List

- Barcode Position Options

Software

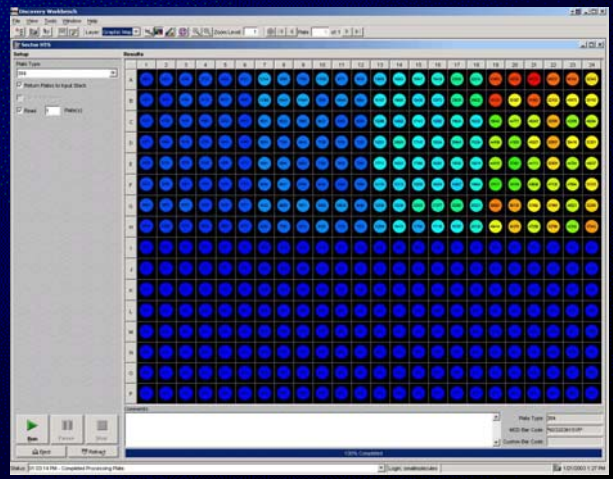
Features

- Stable Software
- Simple/Intuitive GUI
- No Frills
- Plate In/ Data Out

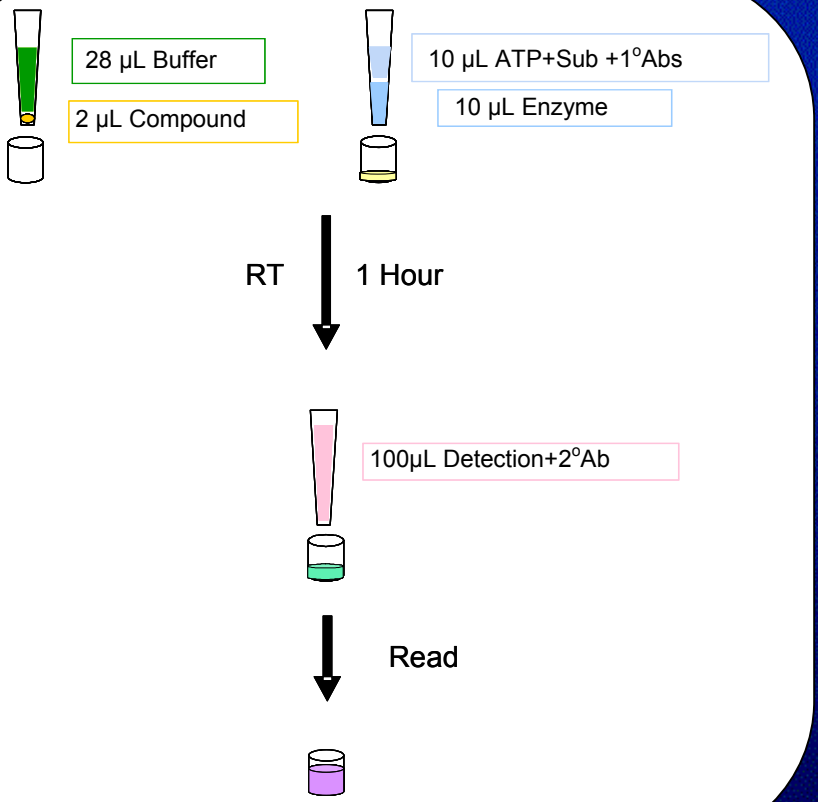


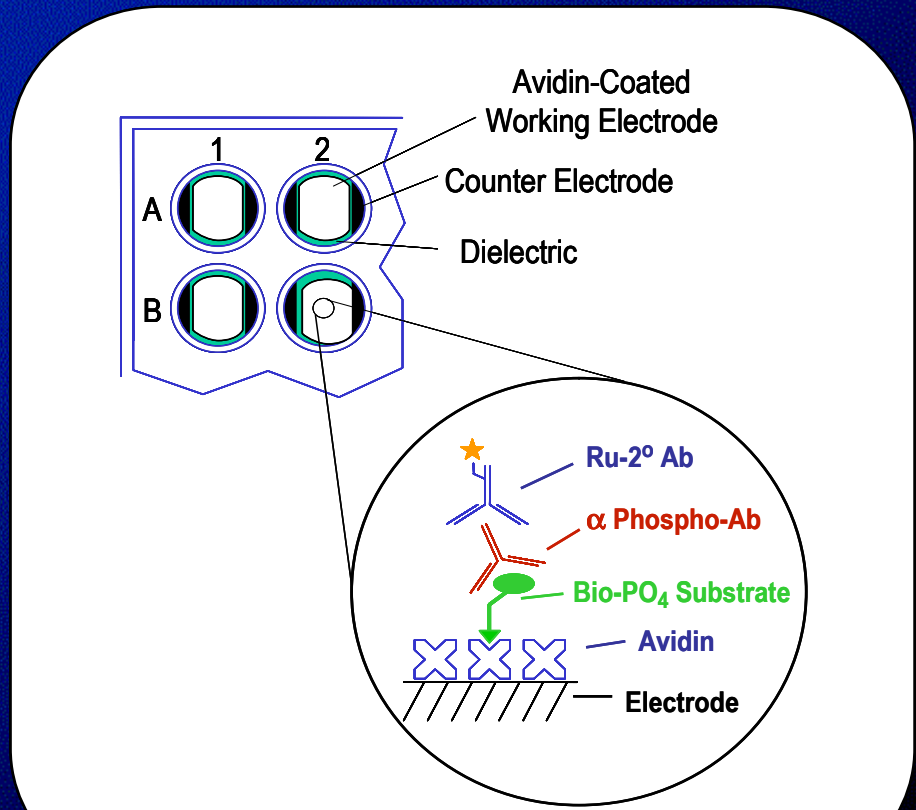
Wish List

- Assay Based Protocol
- Improved File Management



Screening Protocol



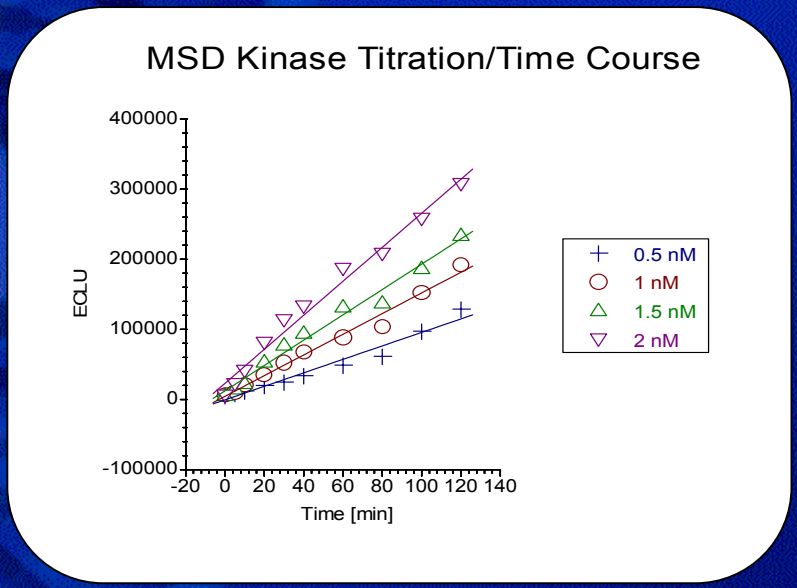


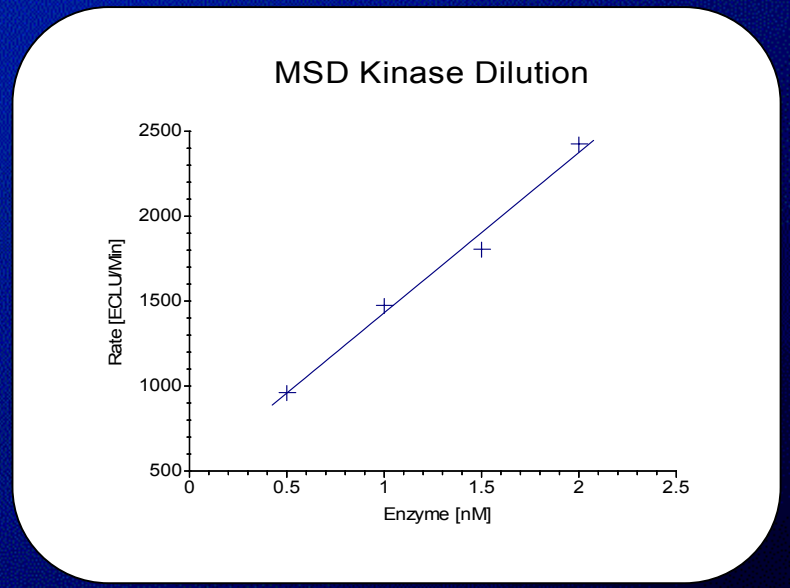
Ser/Thr Kinase

Biotinylated Substrate

Labeled Generic 2° Antibody

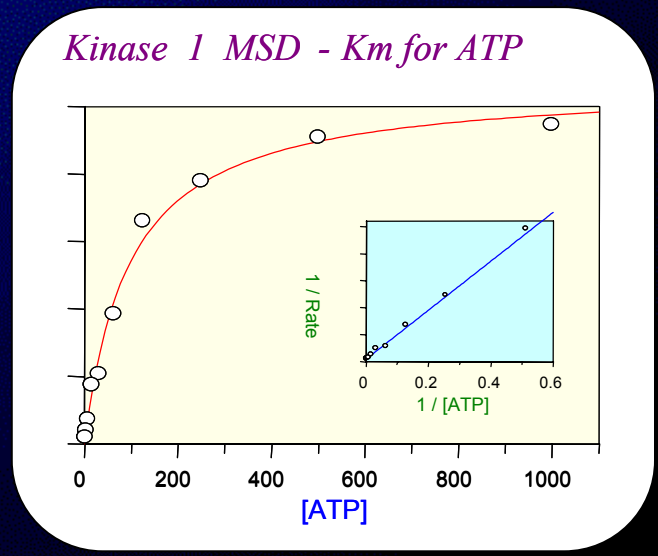
Kinase Assay Development



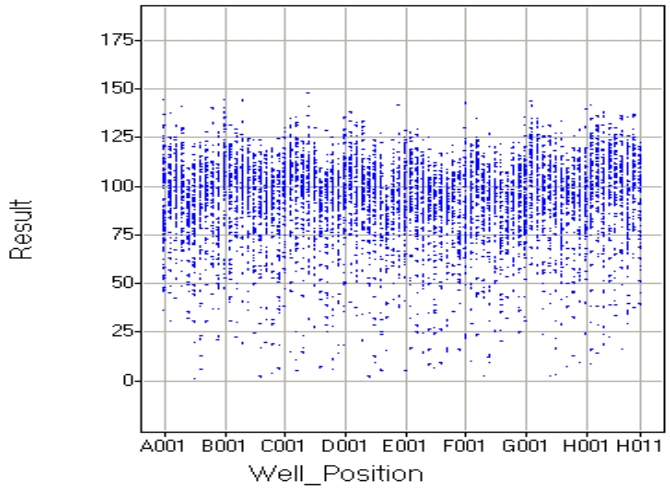


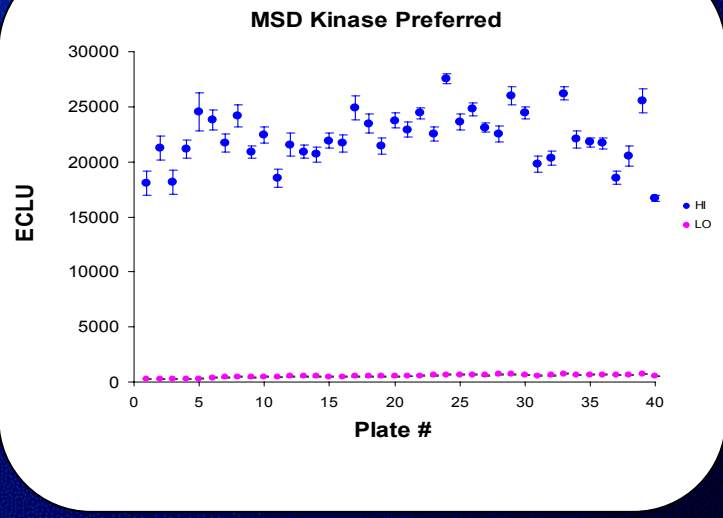
ATP $K_{m\ app}$ [µM]		
Meso Scale	**Lance**	**FBA**
96.56	**99.71**	**100.15**

- *Ser/Thr Kinase*
- *Transferable Assays*
- *Comparable To Other Platforms*
- *Less Enzyme (5-10 fold)*

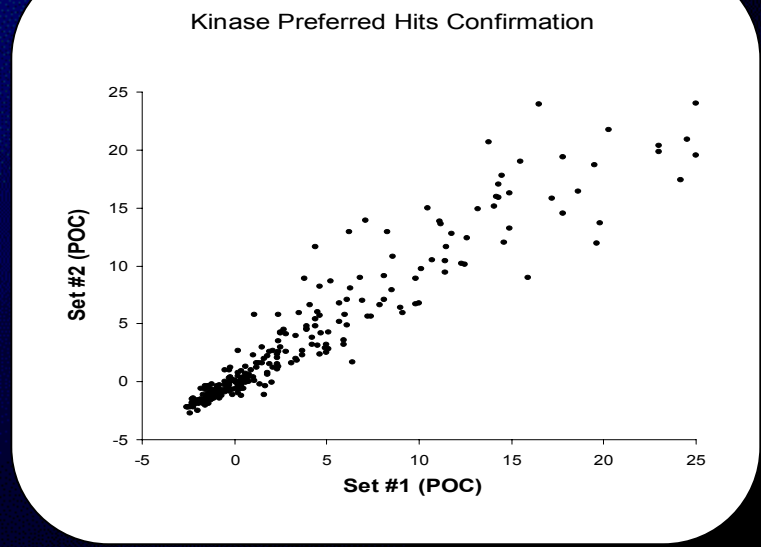


Kinase Preferred

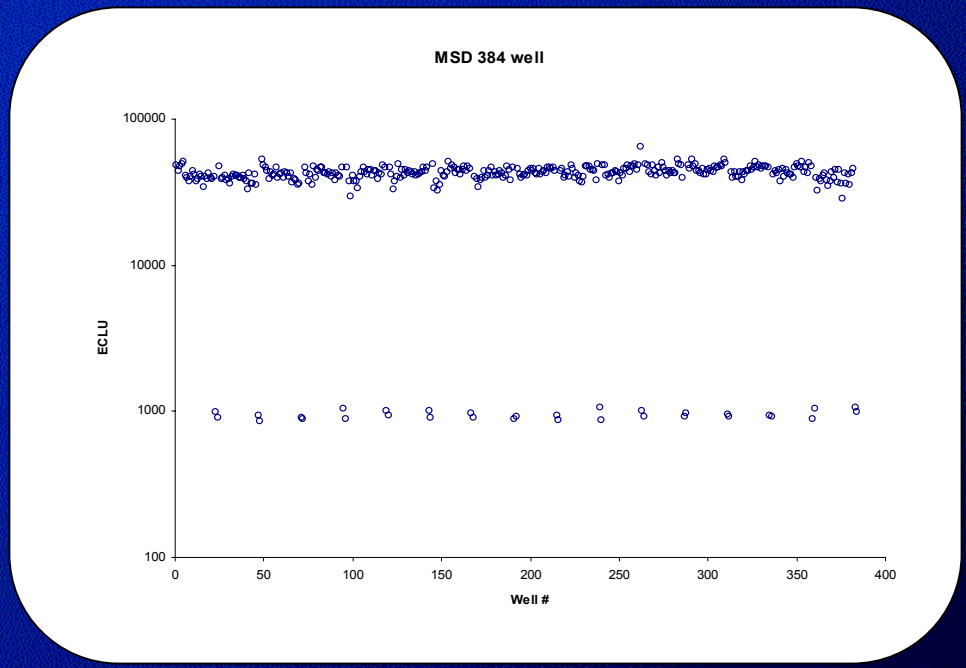




- *Preferred Collection 40 µM*
- *Kinase = 2 nM*
- *S/B= 40*
- *Z' = 0.68*



384 Well

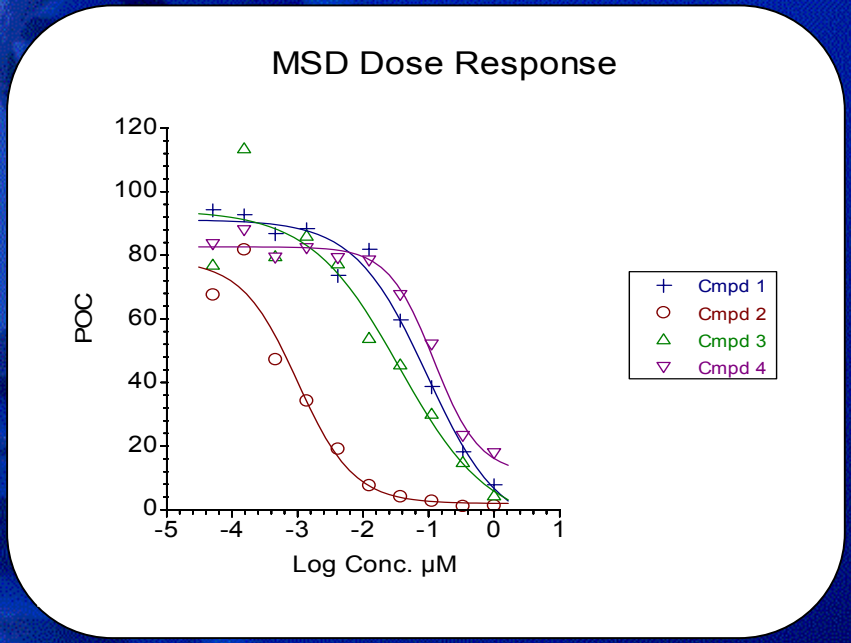


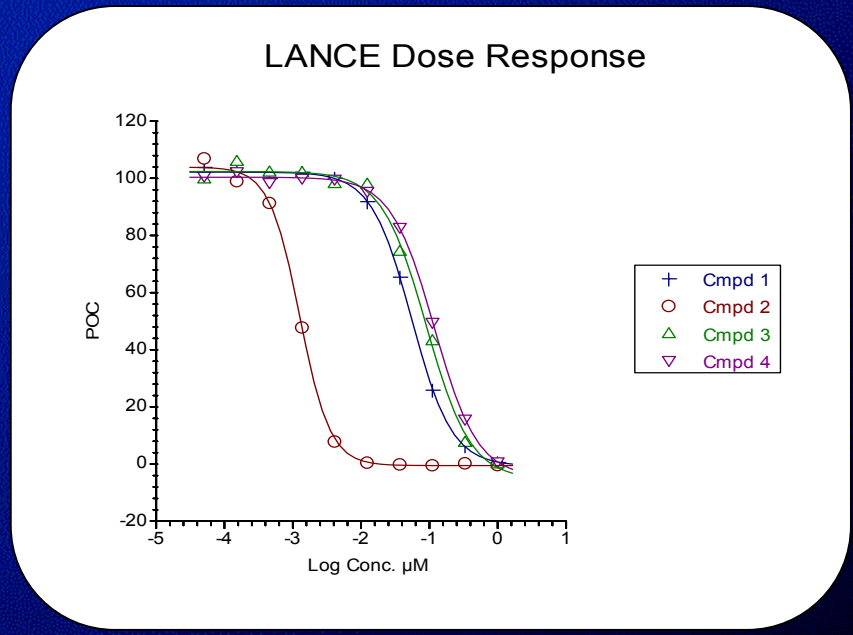
- *Kinase = 5 nM*
- *50 µL Vol.*
- *S/B= 45*
- *Z' = 0.67*

Read Time

Sector HTS	*Discovery*
~ 1 min	*>8 min*

LANCE vs. MSD

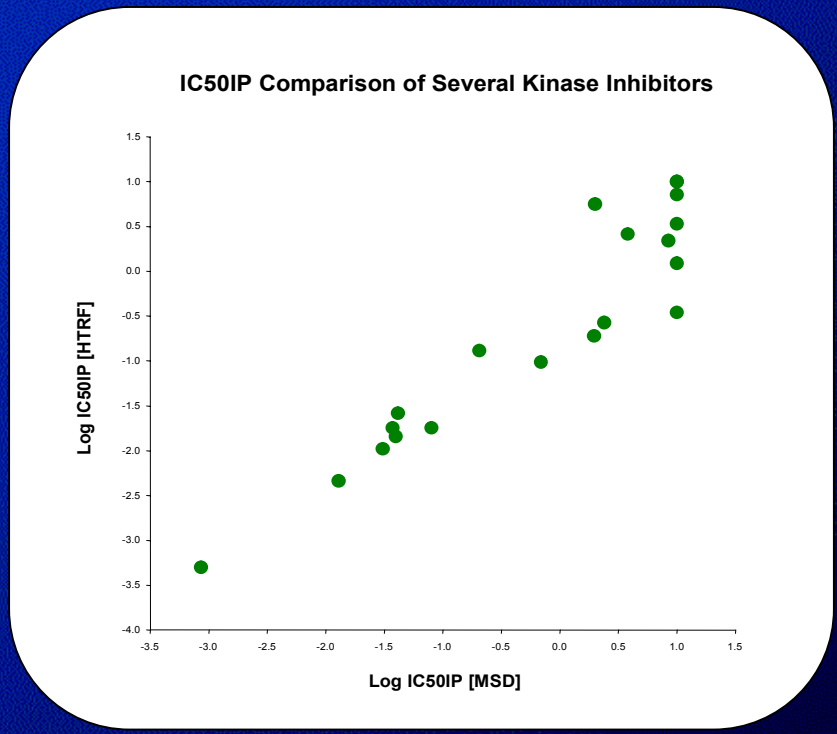




- *Same as TR-FRET*
- *Intrinsic Potency*
- *Selectivity*
- *5x less protein*
- *Lower 'bottom end'*

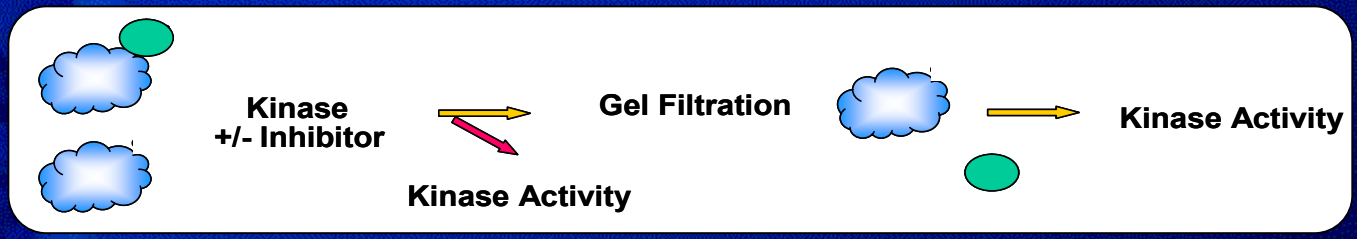
	MSD [IC50 µM]	LANCE [IC50 µM]
Cmpd 1	0.0954	0.0548
Cmpd 2	0.001	0.0013
Cmpd 3	0.0362	0.0892
Cmpd 4	0.12	0.1102
S/B	37	7

LANCE vs. MSD

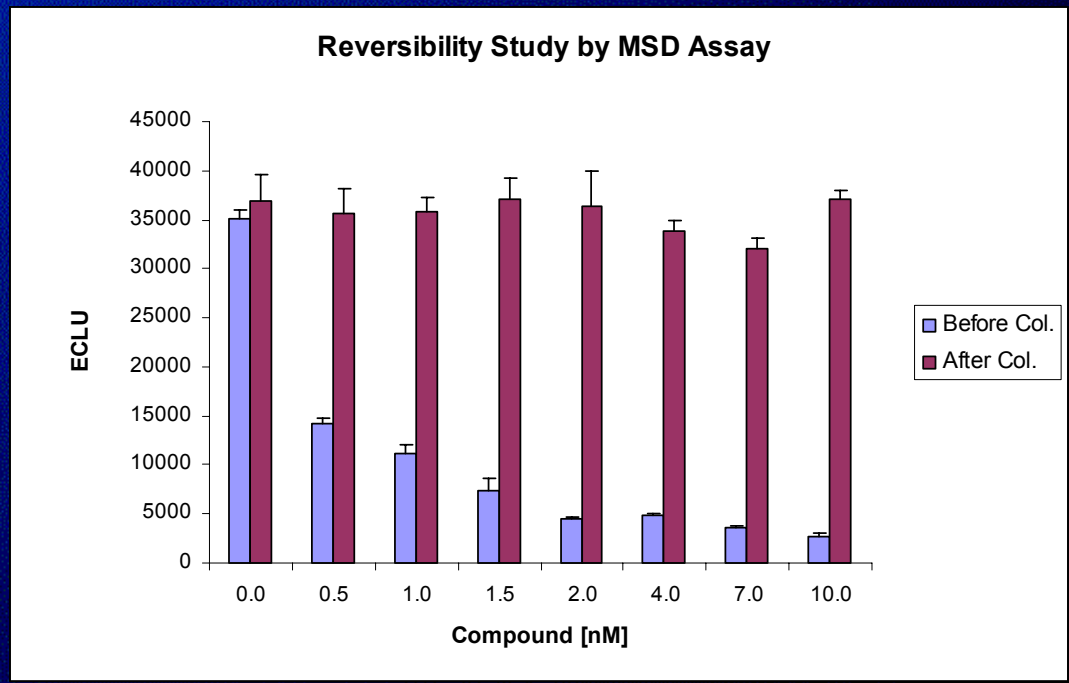


- *20 Inhibitors in side-by-side comparison*
- *IC50 similar across both platforms*
- *Some compounds appear less potent in MSD*

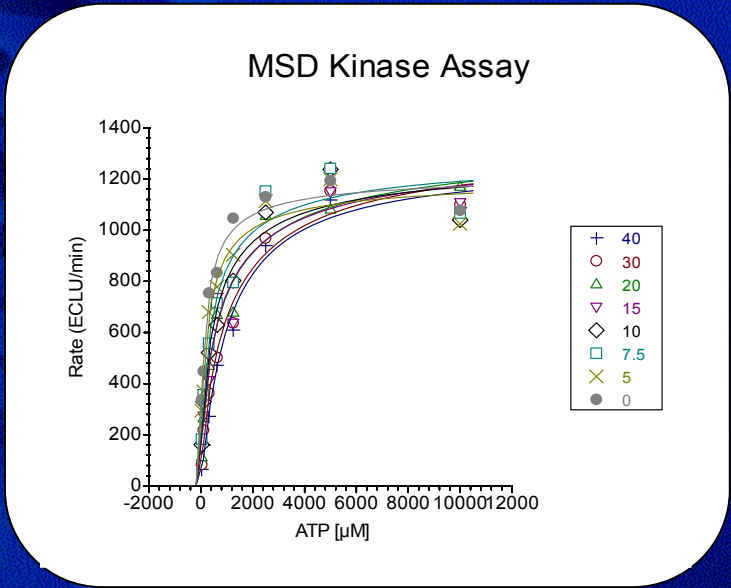
Mechanistic Studies

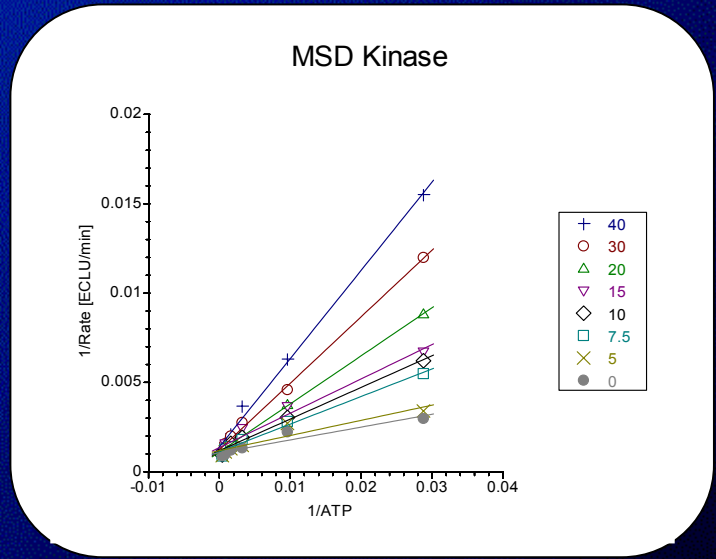


Are Inhibitors Reversible?

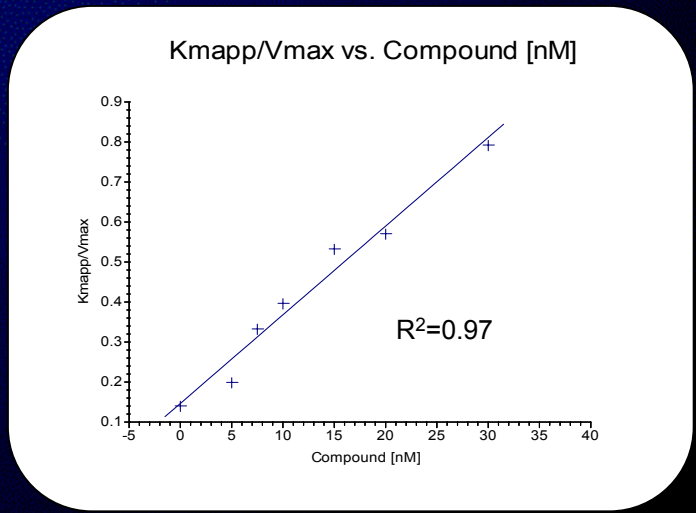


Mechanistic Studies



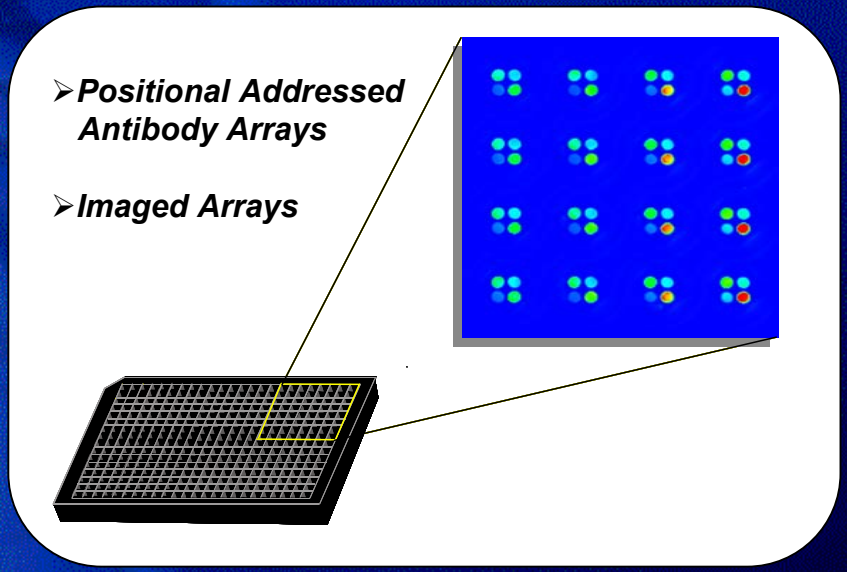


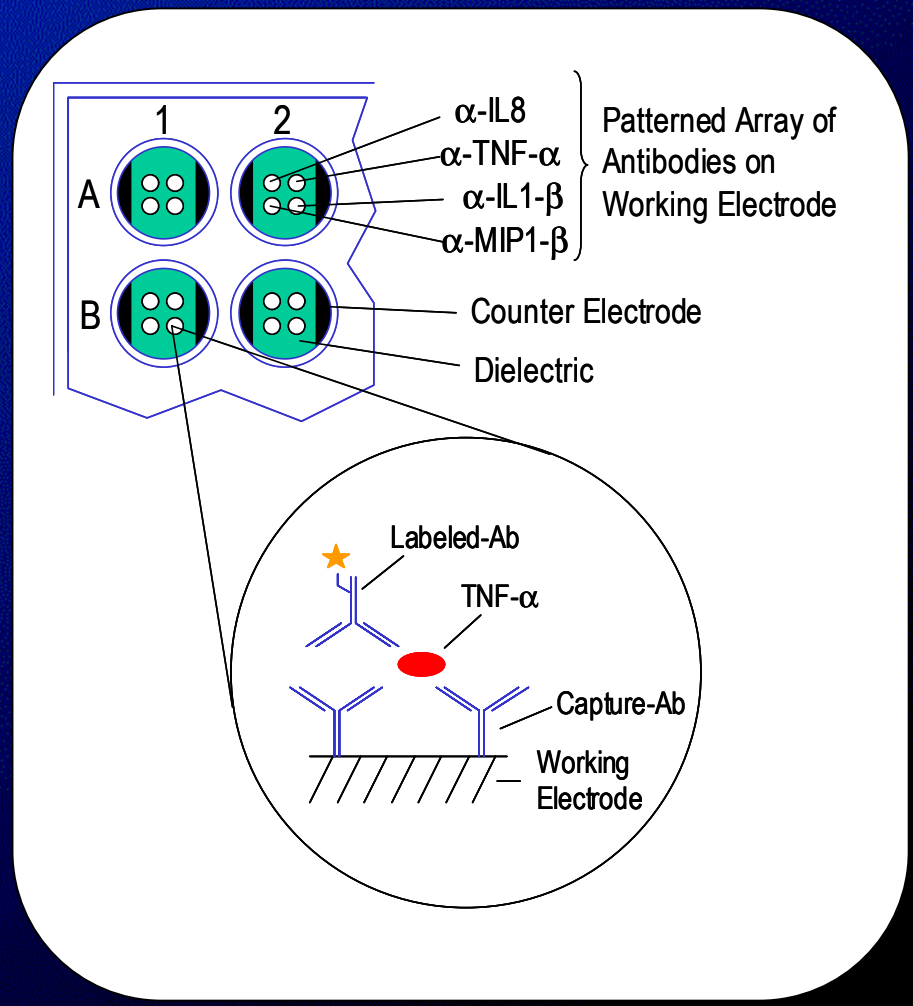
Are inhibitors ATP Competitive?



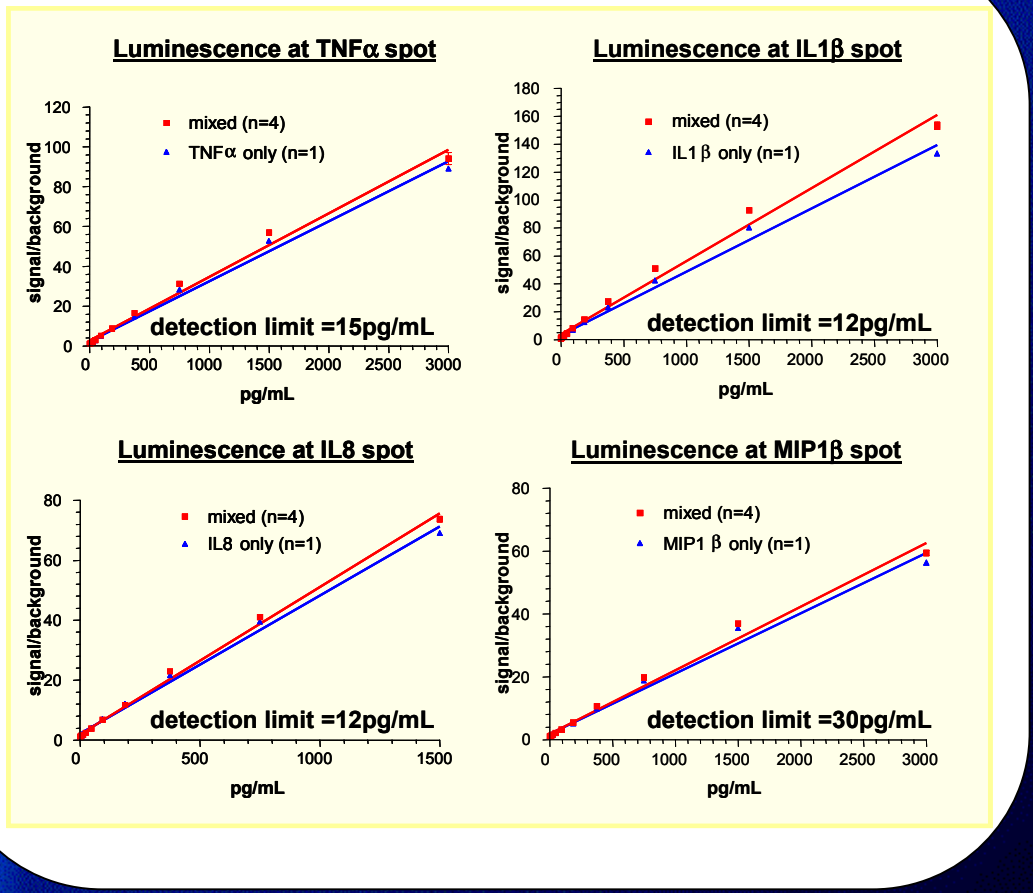
Multiplex Secretagogue Assay

- *LPS Stimulated THP-1 cells*
- *Dispense 50 µL media*
- *Add 100 µL Abs in MSD Buffer*
- *Incubate 2 hours*
- *Read*

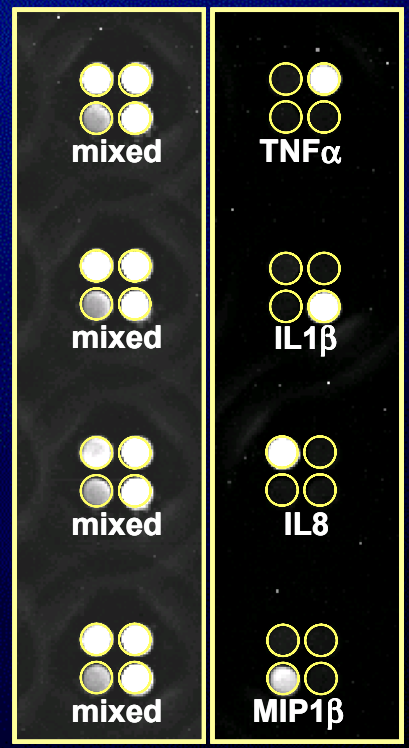




Standard Curves and Detection Limits



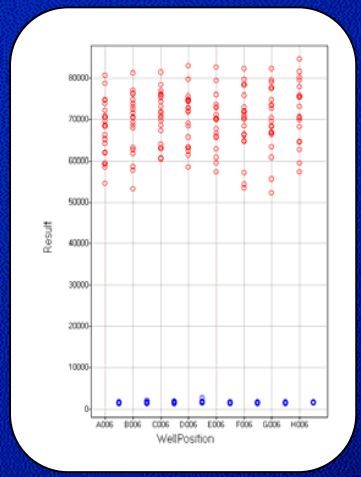
750 pg/mL cytokine:

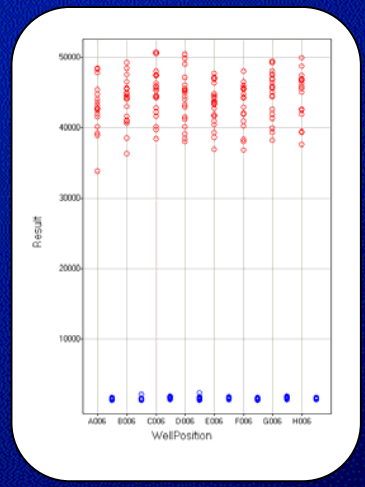


No Ab cross-reactivity or signal cross-talk

HTS Mutiplex

IL8
Z'=0.9 (intra)
Z'=0.8 (inter)

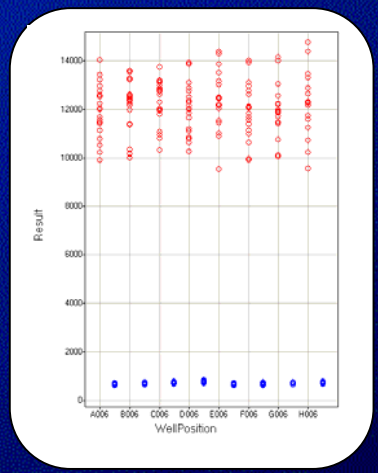


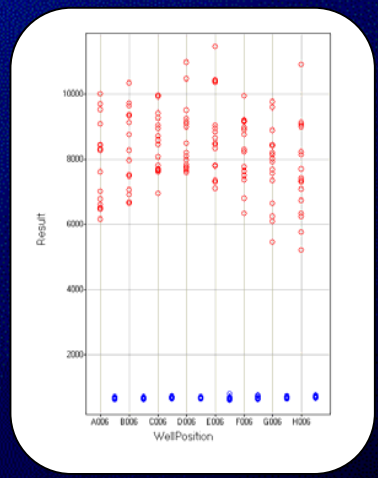


TNFα
Z'=0.8 (intra)
Z'=0.7 (inter)

HI/LO Scatter plots
20 plates

MIP1β
Z'=0.8 (intra)
Z'=0.8 (inter)

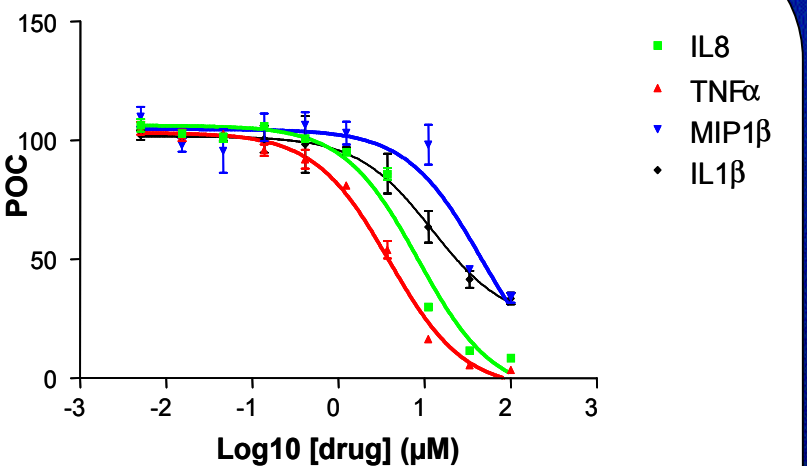




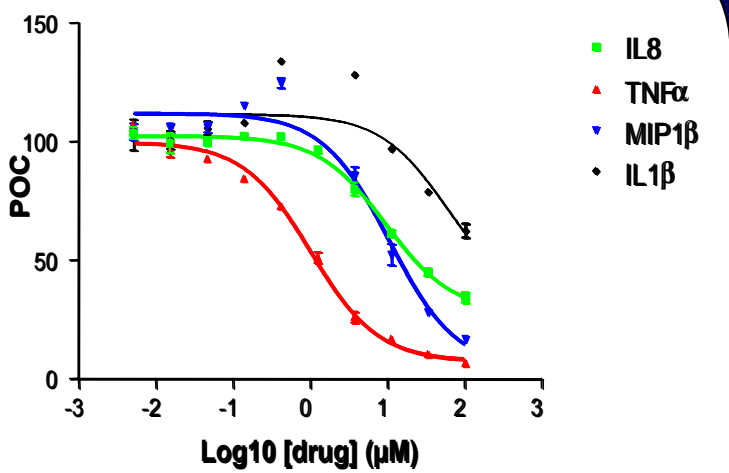
IL1β
Z'=0.8 (intra)
Z'=0.6 (inter)

Total 4320 Compounds Screened @ 10 μM

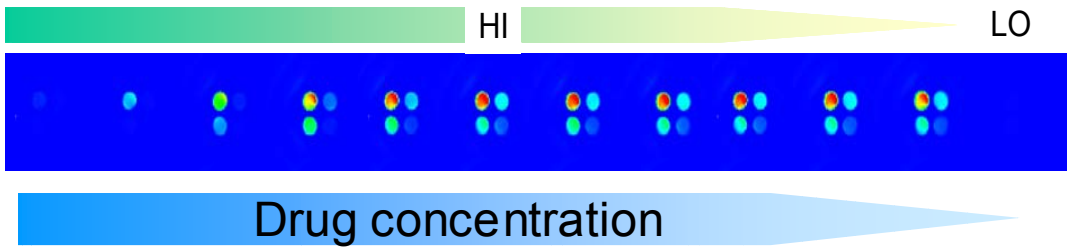
Multiplexed Dose Response

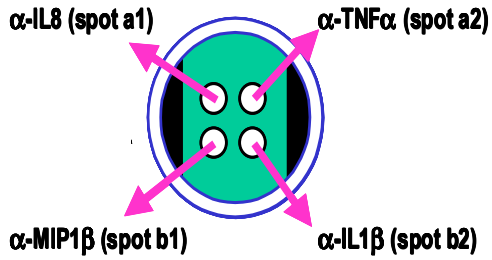


	IC50 IP	STDEV
IL8	6.76	0.30
TNFα	3.70	0.24
MIP1β	23.15	6.07
IL1β	10.60	1.36

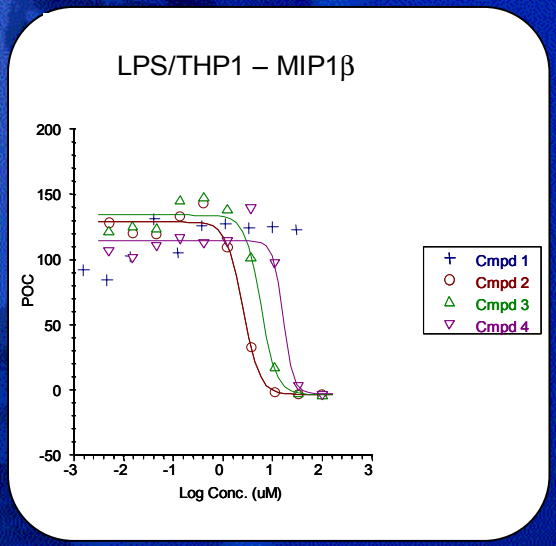


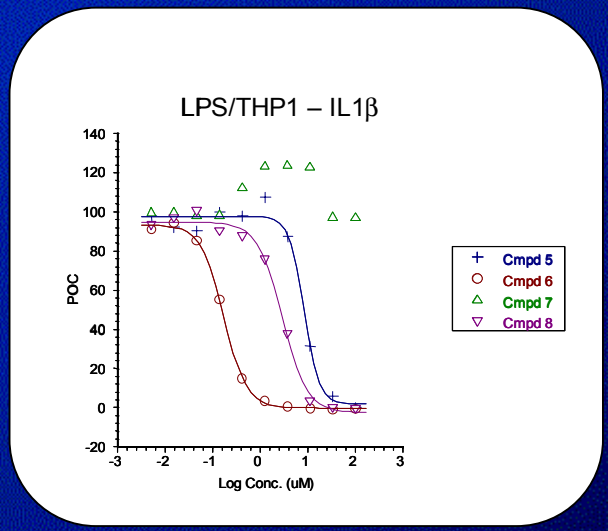
	IC50 IP	STDEV
IL8	8.32	1.64
TNFα	1.00	0.17
MIP1β	7.42	1.15
IL1β	>100	N/A

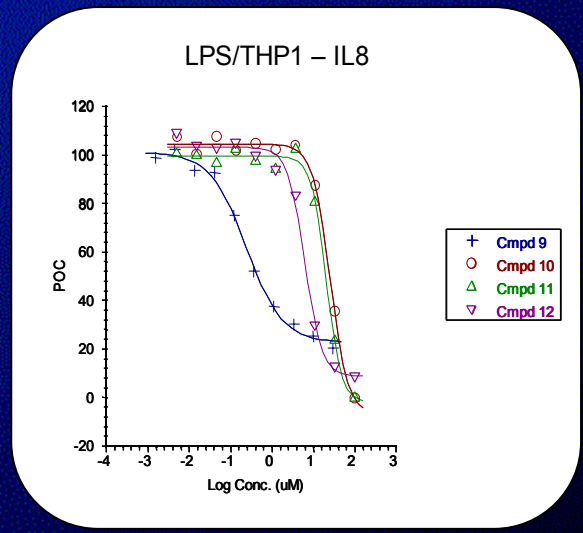


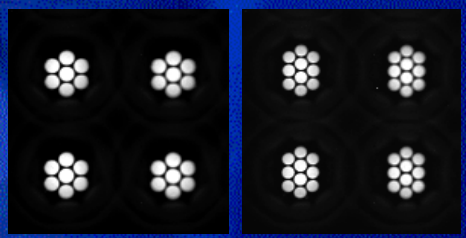


Quantitative Arrays



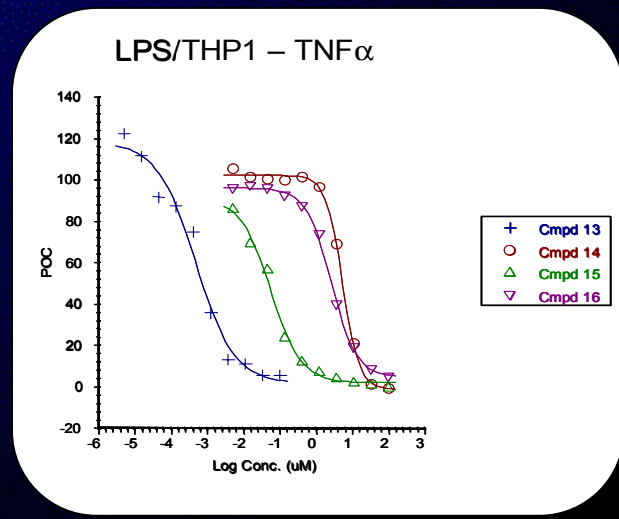






Enabling Capacity

Annotation of Libraries
Multiple Stimulators
Multiple Cell Lines
Multiple Species



…..towards fingerprinting

Lance vs. MSD

		Compound 1		Compound 2		Compound 3	
LANCE	TNFα	3.4 µM	TNFα	0.0007µM	TNFα	0.433µM
MSD	TNFα	5.49 µM	TNFα	0.0005 µM	TNFα	0.84 µM
	IL1β	3.5 µM	IL1β	0.008 µM	IL1β	5.3 µM
	IL8	6.8 µM	IL8	0.007µM	IL8	3.9 µM
	MIP1β	>30µM	MIP1β	>30 µM	MIP1β	>30 µM

Comparable across platforms

Conjugation of Ab pairs limits Lance™ Application

MSD Technology Platform

Pros

- Homogeneous
- Robust Stable Signal
- Short read times 1 min/plate (high density)
- Multiplex-High Content
- 1^{o} Antibody labeling not required

Cons

- Single Read
- Plate Based (logistics, storage)

Future Directions

- Kinetics- Solution vs. Solid Phase
- Carbon Surface Immobilization
- What biology won't work (Binning Targets)

Conclusion

MSD Technology Platform Fits into our Process

- Build assays quickly
- Identify 'hits'
- Assign Potency/Selectivity
- Determine Cellular Efficacy
- Establish *in-vivo* predictors

- Supports Higher Density
- Quantitative HTS Arrays

Identification, Stratification and Advancement of Compounds

Acknowledgments

Amgen

Yanyan Tudor
Violeta Yu

Meso Scale Discovery

Jim Wilbur
Kent Johnson
Charles Clinton
George Sigal